The Falkland Fritillary: Biological and Ecological Factors in the Conservation of *Yramea cytheris cytheris* (Drury 1773)

Nigel Robert Haywood

A thesis submitted in partial fulfilment of the requirements of Bournemouth University for the degree of Doctor of Philosophy

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I have often wondered how such an apparently delicate insect
manages to exist in such a wind-swept locality

Rupert Vallentin, 1853 - 1934

起きよ起きよ我が友にせん寝る胡蝶

Wake up! Wake up, sleeping butterfly! We have a long journey ahead

Matsuo Bashō, 1644 - 1694

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Abstract

This thesis aims to address gaps in the knowledge of the Falkland Islands' only resident butterfly, *Yramea cytheris cytheris* (Drury 1773), and thereby to contribute to an evidence-based approach to its conservation. Sightings had been infrequent, and its distribution, life cycle, habitat requirements and relationship to its Latin American co-subspecies *Y. c. siga* (Geyer 1832) little studied. Research set out here showed it to be an obligate *Viola*-feeder, most commonly on *Viola maculata*, which was widely, but sparsely, spread around the islands in small (< 1 ha) patches around the coast, particularly amongst dwarf shrub heath. *Y. c. cytheris* had a wide geographic spread, but records of only 21 populations had been recorded. For oviposition, it favoured medium-sized *Viola* in warm, sheltered locations, for example north-east facing slopes within a matrix of dwarf shrub heath, grass and patches of bare ground. There was evidence that it chose plants with above-average chlorophyll content. It appeared to be univoltine, laying its eggs singly, with a preference for warmer leaves; the larvae were not gregarious. Female adults were on the wing for an average of four days, the males five, over a flying season from December to February. It showed little mobility, even between adjacent patches. Populations were small, generally <10 on a given day, though catch rates varied considerably. *Y. c. cytheris* and *Y. c. siga* differed little genetically: the commonest haplotypes for each of the genes *COI, EF-1α, wingless*, as well as a concatenation of all three, were shared by both. Latin American butterflies were larger than those from the Falklands, with a lower wing aspect ratio. Morphometric analyses showed Latin American butterflies had more scalloped outer margins to their forewings. *Y. c. cytheris* showed local adaptation in claw shape, with those from the windiest sites being more curved. A draft species action plan sets out recommendations. There are still knowledge gaps in the life cycle, particularly diapause and pupation, and in population sizes and dynamics. An integrated morphometric and molecular approach is advocated in approaching relationships between populations. *Viola* conservation is important, especially in the light of climate change, with greater understanding needed of the role of grazing. Urgent consideration should be given to ensuring reintroductions can be made in response to extinctions. A case is made for *Y. c. cytheris* to become a flagship for insect conservation in the Falklands.

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Preface

The Falkland Island Government's Biodiversity Strategy identified the Falkland fritillary, *Yramea cytheris cytheris* (Drury 1773), as one of the species of concern for which plans should be drawn up to "identify the causes of decline, threatening processes and the specific measures needed to arrest and and reverse the decline, as well as any research, survey or monitoring requirements needed to underpin the action" (Falkland Islands Government 2008, p.13).

Falklands Conservation approached the UK NGO Butterfly Conservation for advice on implementation, and, following further discussions which included the Falkland Islands Government, the South Atlantic Environmental Research Institute (SAERI) and Bournemouth University, this project was established.

I am grateful to the following for their support for this project: Bournemouth University, for a Vice-Chancellor's Scholarship, together with a morale-boosting Sports Scholarship; SAERI, for air fares and logistical support for my three study visits; and the Shackleton Scholarship Fund, for a scholarship for my 2016-2017 study visit.

Ethics statement

Research work in the Falklands was carried out under Licence R19/2015 from the Falkland Islands Government. *Y. c. cytheris* is a protected species in the Falkland Islands (Falkland Islands Government 1999) and lethal collection of specimens was limited by quota. In all cases field work took place with the permission of landowners.

Acknowledgements

I am very grateful to a large number of people for the support I have had in preparing this thesis. Thanks first of all must go to my supervisory team at Bournemouth University, Dr Kathy Hodder, Dr Demetra Andreou, and Dr Emilie Hardouin, for their wisdom, encouragement and guidance. Their expertise in a wide range of disciplines has given my studies a breadth that I have found inspiring.

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Special thanks go to Paula and Steve Fowmes for their kind and generous hospitality in Stanley. Andrea Stanworth and Keith Biles generously loaned me vehicles, only one of which I broke. Danny and Joy Donnelly at Crooked Inlet, Mike Rendell and Phyl Rendell MBE on Bleaker Island, and Tony and Susan Hirtle at Peaks Farm made my time in Camp comfortable and enjoyable. Mike Rendell also generously provided me with five years of data from his weather station, and mixed an outstanding Martini. Micky Reeves's

considerable knowledge of Falklands wildlife informed my stay on Sea Lion Island: I hope this thesis encourages him to buy a strimmer to allow some sunlight to his violets. In the UK, thanks go to Dr Jim McAdam MBE for his help with climate data and the loan of a chlorophyll meter; to Dr James Hogan and Geoff Martin for allowing me access to the Oxford University Natural History Museum and the British Museum of Natural History collections respectively; to Justin Chamberlain at the Met Office; to Robin Woods for personal observations on the Falkland fritillary; to Dr Colin Clubbe of Kew for his help on plants and his infectious enthusiasm; to Professor Jeremy Thomas OBE for his advice and a copy of his unpublished paper on violet-feeding fritillaries; and to Dr Martin Warren OBE and Dr Nigel Bourn for their support and friendship since my days as an intern at Butterfly Conservation: I hope that one day BC, like the RSPB, will extend its operations to the UK Overseas Territories.

I have benefited greatly from correspondence with butterfly specialists overseas: Dubi Benyamini, of the Israeli Lepidopterists' Society; Dr Marjo Saastamoinen, Dr Anne Duplouy and the late Professor Ilkka Hanski of the University of Helsinki; and Professor Niklas Wahlberg of Lund University. Dr Alvaro Zúñiga-Reinoso of the University of Cologne kindly let me have some specimens from his own collection, and Olivier Burri of the Swiss Federal Institute of Technology, Lausanne, wrote a piece of code which solved a morphometric problem for me.

Two people deserve a special mention: Professor Edward Wilson of the Museum of Comparative Zoology, Harvard, who convinced me that it was never too late to become an entomologist, and enthused about the Falklands precisely because there were no ants there; and Dr Emily Heffernan, of New College, Florida, who has been unfailingly helpful when I've been bogged down, and whom I shall always remember for the great rallying cry, "It's the world's most southerly butterfly. Who wouldn't want to work on that?"

I would also like to thank my examiners, Professor Tim Shreeve and Dr Phillipa Gillingham, for their thoughtful and encouraging engagement with the thesis.

Most of all, my thanks go to my wife, Louise, who has cheerfully supported me through the whole process and helped me through the tough bits. As the old music hall song goes, "We've been together now for forty years, an' it don't seem a day too much".

Author’s declaration

This thesis comprises only my own original work, and due acknowledgement has been made in the text to all other material used.

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Chapter 1: Introduction

1.1 The Falkland Islands: an overview

The Falkland Islands archipelago comprises the two main Islands of East and West Falkland, together with over 500 smaller islands and islets. The islands, which have a total land area of about 1,220,000 ha, lie in the South Atlantic 400 km east of the South American continent. The highest point on the islands is Mount Usborne, 705 m. Much of the terrain is covered by acid grassland on poorly drained peat, or dwarf shrub heath where the soil is better drained (Armstrong, 1994; Liddle, 2007).

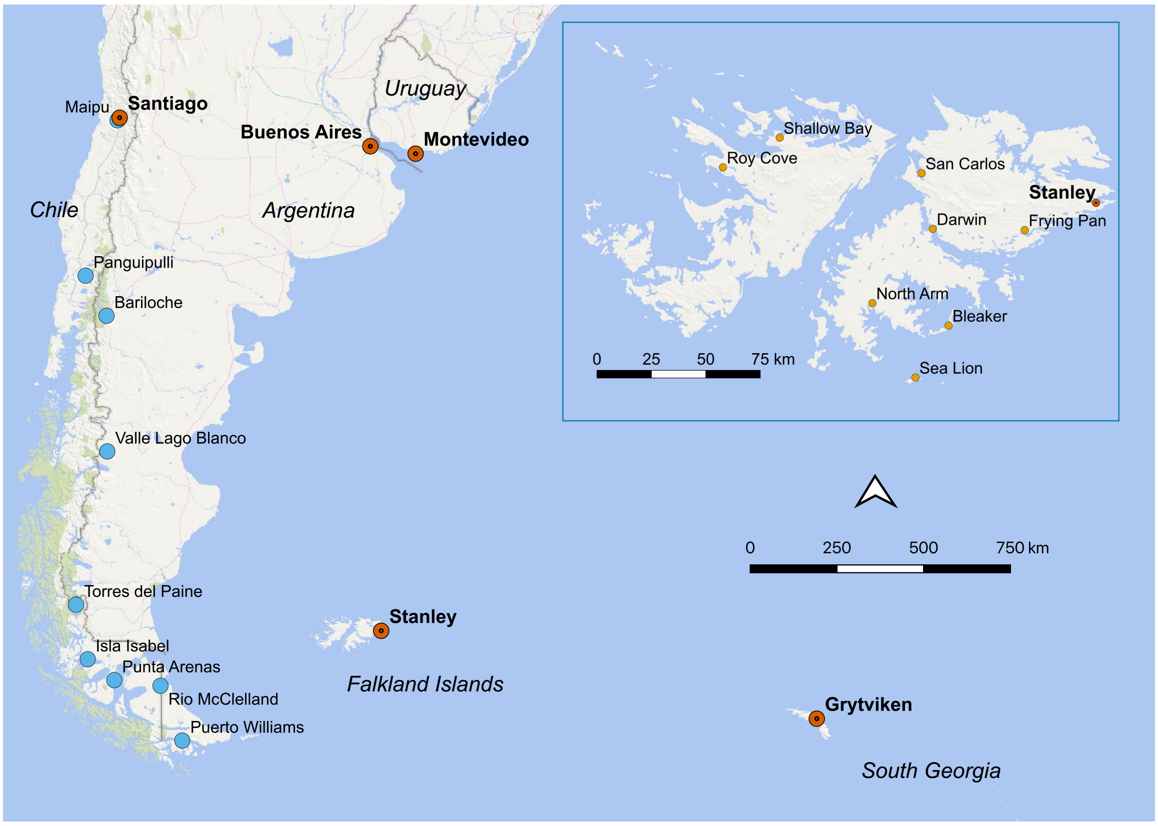

Figure 1.1 The Falkland Islands and Latin America. Country and UK Overseas Territory names are shown in italics; capital cities and major settlements in UK Overseas Territories are marked in bold. Sites from which specimens of *Yramea cytheris* were available for study are marked in blue for *Y. c. siga* and orange for *Y. c. cytheris* Land use: agricultural development and the impact of grazing

Earliest records of the Falklands, dating from the eighteenth century, described a landscape without trees or pasture, covered in heath, with tussac grass, *Poa flabellata,* up to two metres high, fringing the coast and covering small islands (Tourangeau et al.

2019). Cattle were introduced with the first settlers in the mid-eighteenth century, with numbers reaching a recorded peak of 60,000 in 1846; sheep, introduced at the same time, began to predominate in the late nineteenth century, reaching 807,000 by 1898 (Armstrong 1994, Palmer 2004). Grazing has shaped the environment, particularly in those coastal areas which would otherwise have been covered by tussac,

The Falkland Islands outside Stanley are sparsely populated. The 2012 census recorded 2,840 inhabitants, excluding the 1000 strong garrison, of whom 2,140 lived in Stanley (Falkland Islands Government 2013). Grazing remains the main agricultural activity, at a low density (Armstrong 1994, McAdam 2014), with 5,000 cattle and 500,000 sheep recorded in 2016 (Department of Agriculture, Falkland Islands 2017).

The aftermath of the 1982 conflict had an impact on the landscape (Royle 1994, McAdam 2013). The majority of large farm holdings, with owners outside the islands, were broken up, and sold to local farmers; a road network was built up, which gave relatively easy access to the settlements outside Stanley; the garrison was established at Mount Pleasant, serviced by regular flights and ships from the UK, set up in part to help the economic development of the islands; tourism began; a major fishing industry was established, and offshore oil exploration started.

There have been no major changes in land use since the introduction of grazing. Focus since the conflict has been on agricultural development, with improvement of the land to enable better support for livestock a priority. There has been an increasing focus on conservation since the foundation of the NGO Falklands Conservation in 1979, with re-planting of coastal tussac and the establishment of conservation areas.

1.1.1 Climate

Over the five-year period 2013 - 2017, temperatures recorded at the Falklands' main weather station, Mount Pleasant Airport, averaged 10ºC in January and February, and 3ºC in July and August (Figure 1.2) (Valor and López 2017). Snow occurred throughout the year, though seldom settled for long; rainfall averaged 38.9 mm a month, with little monthly variation. The wind was predominantly from the west or south-west, with an annual mean of 8 ms^{-1}, and marked by heavy gusting, averaging 19.3 ms^{-1} (Figure 1.3). At a wind speed of 8 ms^{-1}, the wind chill would make the perceived mean temperature away from shelter -2.4ºC in the winter and 6.7ºC in the summer (Osczevski and Bluestein 2005).

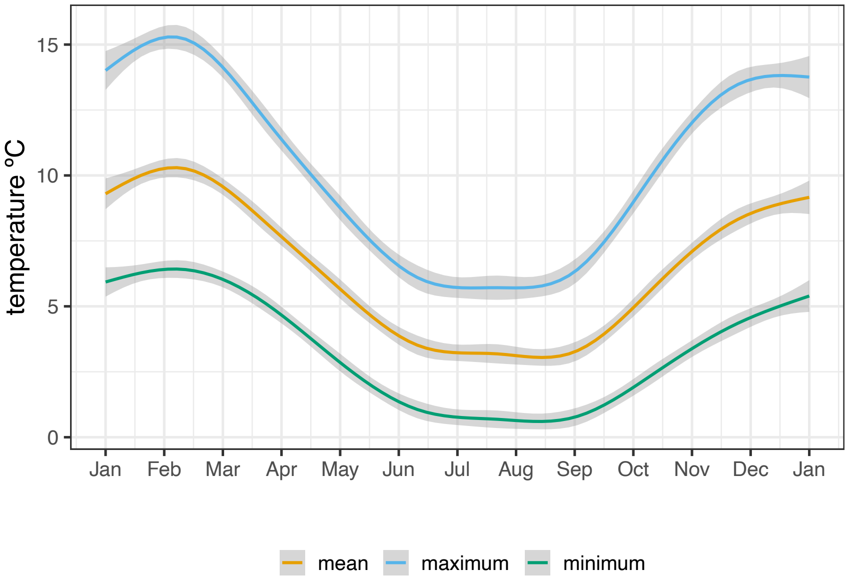

Figure 1.2 Annual temperature range at Mount Pleasant Airport over the five-year period 2013 - 2017. Shaded areas represent the standard error of the fitted regression lines after loess smoothing. Maximum and minimum temperatures ranges are based on weekly means.

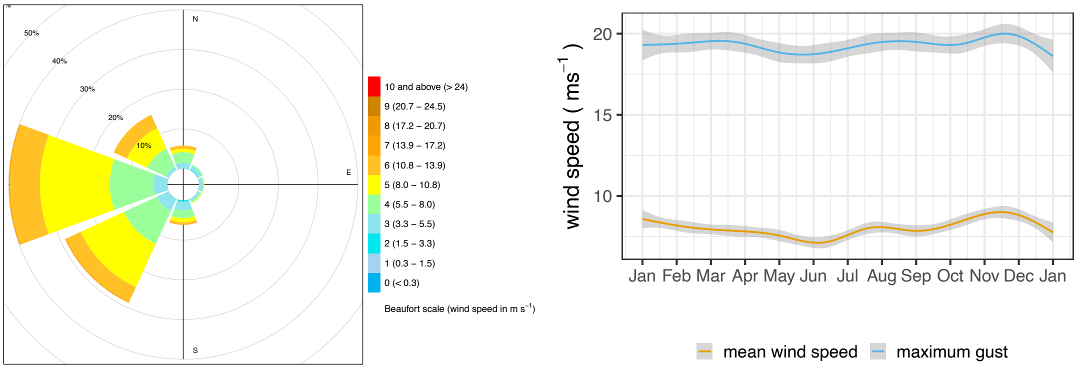

Figure 1.3 Annual wind directions and wind speed ranges from Mount Pleasant Airport. Data are from the five year period 2013 - 2017. The air flow was predominantly from the west and south west, with an annual mean speed of 8 ms^{-1}. The maximum gusts represent the weekly average of the highest wind speeds, with an annual mean of 19.3 ms^{-}1. Shaded areas represent the standard error of the fitted regression lines after loess smoothing.

1.2 Butterflies in the Falkland Islands

The Falkland fritillary, *Yramea cytheris cytheris* (Figure 1.4) is, as far as is known, the only butterfly resident in the Falklands. A southern monarch, *Danaus erippus*, caught on the islands, was exhibited in 1892 (South London Entomological and Natural History Society 1892). Robinson (1984) listed the southern painted lady, *Vanessa carye,* to which Jones (Jones and Lewington 2004) added the Brazilian painted lady, *Vanessa braziliensis*, speculating that the two species might sometimes breed on the islands.

Figure 1.4 The Falkland fritillary, *Yramea cytheris cytheris* , on a patch of wild celery, *Apium australe,* on Bleaker Island. Photograph by the author.

Jones also recorded a butterfly, the Falkland blue, which had yet to be collected and identified, although an example had been photographed on West Falkland in 1987 (Figure 1.5). This butterfly, which continues to be the subject of speculation in the Islands, was first reported at second hand by Vallentin (1901) as occurring in Stanley, though in 1904 he reported "it appears to be quite extinct" (Vallentin 1904, p. 22). Elliott (1927) recorded reports from Port Louis. Bálint et al (2013), on the basis of the 1987 photograph, placed the butterfly in the Latin American Lycaenid genus *Pseudolucia.*

The inclusion of the Falkland blue butterfly in the Falklands fauna is problematic. Most Lycaenid larvae have some form of association with ants, albeit obligate for only a small proportion (Malicky 1970, Fiedler 2006, Schär et al. 2018); ants, other than those found in biosecurity checks (e.g. *Ienipethima humile*, *Ochitellus* spp.), have not been reported in the Falkland Islands (Wetterer et al. 2007, James 2016). Lycaenid larvae also have a preference for Leguminosae as host plants (Downey 1962, Munguira et al. 2009), a family which only exists in the islands through introductions. These factors diminish the likelihood of the butterfly's being a long-term resident. One possibility is accidental introduction: for example a larva which subsequently hatched into a Lycaenid, *Lampides boeticus*, was found in a pack of imported peas (Penguin News 2011).

Figure 1.5 An unidentified blue butterfly photographed at Hill Cove in November 1987. The site was 10 km to the east of the settlement, at 350 m. Photograph by Chris Samson supplied by Dubi Benyamini of the Israeli Lepidopterists' Society.

Elliott (1927) reported sightings of yellow butterflies at Port Louis and Darwin without speculating on their species. No other records of yellow butterflies in the Falklands have been found. Darwin's (2005) and Fitzroy's (Darwin et al. 1839) observation of large numbers of butterflies about 800 km north east of the Falklands, off San Blas, in a strong north-westerly breeze, during the 1831 voyage of the Beagle showed mass movement, driven by wind, was possible. Darwin noted "infinite numbers of Lepidoptera" of various species, chiefly yellow; Fitzroy observed that they filled a space not less than two hundred yards in height, a mile in width and several miles in length. Williams (1930) suggested the yellow butterflies were *Colias lesbia*, a migratory species found in the Andes, with a range extending from Brazil to southern Argentina and Chile. These would appear a strong candidate for the yellow butterfly reported by Elliott.

1.3 *Yramea cytheris*: questions of classification

1.3.1 Position among the fritillaries

Yramea cytheris is a member of the tribe Argynnini (Nymphalidae: Heliconiinae: Argynnini), the fritillaries, mainly found in the northern hemisphere (Lamas and Grados 2004, Simonsen 2006). The species name *cytheris* had variously been ascribed to *Brenthis*, *Argynnis* and *Issoria* before settling in *Yramea* (Reuss 1921), a genus currently comprising six species, all, other than the Falkland population of *Y. cytheris,* found in Latin America, mainly in Chile, Argentina, Peru and Bolivia.

Simonsen et al. (2006) drew together work on Argynnini, investigating larval host plants, morphology and DNA. They concluded that it was a robust, monophyletic clade, which split into two further clades, the Euptoietina and a grouping of Yrameina, Boloriina and Argynnina. Yrameina only comprised the genus *Yramea*, Boloriina the genus *Boloria*, and Argynnina the genuses *Issoria*, *Brenthis* and *Argynnis.*

Y. cytheris was first described, illustrated by a male, as *Papilio nymphalis cytheris*, from a specimen "from one of the Falkland islands, situated near the entrance of the streights [*sic*] of Magellan" (Drury 1773, p. 7). Two sub-species are currently accepted: the nominate, *Y. c. cytheris*, the Falkland fritillary, found in the Falkland Islands, and *Y. c. siga* (Geyer, 1832) found in South America, principally Chile and Argentina. The basis for regarding them as co-subspecies is considered at 1.3.3.

1.3.2 Early classification

The first mention of what appears to be *Y. cytheris* as a South American species was as *Argynnis siga*, in Geyer's continuation of Hübner's *Zuträge zur Sammlung exotische Schmetterlinge* (Geyer 1832). The illustration, like Drury's, shows a male, in this case recorded, presumably in error (Burmeister 1878), as being taken in Java (present day Indonesia).

Y. cytheris is sexually dimorphic (Figure 1.6) the female resembling *Y. lathonioides* (Blanchard 1852), the sexes of which are alike. Blanchard (1852) and Reed (1877), perhaps assuming they were dealing with a similarly monomorphic species, recorded the female as *Argynnis anna* (which Reed had considered renaming *Argynnis chilensis*).

The species name lives on in the Spanish name for the butterfly, ana del sur (Klimaitis 2000).

Figure 1.6 Ventral surfaces of the right hind wing of *Yramea cytheris* showing sexual dimorphism. The female is on the left, the male on the right. Photograph by the author.

Butler (1881 pp 465-6) unpicked the confusion to some extent, although putting *cytheris* in the genus *Brenthis* and supporting the addition of a separate species, *Argynnis montana* (Reed, 1877) with a "much more vivid coloration of the under surface". By the time of Elwes (1889), who noted "The synonymy of the Chilian [*sic*] species of *Argynnis* is somewhat involved", and Staudinger (1899), who went over the previous literature and examined a range of new examples, the situation started to settle. Staudinger rejected *anna* as a separate species, stating that he had received many hundreds of specimens from Chile, and that, if the two species were commonly found, he would not have expected to find only *Y. cytheris.* Enderlein (1912) finally brought together as synonyms *cytheris, siga, anna* and *lathonioides*, although the latter is now recognised as a separate species. Herrera et al. (1958) summarised the various changes, and gave a useful timeline.

The differentiation between *siga* and *cytheris* was not, however, settled. Butler (1893) and Vallentin (1904) both described butterflies caught in the Falklands as *Argynnis siga.* Reuss (1921), who first proposed the genus *Yramea,* in which he placed it, used *cytheris* for the South American butterfly.

1.3.3 Basis for two subspecies

Watkins (1924) was the first to propose a subspecies, *Argynnis cytheris falklandica,* for the Falklands butterfly. This was unconventional, as the type of *cytheris* was caught in the Falklands, so should, as nominate, have been *A. cytheris cytheris.* The South

American subspecies would then have been given a different subspecies name. This might be because of the misconception that Drury's specimen was from South America: Butler (1881, p. 466) wrote "Drury's type was from the Straits of Magellan, and an example in the British Museum of Natural History, London (BMNH) from Port Famine agrees well with it: none of the Magellan males are quite so brilliantly coloured as the Chilian [*sic*] variety *B. siga.*" Watkins's proposed name, however, was not accepted, nor was a further sub-species proposal, with a new genus name, for a Chilean specimen, *Chilargynnis cytheris subtusviola* (Bryk 1944). The principle of subspecies was, however, accepted and the present-day situation was arrived at, with the nominate *Y. c. cytheris* representing the Falklands butterfly, and *Y. c. siga* the Latin American (Lamas and Heppner, 2004, Benyamini et al., 2014).

Just as much of the discussion about species was based on colour, so was the division into subspecies. Bryk's (1944) proposal made that explicit, with *subtusviola* suggesting a violet underwing. Watkins (1924, p.456) set out a clear differentiation for the Falkland subspecies:

> "This, the Falkland Islands race of *cytheris*, differs from the typical form from the Magellan Straits in the greater whiteness of the pale markings below (the costal marks of both wings and the long central dash of the hind wing), and also in the general tint of the light areas of the underside in the ♀, which have no trace of ochreous or brownish, but are whitish pink as a background to dark purplish markings."

1.3.4 Naming conventions in this thesis

(i) **English**

Y. c. cytheris is sometimes referred to as the Queen of the Falklands fritillary (Strange, 1992; Jones and Lewington, 2004). The earliest usage appears to be on a Falkland Islands postage stamp of 1984, designed by Ian Strange (Gibbons 2016). It possibly originated in *Y. c. cytheris's* earlier attribution to the genus *Issoria*, represented in Europe by *I. lathonia*, the Queen of Spain fritillary. In accordance with the Falkland Island Biodiversity Strategy (Falkland Islands Government 2008) the name *Falkland fritillary* is used in this thesis.

(ii) **Scientific names**

Yramea cytheris is used in the following chapters for both populations of *Y. cytheris* when it is obvious whether the Falkland or the Latin American butterfly is being considered.

The terms *Y. c. cytheris* and *Y. c. siga* are used when further clarity is required. This does not prejudge the question of whether the two populations constitute two separate subspecies.

1.4 *Yramea cytheris*: range and distribution

1.4.1 Latin American populations

Yramea cytheris has a wide distribution in Latin America, from Mendoza to Tierra del Fuego (Dapoto et al. 2003), a north-south range of over 1700 km. There is one possible mention for Ecuador, as "*A. cytheris*", though probably with "*A.*" for "*Adelpha*" rather than "*Argynnis*" (Brown 1950). If the latter, it would be very much an outlier. The attribution to Java (1.3.2), in present day Indonesia (Geyer 1832), is almost certainly an error. Klimaitis (2009) gives its range in Argentina as the provinces of Río Negro, Neuquén, Chubut, Santa Cruz and Tierra del Fuego. Benyamini et al. (2014) give its Chilean distribution as all the provinces from Metropolitan Santiago to Magallanes. This overall distribution is confirmed by the origins of specimens in the BMNH (*n* = 49: Huertas (2007)) and Oxford University Museum of Natural History (*n* =24: author's record), and through searches on the internet (Figure 1.7). Particularly helpful web sites for observations, supported by photographs, were *EcoRegistros.org* (*n* =22), and *iNaturalist.org.* (*n* =64).

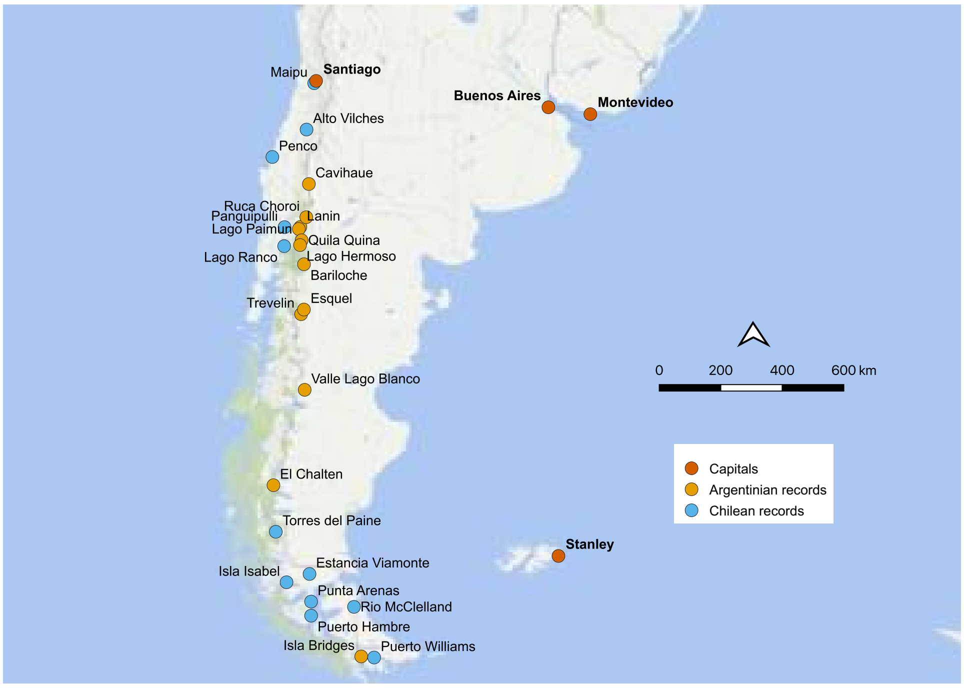

Figure 1.7 Latin American records of *Y. cytheris*, compiled from museum specimens and the results of literature searches. Capital cities, including Stanley, the principal settlement in the Falkland Islands, are shown for reference.

1.4.2 Falkland Island populations: 19th and 20th century records

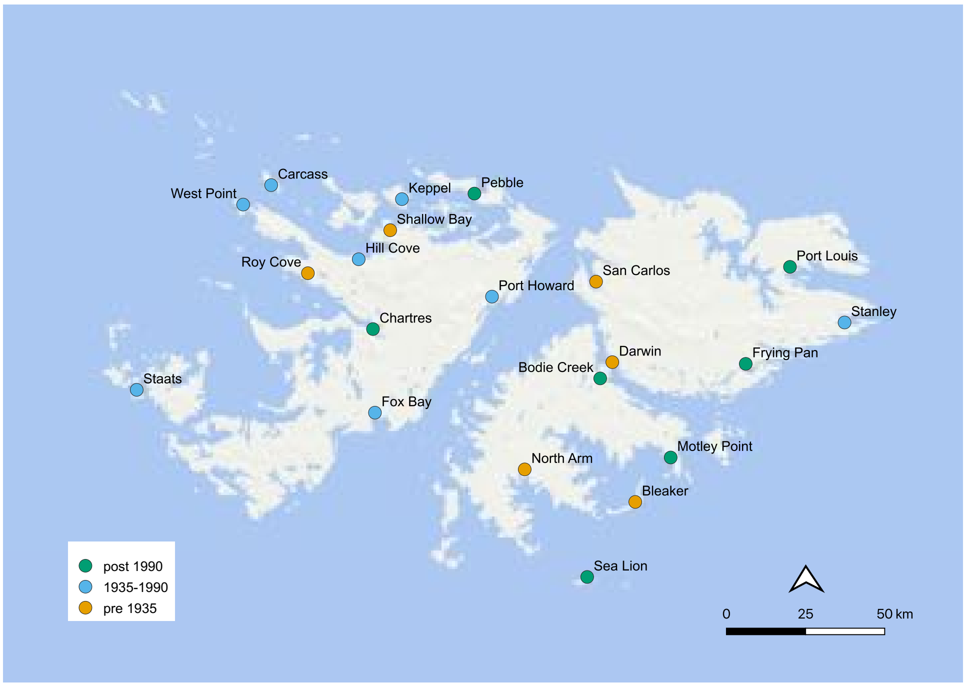

Figure 1.8 Earliest records of *Y. cytheris* in the Falkland Islands, divided into: pre 1935, drawing on museum specimens and the results of literature searches; between 1935 and 1990, drawing on Carstairs (1990); and post 1990, drawing on personal communications and observations.

After the specimen described by Drury, the next record of *Y. cytheris* in the Falklands was a single specimen caught at Darwin Harbour (Butler 1893), although the butterfly was already known on the islands by 1892 (South London Entomological and Natural History Society 1892). Vallentin recorded specimens of *Y. cytheris* at Roy Cove in January 1900, which were subsequently passed to the BMNH. He observed (Vallentin 1904, p. 22), "I noticed numbers [...] round the house, and also in the vast enclosures of Mr. Bertrand's property. The bright flowers growing in the sheltered corners of the garden seemed very attractive to these butterflies, and without any difficulty I captured a nice series of them". He added that the butterfly had been seen in various places in the West Falklands, but that Butler's example was the only record from the East Falklands, and "must have been a stray specimen blown thither by the wind". He noted that the best of Butler's specimens were incorporated with the national collection: they are now to be found at the BMNH (Huertas 2007).

Vallentin, in *Notes on Insects* in *The Falkland Islands* (Boyson 1924) recorded that he had collected further specimens from West Falkland in 1909-10. He gave two further records: of Dr Wace, who had found *Y. cytheris* "fairly commonly" in the Darwin area, and Colonel Reid, who had caught 14 specimens of the butterfly in Darwin between October 1908 and February 1909. Some of Reid's collection are also in the BMNH (Huertas 2007). Vallentin added that it was possible to ride for a whole day over West Falkland and never see more than two or three specimens.

Further probable records from the early 20th century are to be found in the papers of Arthur Cobb (Cobb 1996). He recorded a "red butterfly" at Hill Cove on 1 February 1910, two "buttermoths, rusty with sparks on" on Bleaker Island on 14 December 1910, and his "first red butterfly, during a heatwave" on 7 December 1922. One of the difficulties of assessing historical records is that migrant *Vanessa* spp. on the islands, especially the southern painted lady, *V. carye*, could be mistaken at a distance for *Y. cytheris.* These, however, usually appear from January to March (Strange 1992), so the two Bleaker sightings are most likely to be *Y. cytheris.* No historical specimens of *Y. cytheris* from Bleaker have been discovered.

Records are sparse later in the 20th century, although the BMNH have specimens from an unspecified location in the Islands donated by Elliott in 1934, and from San Carlos collected by Bonner in 1935 (Huertas 2007). Elliott was the Falkland Island Company's manager at North Arm. In a letter to the Company (Elliott 1927) he wrote that he believed the fritillary was common everywhere in the Falklands. He added that he did not get much chance of collecting away from North Arm, which suggests that his butterflies had been caught there.

Carstairs (1990) reviewed records of sightings of *Y. cytheris* in the Falklands. He noted that Robin Woods, an ornithologist who travelled throughout the islands from 1956 to 1963, recorded only two sightings over that period, in Stanley in the summer of 1961-2, and on West Point in February 1963. Carstairs himself, living on the islands from 1972-5, and travelling widely as a peripatetic teacher, recorded the butterfly only once, on Staats Island on 27 December 1973. He gave other reported sightings following the 1982 conflict, some in response to a radio appeal, all from West Falkland: one on Keppel Island in October 1983, and one on Carcass Island in January 1988, as well as reports from Hill Cove, Fox Bay and Port Howard between 1983 and 1989.

In a short follow-up report, Carstairs (1992) recorded that, to September 1991, substantiated sightings of the butterfly were known for 14 localities, in 13 ten-kilometre grid squares (the Falkland Islands, as a UK Overseas Territory, having been mapped by the Overseas Directorate of the Ordnance Survey). Ten localities were on West Falkland

and four on the East. Records were spread from November to February, with the majority (21%) reported in December. He observed that both elevation and habitat preferences appeared to be wide, with butterflies being recorded to 1000 feet above sea level and over a variety of site types, including diddle dee camp, white grass, re-seeded grassland and boulder fields.

Reports of butterflies from 1890 to 1999 show a patchy pattern, although there was a gap from 1935 to 1961 which gives some support to the view ascribed by Carstairs (1990) to those living outside Stanley that numbers had declined (Figure 1.9).

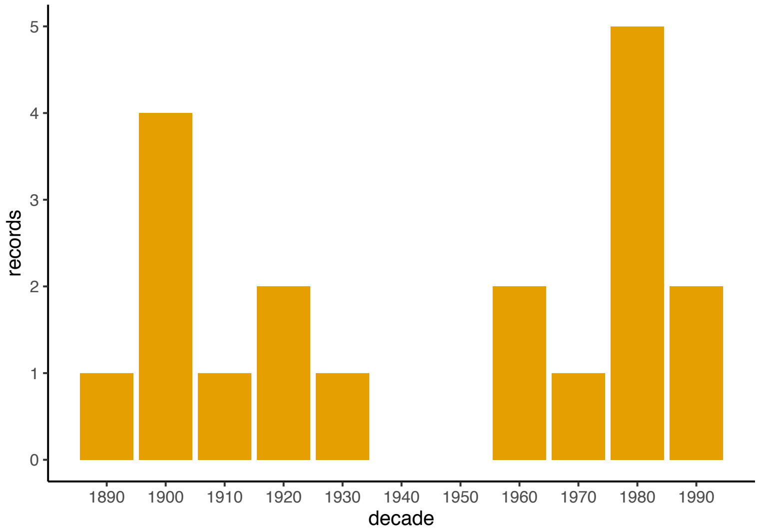

Figure 1.9 Recorded sightings of *Y. cytheris*, 1890 - 1999. Numbers refer to records, rather than to individual butterflies. Equality of search effort cannot be assumed. There appears to be no clear pattern, although there was a gap in records from 1935-1961, supporting a view, ascribed by Carstairs (1990) to those living outside Stanley, that numbers had declined.

1.4.3 Life cycle

The records in 1.4.2 gave no details of the life cycle of *Y. cytheris*, nor did they contain any observations of its eggs, larvae or pupae in the Falklands. Carstairs (1990) stated that the food plant of the larvae was not known, but might be the common violet, *Viola maculata* or gorse, *Ulex europaeus.* The Latin American population had already been associated with *V. tricolor*, with larvae feeding on *V. odorata* in captivity (Herrera et al. 1958), and with unspecified *Viola* spp. (Hovanitz 1970, Hayward 1973, and Dapoto et al. 2003). Woods (2000) said it was "highly probable" that the larval food plant of the

Falklands butterfly was *V. maculata,* which Jones and Lewington (2004) reflected. The earliest recorded observation of eggs and larvae in the Falklands was by Robin Woods, a visiting ornithologist (2010, personal communication, 1 November) in December 1996 on Carcass Island. He found eggs and a single larva of around 3 mm on *V. maculata* leaves. He added that he had observed the butterfly on Sea Lion Island, which had populations of *V. magellanica* but not *V. maculata,* and speculated that the former might also be a larval host plant. Dubi Benyamini, of the Israeli Lepidopterists Society (2012, personal communication, 1 April) recorded reports of eggs on *V. maculata* at Port Louis, and on cultivated *V. tricolor* in Stanley. No record was found of eggs laid on any genus other than *Viola*, although Shapiro (1992) observed, without supporting evidence, that in Latin America larvae also fed on Rosaceae (*Acaena*); nor was there any evidence of eggs being laid on bare ground or litter.

While there are inadequate records to set out a phenology, Vallentin gave his earliest sighting of the imago over the summer of 1909-1910 as 7 November, and the latest as 15 March (Boyson, 1924).

1.5 Larval host plants: *Viola* spp. in the Falkland Islands

The *Viola* genus comprises around 550 species worldwide (Ballard and Sytsma 2000). Their phylogeny is outlined by Ballard et al. (1999), with the position of *V. maculata* analysed by Marcussen et al. (2011). Six species of Viola have been recorded in the Falklands:

Viola maculata Cav. (Figure 1.10)

- range: Central and southern Chile and Argentina, northwards to 33ºS in the Andes where it is found at elevations up to 2500m;
- Falklands status: East and West Falkland. Fairly common (Vallentin and Cotton 1921); common and widespread (Broughton and McAdam 2005). Moderate vulnerability (Upson et al. 2016). Elevation 0 - 250 m (Broughton and McAdam 2005).

V. magellanica Forst. f.

- range: Southern Patagonia, northwards to 39ºS (Moore 1974);
- Falklands status: Sea Lion Island (Woods 2000) and a few isolated sites on West Falkland (Falklands Conservation, unpublished). Very rare (Upson 2012). High vulnerability (Upson et al. 2016). Elevation 1 m (Broughton and McAdam 2005).

V. tridentata Menz. ex Ging.

- range: Southern Patagonia, northwards to 47ºS (Moore 1968);
- Falklands status: Mountain species, very scarce (Vallentin and Cotton 1921); locally distributed, particularly on uplands (Broughton and McAdam 2005). Moderate vulnerability (Upson et al. 2016). Elevation 15 - 610 m (Broughton and McAdam 2005).

V. arvensis Murray.

- range: Eurasia and North Africa, widely naturalised elsewhere (Moore 1968);
- Falklands status: East and West Falkland. Cultivated and waste ground near settlements. Rather common (Moore 1968).

V. tricolor L. and *V. x wittrockiana* Gams. have also been recorded, as cultivars, by Falklands Conservation (unpublished).

Figure 1.10 *Y. cytheris's* main larval host plant on the Falklands, the common violet, *Viola maculata*. The example here is shown growing amongst wild celery, *Apium australe*, on Bleaker Island. Photograph by the author.

1.5.1 Distribution and dispersal

Data supplied from Falklands Conservation showed that *Viola* spp. were widely spread throughout the islands (Figure 1.11). *V. maculata* was the commonest, found most frequently in small patches amongst dwarf shrub heath on coastal slopes (Moore 1968, Woods 2000, Broughton and McAdam 2005, Liddle 2007). From the references in Vallentin and Cotton (1921) they appear to have been widespread for over a century.

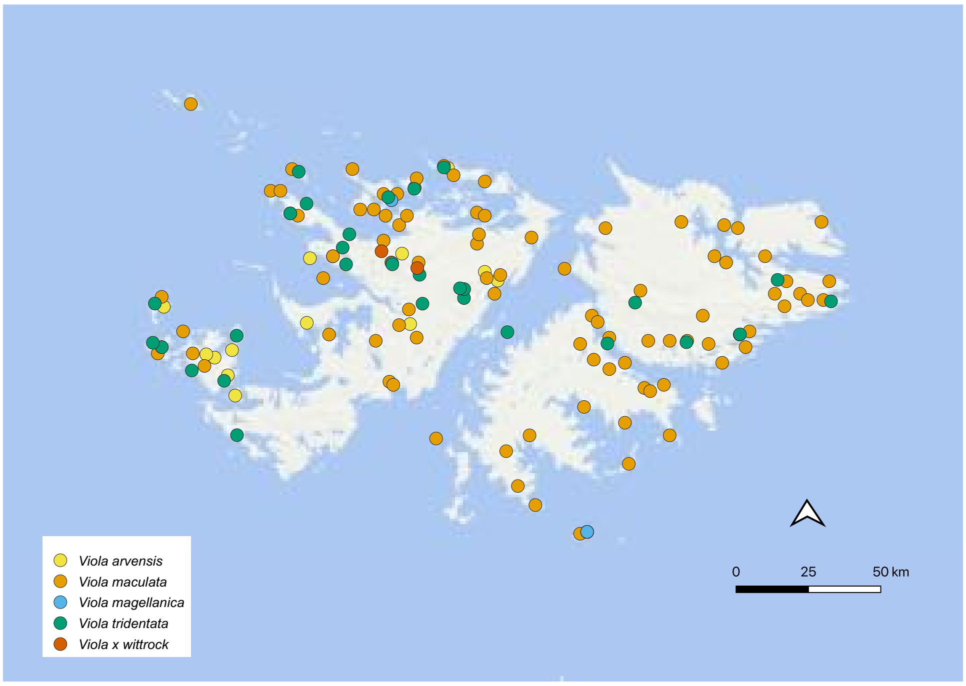

Figure 1.11 Distribution of V*iola* spp. in the Falkland Islands. Most of the sites were around the coast, but *V. arvensis, V. maculata* and *V. tridentata* were all found at elevations of up to 250 m. Data (current to 2013) were supplied by Rebecca Upson of Falklands Conservation.

The dispersal mechanism of *Viola* in the Falklands is not clear. There are three types of *Viola* dispersal generally recognised: myrmecochorous, or spreading by ants; autochorous, explosive ejection of seeds by the parent plant; and diplochorous, explosive ejection of seeds, followed by spreading by ants (Beattie and Lyons 1975). The role of ants in *Viola* dispersal, and the implications for the range of *Boloria* fritillaries in the UK, has been examined by Randle (2009). While the methods used by *V. maculata* and *V. magellanica* have not been recorded, the absence of ants in the Falklands rules out all but autochorous dispersal. This is a slow process: Beattie and Lyons (1975) gave the example of *V. odorata*, with a mean dispersal distance of 0.01 m. On that basis, were there local extinctions of *Viola*, recolonisation would be slow.

The possibility of endozoochorous dispersal of *Viola*, through birds or sheep, which would be faster, has not been studied. It would be particularly instructive to consider the possible role of sheep, as sheep tracks through dwarf shrub heath, for example, have, by definition, a large number of sheep passing along them, and also provide sheltered environments for *Viola* germination and growth.

1.5.2 Threats

The principal threats to *Viola* in the Falklands are climate change and changing land use.

(i) **Climate change**

Jones et al. (2013), compared eight regional climate models drawn up under the EU-funded CLARIS LPB Project. These projected a 1.8°C (± 0.34 *SD*) increase in the Falklands' mean annual maximum temperature by 2071-2100 compared with the period 1961-90, but little change in annual rainfall (20.8mm ± 39.0 *SD*). They did not model seasonality of change. A manipulation study of *Empetrum* heathland in the Falklands (Bokhorst et al. 2008), using climate warming scenarios of up to 1°C, showed little change in cover and biomass over its 12 year duration, although there was a 37% decline in soil arthropod abundance, possibly in response to higher temperatures and increased evaporation. Upson et al. (2016) identified a range of threats to Falklands dwarf shrub heath as a result of higher temperatures. These included changes in soil moisture levels, with consequent drying out and resistance to rewetting; an increase in invasive plants more suitable to higher temperatures, particularly gorse, *Ulex europaeus*; increased fire risk in dwarf shrub heath; and changes in soil organic carbon, with particular damage to peatlands, including risks of compaction, erosion and flooding. Assessing the responses of Falklands plants to this, they placed *V. magellanica* in the highest category of vulnerability, and *V. maculata* and *V. tridentata* in the medium, based on their exposure level and sensitivity (Williams et al. 2008).

(ii) **Land use**

Climate change would have an impact on stock density and forage resources. Grazing land, at present, has a low capacity for stock. Sheep density is approximately one sheep per two hectares (McAdam 2014); by comparison, upland farms in the UK carry upwards of 50 sheep per hectare. Landowners are constantly looking for greater efficiency: a warmer climate would change their decision-making process, with potential change in grazing patterns (Kerr 2002). A change in grazing patterns would change the potential habitat for *Viola,* with the impact unknown.

Grazing remains an important consideration in conservation work on the Falklands (McAdam 1980). Much of the tussac grass (*Poa flabellata*) which surrounded the Islands' coastline was destroyed over the past 150 years by overgrazing, with a deleterious effect on a wide range of native species which depended on it for shelter (McAdam 1980, Tourangeau et al. 2019). Moore (1968) considered that grazing could have had an impact on *Viola* spp., causing them to be restricted to the more lightly grazed coastal slopes.

1.6 Aims and research questions

The following research questions (RQs) formed the basis of the project.

RQ1. Do morphological and genetic comparisons support the classification of *Yramea cytheris cytheris* and *Yramea cytheris siga* as separate sub-species of the same species?

RQ2. What are the characteristics of suitable habitat for *Y. cytheris* both at patch and oviposition location level?

RQ3. Do the population dynamics of *Yramea cytheris* and its habitat requirements suggest that it can adapt to predictable climatic and other environmental changes on the Falkland Islands?

1.7 Statistical power and effect sizes

The data sets from the Falklands and Latin America were small, particularly when subsetting was necessary. This was an inevitable concomitant of working with small populations of an uncommon species (Cardini and Elton 2007), the butterfly's protected status in the Falklands, and the difficulty of obtaining samples from Latin America. This had implications for statistical power.

To enable assessment of results in these circumstances both effect sizes, and correlation strengths, using the appropriate metric together with associated *p*-values, have been given, together with confidence intervals (Anderson et al. 2000, Tan and Tan

2010). Effect sizes and correlation strengths have also been given a descriptor, such as "small", or "weak". The basis for these is Cohen (1988), modified in Sawilowsky (2009) and Fowler et al. (2013). Cohen's "t-shirt size" approach (Glass et al. 1981) has been criticised as detracting from the data (for a discussion, see Ellis 2010). Readily understandable descriptors can, however, be helpful where measurement scales vary, and are used here, in the form shown in Table 1.1, as an aid to interpretation (Lajeunesse 2013, Brenner 2019). In using this approach, Thompson's (2001) warning has been borne in mind: "If people interpreted effect sizes with the same rigidity that $\alpha = .05$ has been used in statistical testing, we would merely be being stupid in another metric".

The usage of descriptors has been even more controversial in the case of p-values: for a review see Hubbard (2015), and for an uncompromising adherence to significance equalling $p < 0.05$ see Hankins (2013). Descriptors have been used here, as with effect sizes and correlation strengths, as an aid to interpretation and also concision, "significant" meaning, for example, "significant at $p < 0.05$". They have not been invested with a pass/fail power: in the words of Rosnow and Rosenthal (1989), "surely God loves the .06 nearly as much as the .05".

Table 1.1. Effect sizes, correlation strengths, significance and their descriptors. Effect sizes are based on Cohen (1988), correlation strengths are based on Fowler et al. (2013), significance on Hubbard (2015 p. 203) and the usage of the R programme (R Core Team 2018). These conventions have been followed throughout the chapter as an aid to assessing the strength of a given effect, though they are supplementary to, rather than a replacement for, the calculated measures.

effect size			correlation strength			significance		
desc	d	η_p^2	desc	r	ρ	desc	p-value	R
very small*	0.1		very weak	0.1	0.1	significant	<0.05	*
small	0.2	0.01	weak	0.2	0.2	very significant	<0.01	**
medium	0.5	0.06	moderate	0.4	0.4	highly significant	<0.001	***
large	0.8	0.14	strong	0.7	0.7			
very large*	1.2		very strong	0.9	0.9			
huge*	2.0							

*The categories 'very small', 'very large' and 'huge' for d were proposed by Sawilowsky (2009)

1.8 Conventions

The following stylistic conventions have been observed:

(i) citations and bibliography follow Bournemouth University's Harvard-style recommendations (Bournemouth University 2018);

(ii) orthography, punctuation and the setting of formulas follow the guidance in *New Hart's Rules* (Waddingham 2014);

(iii) graphics employing more than one colour use the colour-blind friendly palette advocated by Chang (2012). When other colour palettes are in widespread use, e.g. for Phase 1 habitat surveys or Beaufort scales, conventional practice is followed;

(iv) *p*-values are given as 0.xxx, the fact of their being, by definition, <1 notwithstanding, to improve the appearance of tables.

1.9 References

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Chapter 2: Study sites

This chapter gives an overview of the study sites, providing background to the analyses in Chapters 3 to 7. It includes maps (sources at 2.2) recording oviposition sites and adult butterflies. The methodology behind the data points is covered in subsequent chapters: for oviposition sites see 4.2.1 and for adult butterflies see 3.2.2. Locations of *Viola* spp. shown on the maps were noted during the Phase 1 survey, and, while recording *Viola* presence, should not, with one exception, be regarded as exhaustive at the level of individual plant. The exception is for Sea Lion (2.3.4 (iii)), where the data points for January 2018 *Viola* in Figure 2.16 represent each individual *Viola* plant found in a three-hour search of the entire site.

2.1 Selection of study sites

The aim was to identify four study sites, the maximum possible given time, distance and resource constraints: one study site on East Falkland, one on West Falkland, and two others, including at least one island, to provide a broad spread on both the north-south and east-west axes. This would enable investigation of the genetic and morphological contrasts between populations and its relationship with geographical distance. Variation between the sites in topography and vegetation was also sought to assess any impact on oviposition preferences. There were two overriding considerations: a site had to have recent records of *Viola* spp. to make it worth investigating, based on the unpublished records held by Falklands Conservation (1.5.1, Figure 1.11); more important, during initial investigation, there had to be sufficient evidence of *Y. cytheris* - at the minimum adults in flight - to suggest that adequate data could be collected. An analysis of existing knowledge suggested seven candidate sites (Figure 2.1).

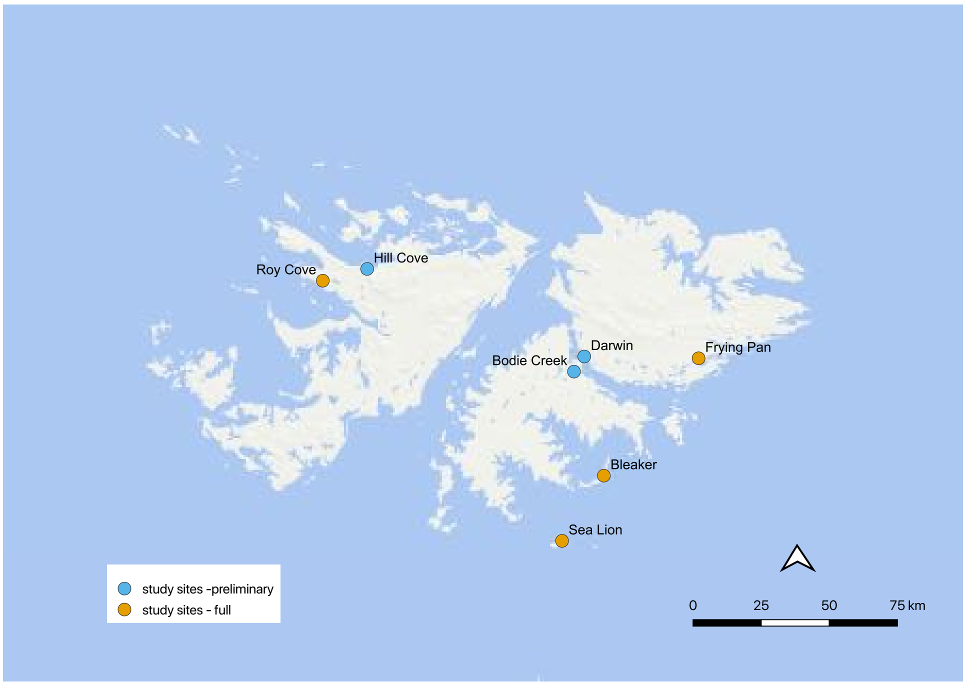

Figure 2.1 Falkland Island study sites. Those marked "preliminary" were investigated, but showed insufficient numbers of *Y. cytheris* to justify a full investigation. The four sites selected for full investigation provided the data for all subsequent analyses.

These were:

Bleaker Island: personal observation of adults in 2013 suggested a thriving colony. There was an historical dimension, from Cobb's observations in 1910 and 1922 (Cobb 1996). It was unusual in having only one known colony, which was on a raised beach. There were no other patches of *Viola maculata* on the island;

Bodie Creek: personal observation of an adult in 2013, and communications from Falkland Islanders who had seen numbers of adults, suggested there was a colony, with a number of smaller colonies nearby, which might be helpful for population structure analyses;

Darwin: the first record of *Y. cytheris* from a named Falkland Island location was from Darwin Harbour (Butler 1893). There are further records of captures by Reid and Wace in the early 20th century (Boyson 1924). Specimens collected by Reid are held by the Oxford University museum of Natural History (OUMNH) and British Museum of Natural History, London (BMNH);

Frying Pan: personal observation of small numbers of adults in 2010 had suggested the presence of small colonies of butterflies in a 1.5 km strip along a low cliff; eggs and larvae had also been found;

Hill Cove: Cobb (1996) recorded an adult in 1910. Carstairs (1990) reported sightings of adults in the 1980s. Falklands Conservation's unpublished records showed *Viola* were widespread, and at a range of elevations. It was one of two possible sites on West Falkland;

Roy Cove: Vallentin caught a series of adults in 1900 which are held by BMNH. This was the other possible site on West Falkland;

Sea Lion Island: personal observation of a single egg in 2013, together with communications from Falkland Islanders, suggested a small colony. This was the only location holding *V. magellanica* rather than *V. maculata.*

There were exploratory visits to Bleaker, the Frying Pan, Hill Cove, Roy Cove and Sea Lion in January and February 2016, and to Darwin and Bodie Creek in December 2016, during *Y. cytheris's* observed flying period. Each site was mapped following the guidelines for Phase 1 Surveys set out by the Joint Nature Conservation Committee (JNCC) (JNCC 2010). JNCC numbering, description and colouring of habitat types was followed, with the Falkland habitat numbering proposed by Upson (2012) included in the legends. This initial survey formed the basis for subsequent searches for *Viola* spp.

No evidence of *Y. cytheris* was found at Bodie Creek; two eggs, 4 km apart, were found at Hill Cove. Both sites were rejected as unlikely to provide enough usable data. This left Bleaker (31 adults, 59 eggs, 3 larvae), Frying Pan (2 adults, 23 eggs, 12 larvae), Roy Cove (12 adults, 31 eggs, 13 larvae), Sea Lion (6 adults, no eggs or larvae) and Darwin (1 adult, 3 eggs). Sea Lion was selected rather than Darwin, as it provided a contrast with the other sites, particularly in having a different larval host plant, *V. magellanica*.

2.2 Site descriptions: methodology

The study sites are described in a common format; the rejected sites are covered in less detail. The overview and land use sections were based on discussions with landowners, supplemented by guidebooks, particularly Wagstaff (2003), Wheeler (2004) and Summers (2005). Agricultural data were taken from the Falkland Island Government's website (Department of Agriculture, Falkland Islands 2020). Vegetation was recorded during the Phase 1 Survey, and identified principally through Liddle (2007), although Vallentin and Cotton (1921), Woods (2000) and Broughton and McAdam (2005) were also helpful.

Wind direction and speed data were taken over five summers (November to February), from 2013 to 2018 from the WMO weather stations nearest to each of the four study sites. The exception was Bleaker Island, which has a non-WMO weather station, a MetPak II (Gill Instruments, Lymington, Hants, UK) using PC200W software (Campbell Scientific, UT, USA). WMO weather station data were accessed from the OGIMET website (Valor and López 2017). Bleaker data were supplied by the landowner, Mike Rendell. Records were complete for all weather stations other than Sea Lion, for which data were available for only 291 out of a possible 596 days.

Each site description includes a photograph of the site, with features referenced by letters to a separate aerial map, and a Phase 1 Survey map. All maps were drawn up in QGIS 3.4 (QGIS Development Team 2018). The base maps were accessed through QGIS XYZ tiles from Bing Aerial (for Bleaker, Bodie Creek, Darwin, Hill Cove, Roy Cove and Sea Lion) and Google Satellite (for Frying Pan).

Wind roses were produced from weather station data using the package *openair* (Carslaw and Ropkins 2012) in R (R Core Team 2018).

2.3 Study site descriptions

2.3.1 Bleaker Island

(i) **Overview**

Bleaker Island (52.21° S, 58.85° W) is 26 km long, with a maximum width of 3 km, covering 2,070 ha. It is low lying, with the highest point being Semaphore Hill, 89 m (Figure 2.3). Bleaker lies off East Falkland, from which it is separated at its southern end by an 800 m channel, the nearest settlement being North Arm, 32 km to the west. It is a working farm and tourist destination. The northern end of the island is a National Nature Reserve.

(ii) **Vegetation**

The predominant vegetation of Bleaker is dwarf shrub heath, principally diddle dee (*Empetrum rubrum*) and Christmas bush (*Baccharis magellanica*), together with semi-improved neutral grassland, to a great extent fertilised by droppings from livestock and birds (Figure 2.2). Abandoned nesting sites, particularly those of king cormorants (*Phalacrocorax atriceps*), provide fertile substrate for groundsel (*Senecio vulgaris*). There are large stands of tussac (*Poa flabellata*) around the coast.

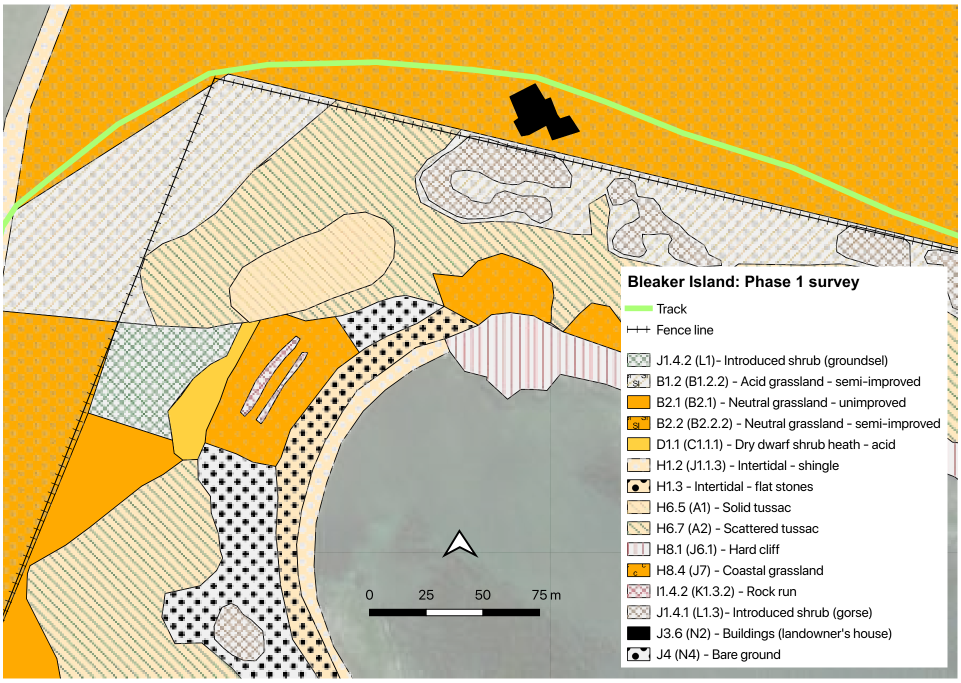

Figure 2.2 Bleaker Island: Phase 1 survey, January 2016. The main oviposition sites are around the edges of the two rock runs, with occasional sites on the edge of the neutral grassland where it meets the dwarf shrub heath.

(iii) **Violets and butterflies**

The main *Viola* patch, of *V. maculata*, is on the raised beach above Pebbly Bay. The patch is approximately 0.25 ha. Land within 5 km of the patch was surveyed over three field visits: the only other *Viola* spp. were found in a small clump north of Long Gulch (Figure 2.3) The land owners had not seen any others on the island.

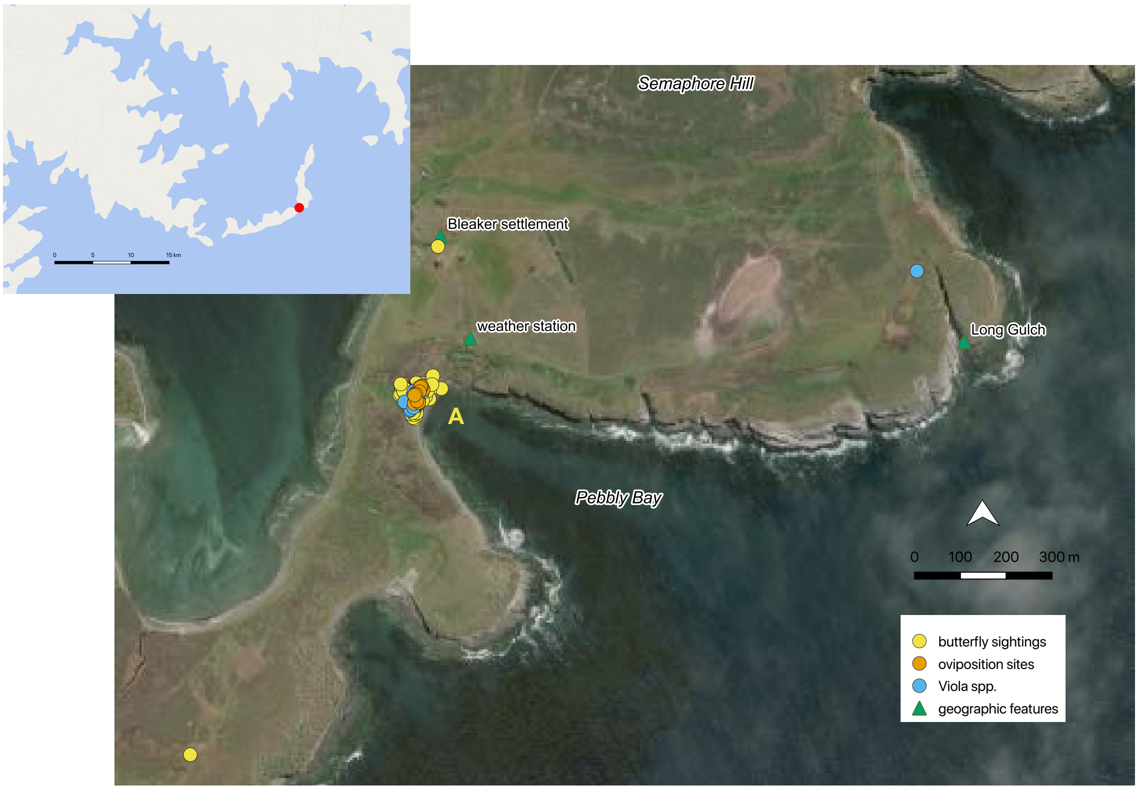

Figure 2.3 Bleaker Island *Viola* and butterfly sites, 2016 - 2018. The oviposition site, marked in red on the smaller scale map derived from Figure 2.1, covers an area of approximately 0.25 ha. The *Viola* there were thought, until 2018, to be the only ones on the island: a small clump, however, was found at the north end of Long Gulch in 2017. The "A" marked in the middle of Pebbly Bay corresponds to that in Figure 2.4. Figure 3.1 shows the butterfly site at a larger scale.

The butterfly site has two distinct rock runs (Figure 2.4) with *Viola* along their edges, and a large area of relatively bare ground at its southern end with a number of very bushy *Viola* plants. Vegetation includes wild celery (*Apium australe*), diddle dee (*Empetrum rubrum*), daisy (*Bellis perennis*), prickly burr (*Acaena magellanica*), sea cabbage (*Senecio candidans*), sheep's sorrel (*Rumex acetosella*) and acid grassland, both unimproved and semi-improved (Figure 2.2). There are scattered patches of tussac (*Poa flabellata*) around the patch.

Figure 2.4 Bleaker Island, Pebbly Bay, looking south. There is a wide rock run on the right of the picture, with a narrower one towards the middle. The main concentrations of oviposition sites are around the edges of these runs, on *Viola* sheltered either by stones or by wild celery (*Apium australe*). The "A" marked in the middle of Pebbly Bay corresponds to that in Figure 2.3. Photograph by the author.

(iv) **Land use**

Agriculture

Bleaker has been run as a farm from the late 19th century, at one stage with over 3000 sheep. It now has approximately 1000 sheep and 70 cattle. Bleaker's tussac is managed sustainably to provide winter feed for the cattle. The farm is organic.

Other

Bleaker is a popular destination for wildlife tourists who access it via its own air strip. While there is a landing stage for small vessels, it is not used for passenger traffic. There is accommodation for 12 and there are occasional day visitors. The footfall is not heavy, and all visitors are briefed by the owners to ensure they have as little impact on the environment as possible.

(v) **Wind**

The small weather station on Bleaker is run by the landowner, Mike Rendell, who provided wind data for five summers (November to February), from 2013 to 2018 (Figure 2.5). The wind was predominantly from the north-west, west and south-west with a mean speed of 6.85 ms^{-1}. The flat, pebbly nature of the butterfly patch, with little shelter other than *Apium australe*, means that the oviposition sites are very exposed

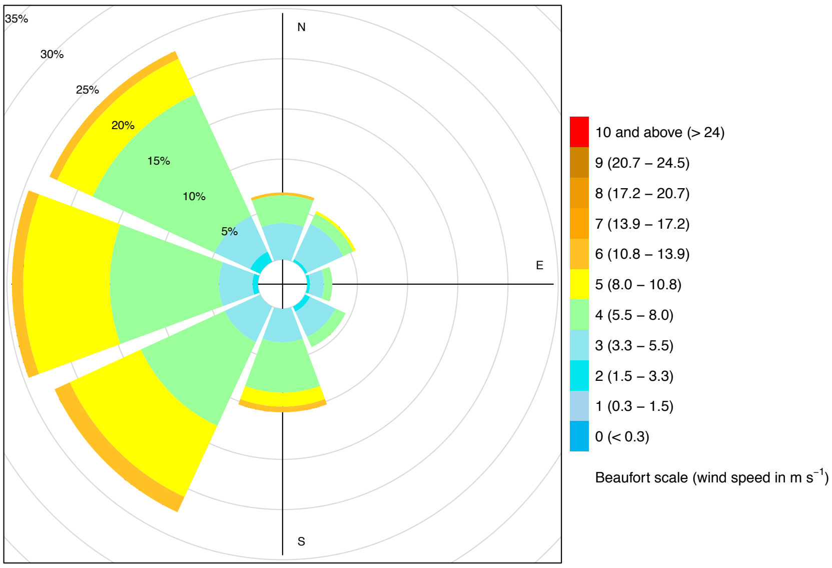

Figure 2.5 Wind rose for Bleaker Island, November - February, 2013 to 2018. The spread across the three westerly octants is unusual: none of the other sites has a strong northwesterly component. The oviposition site's location on a raised beach affords it little protection from the prevailing wind.

2.3.2 The Frying Pan

(i) **Overview**

The Frying Pan (51.81° S, 58.33° W), named from the shape of the creek (Munro 1998), opens into Island Harbour. It is on the Stanley - Mount Pleasant Airfield (MPA) road, the busiest in the Falklands. The creek has rock cliffs on either side, the north side being the site of a disused quarry. It is approximately 8 km east of MPA. The Frying Pan is part of Fitzroy lands, some 27,250 ha, held by Falkland Island Landholdings.

(ii) **Vegetation**

The predominant vegetation is dwarf shrub heath on a sand/gravel substrate (Figure 2.6) There is some marshy and neutral grassland, although most grass is in a mosaic with dwarf shrub heath and sand or gravel. Vegetation on cliffs is principally diddle dee (*Empetrum rubrum*) and Christmas bush (*Baccharis magellanica*) growing in crevices or on thin soil substrate.

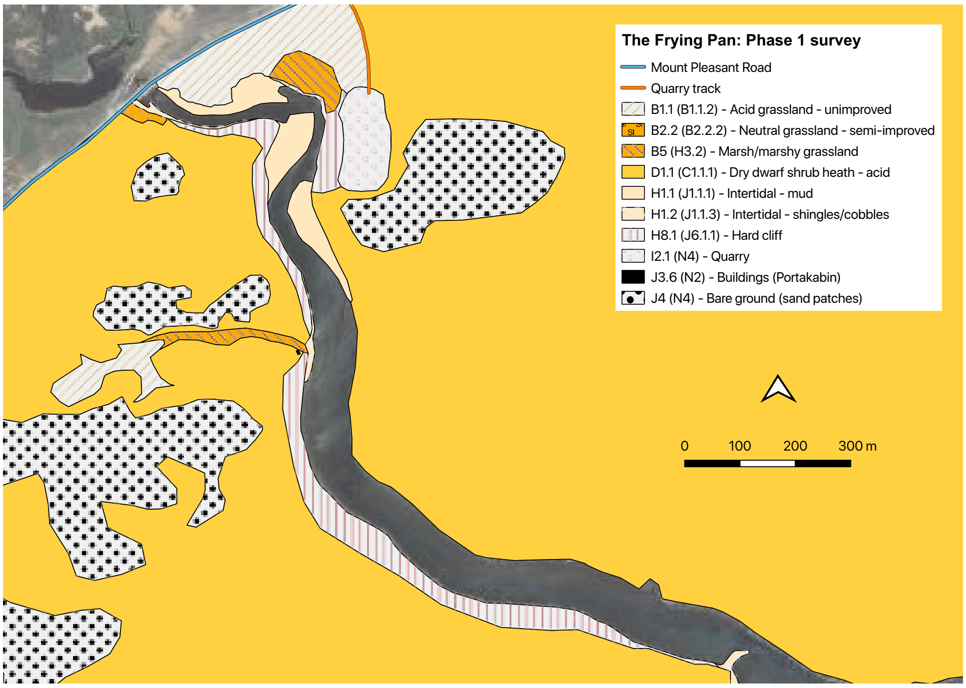

Figure 2.6 The Frying Pan, south cliff: Phase 1 survey, February 2016. The main oviposition sites are around the hard cliffs and in the areas where the cliffs meet the dwarf shrub heath. While much of the vegetation is shown as dwarf shrub heath, it takes the form of a mosaic with grass and bare sand or gravel.

(iii) **Violets and butterflies**

Viola spp. are widespread over the west bank of the creek (Figure 2.7), in groups of separate, distinct patches extending for over 1500 m. Only two small patches were found on the east bank. Individual plants are found on the flat, gravelly ground above the creek, but the greatest concentrations are on the steep slopes leading down to the water. Oviposition sites are concentrated in three main areas: the north-facing cliffs near the road; a small patch sheltered by rocks 300 m further south; and a grazed area of dwarf shrub heath by the sand bar at the mouth of the creek.

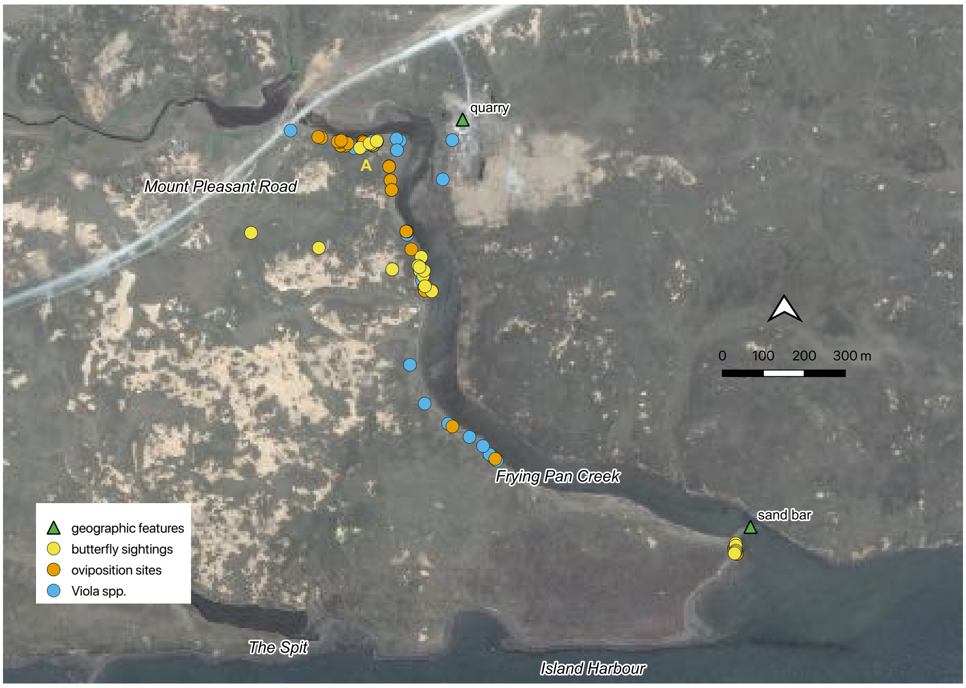

Figure 2.7 Frying Pan *Viola* and butterfly sites, 2016 - 2018. *Viola* plants are widespread, in patches, along the west bank of the creek. Oviposition sites are also spread, but concentrated in three main areas: the cliffs at the north end marked "A", corresponding to that in Figure 2.8; a small patch sheltered by rocks 300m further south; and a grazed area of dwarf shrub heath by the sand bar at the mouth of the creek.

(iv) **Land use**

The 16,000 strong flock of sheep at Fitzroy is the fourth largest in the Falklands. The Frying Pan is only sporadically grazed, including by a few horses around the sand bar (Figure 2.7). The Frying Pan is a popular, easily accessible angling location, particularly amongst the approximately 1000 service personnel at MPA. The north-facing cliffs on the southern bank (marked "A" in Figure 2.7) have a particularly heavy footfall.

Figure 2.8 The northern end of the Frying Pan. The flags mark oviposition sites, which are usually on small plants growing on shallow soil pockets between rock outcrops. The intertidal stones provide basking sites for the butterflies. The letter "A" corresponds to that in Figure 2.7. Photograph by the author.

(v) **Wind**

The nearest weather station is at MPA (WMO ID 88889). It is the only station on the islands with comprehensive coverage of all weather metrics. Mean summer (November-February) wind speed 2013 - 2018 was 8.38 ms^{-1}, with wind predominantly from the west to south-west (Figure 2.9).

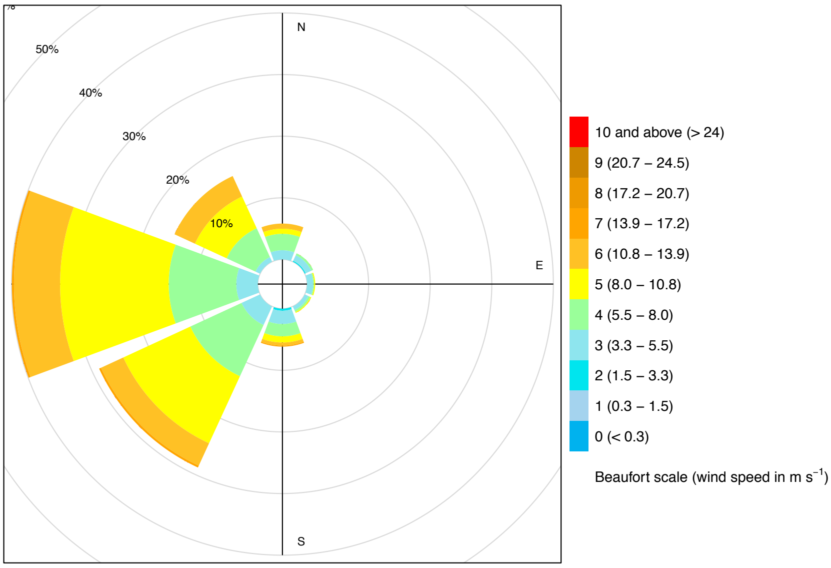

Figure 2.9 Wind rose for Mount Pleasant Airport, November - February, 2013 to 2018. The cliffs on the west bank of the Frying Pan afford shelter from the predominantly westerly and southwesterly winds, although the north-facing section of bank is exposed to strong westerlies.

2.3.3 Roy Cove

(i) **Overview**

Roy Cove (51.55º S, 60.38º W), established as a settlement in 1872, is on the north-west coast of West Falkland. The study site is mostly within the boundaries of Crooked Inlet farm. It is hilly countryside, dominated by Cooke Hill, 282 m. It is accessible by road from Port Howard, and has its own airstrip.

(ii) **Vegetation**

Roy Cove is a mixture of unimproved and semi-improved acid grassland, dwarf shrub heath, rock outcrops and bare sandy patches (Figure 2.10). Shelter near the settlement is provided by gorse hedges (*Ulex europaeus*). The dwarf shrub heath mainly comprises diddle dee (*Empetrum rubrum*) and Christmas bush (*Baccharis magellanica*) with some scurvy grass (*Oxalis enneaphylla*). There are patches of small fern (*Blechnum penna-marina*) and native yarrow (*Acaena lucida*) in rock runs and on the edges of dwarf shrub heath, together with dandelions (*Taraxacum* agg.) which provide a nectaring resource. Pig vine (Gunnera magellanica) is common in marshy grassland.

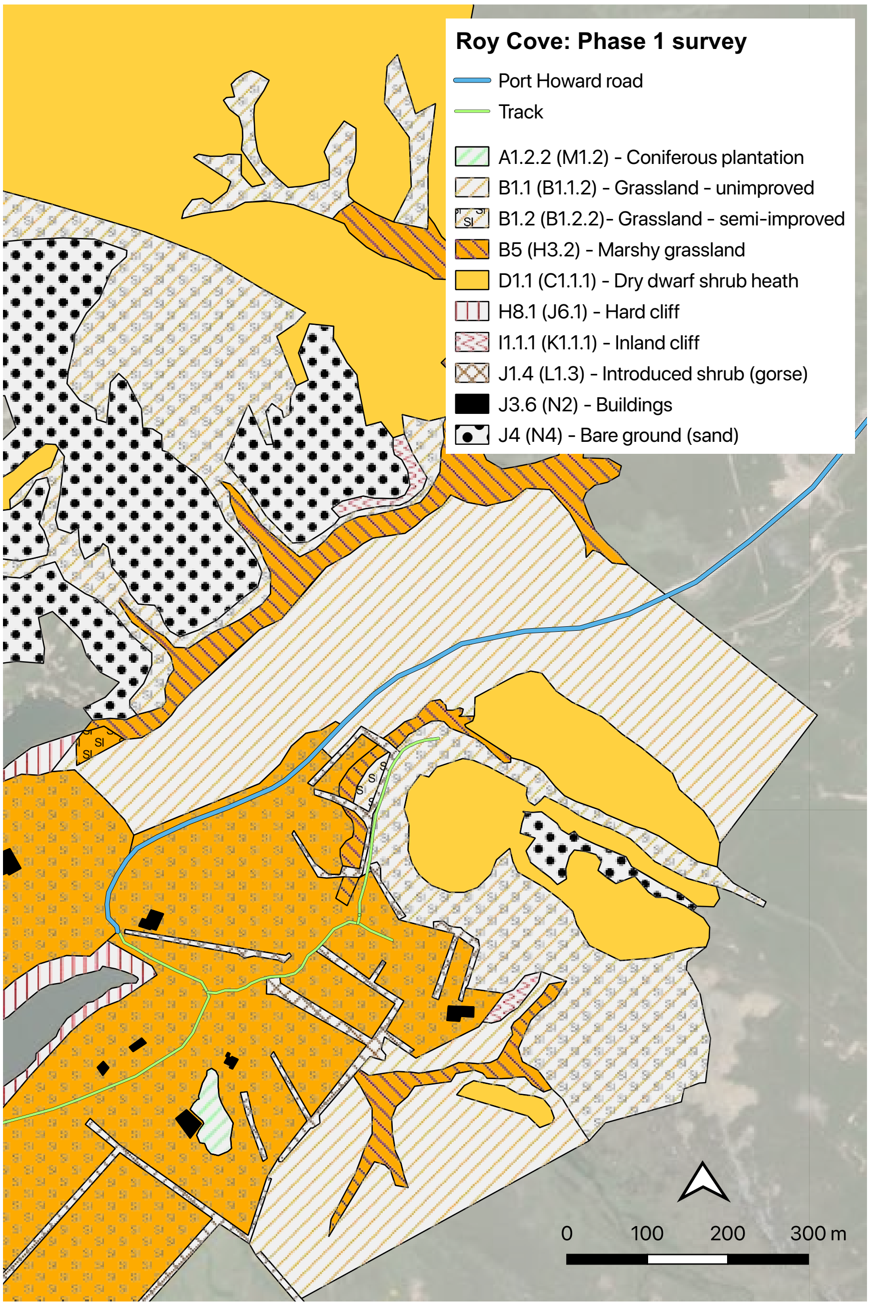

Figure 2.10 Roy Cove: Phase 1 survey, January 2016. The main oviposition sites on the edge of the semi-improved grassland where it meets the dwarf shrub heath and around the inland cliffs.

(iii) **Violets and butterflies**

Roy Cove was the source of historical specimens of *Y. cytheris,* now in the BMNH and the OUMNH, collected by Vallentin (1904, and in Boyson, 1924) from the enclosures around Bertrand's former house (Figure 2.11).

Viola spp. are spread widely around Roy Cove, mostly in a 1500 m strip from north to south, in the lee of hills providing shelter from the prevailing southwesterly wind.

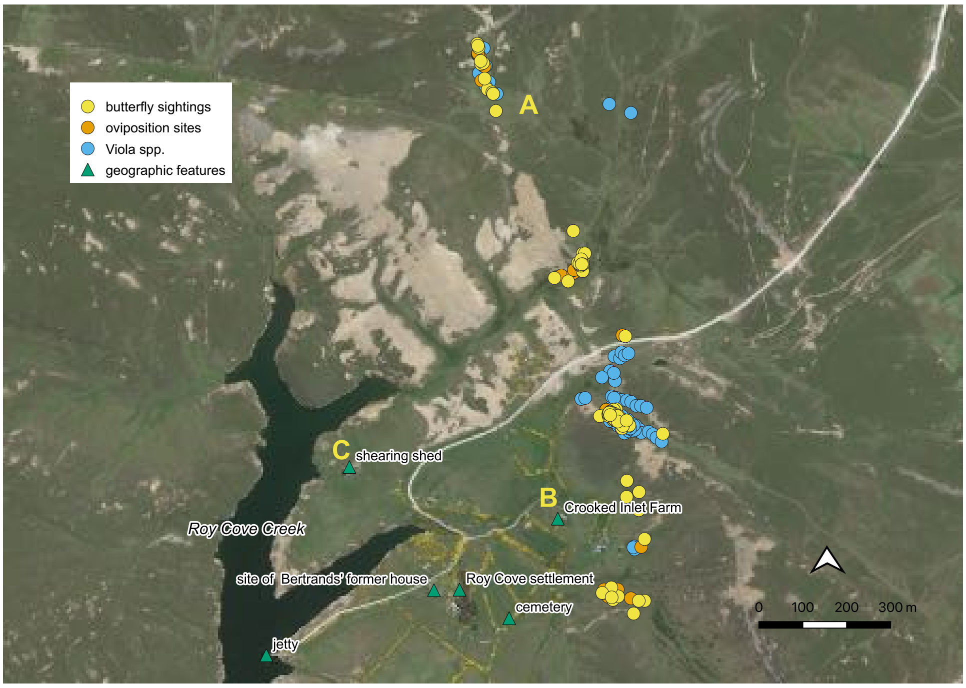

Figure 2.11 Roy Cove *Viola* and butterfly sites, 2016 - 2018. The letters A - C correspond to those in Figure 2.12. The yellow straight lines show gorse shelter, planted to protect sheep from the wind. The site of Bertrands' former house shows where Vallentin (see Vallentin 1904) stayed while collecting *Y. cytheris* in the house's enclosures. The bare patches of coarse sand are clearly visible.

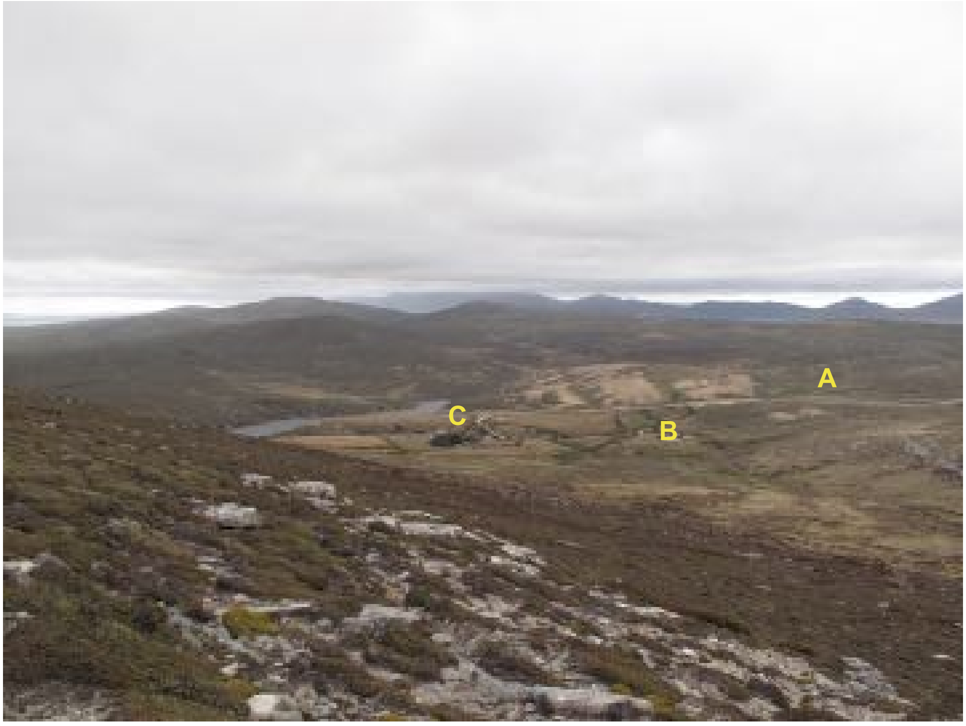

Figure 2.12 Slopes above Roy Cove seen from Cooke Hill. *Viola* typically are found in small groups in areas with a mixture of rock, bare ground and dwarf shrub heath. The letters A - C correspond to those in Figure 2.11. "A" is the upper meadow, the furthest extent north of the butterfly colonies. "B" is Crooked Inlet farm, and "C" the main shearing enclosure and shed for neighbouring Pickthorne farm. Photograph by the author.

(iv) **Land use**

Agriculture

Crooked Inlet farm, the major landholder at Roy Cove, farms 5,700 sheep and 30 cattle. It is certified organic. The sheep graze widely, including over butterfly oviposition areas. The neighbouring Pickthorne farm has 1000 sheep (Department of Agriculture, Falkland Islands 2020).

Other

Crooked Inlet has a single self-catering cottage, but tourism is otherwise undeveloped. There is no other land use.

(v) **Wind**

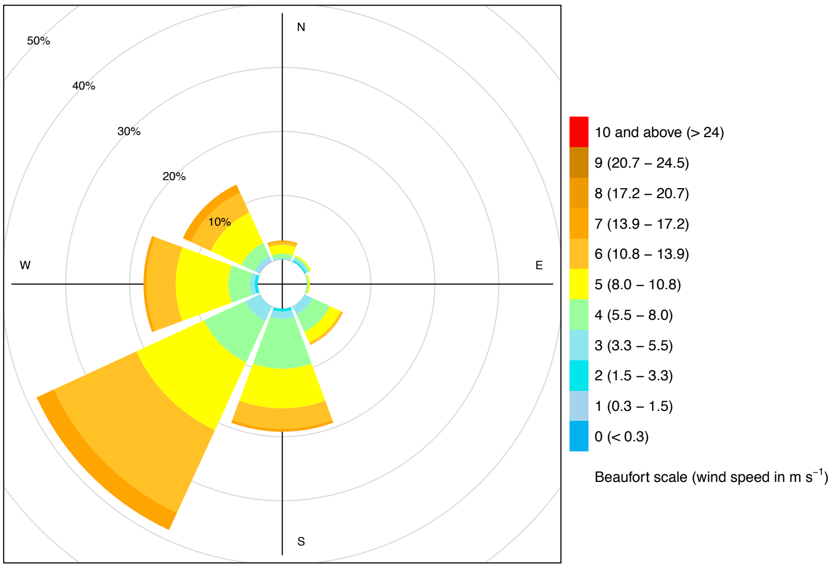

Figure 2.13 Wind rose for Mount Byron, the nearest meteorological station to Roy Cove, November - February, 2013 to 2018. The southwesterly prevailing wind averaged 9.35 ms^{-1}. The butterfly sites are predominantly in north-east facing areas sheltered by hills or cliffs.

The nearest weather station is at Mount Byron (WMO ID 88870). Mean summer wind speed 2013 - 2018 was 9.35 ms^{-1}, with wind predominantly from the south-west. Mount Byron has the highest mean wind speeds of the Falkland Island weather stations. The station is at 480 m, with no land between it and Latin America. The Roy Cove sites, however, showed wind speeds on average, 47% lower than those of Mount Byron (7.2.3 (i)), due to the lower vertical shear effect and the rolling countryside.

2.3.4 Sea Lion Island

(i) **Overview**

Sea Lion Island (52.42º S, 59.08º W), covering 905 ha, is the most southerly inhabited island in the Falklands archipelago (Figure 2.1), lying 17 km off the southern tip of East Falkland. It is low-lying, with the highest point, Bull Hill, at 46 m, at its southern end, where there are also vertical hard cliffs of 24 m. A Ramsar site, it became a national nature reserve in 2017 and is one of the Falklands' major tourist destinations. It was formerly a sheep farm, but all agricultural work has now ceased.

(ii) **Vegetation**

The island is a mosaic of dwarf shrub heath, marshy and semi-improved grassland, with expanses of cinnamon grass (*Hierochloe redolens*) (Figure 2.14). There are also extensive areas of tussac (*Poa flabellata*) around the coast, which were protected from overgrazing when the island was farmed. Old penguin and cormorant rookeries provide a substrate for patches of groundsel (*Senecio vulgaris*).

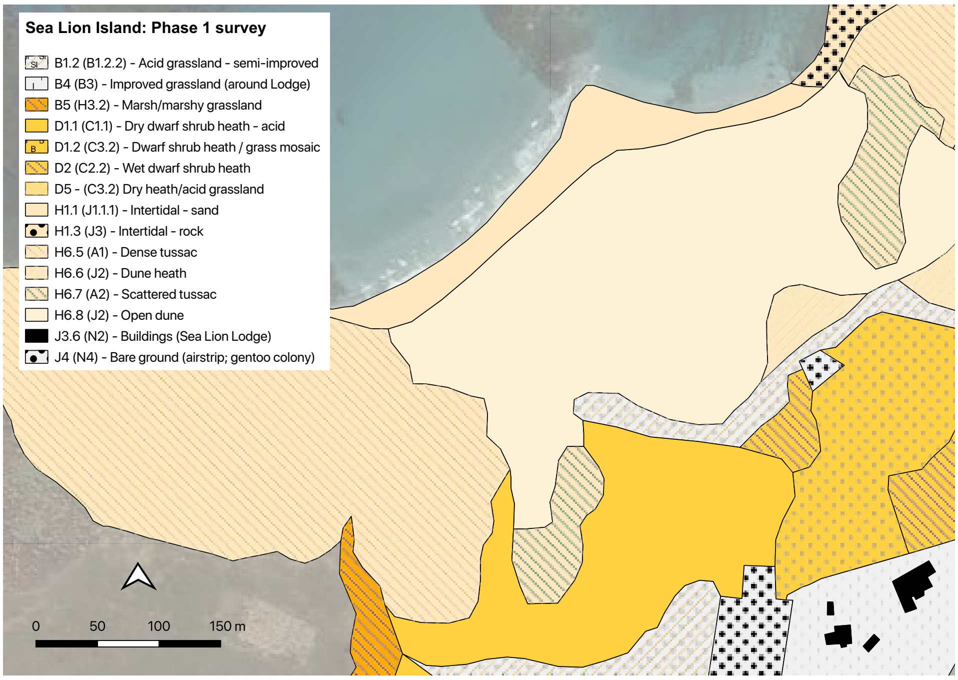

Figure 2.14 Sea Lion Phase 1 survey, January 2016. The main oviposition sites are on edges where dwarf shrub heath, whether wet or dry, or tussac, which provide shelter, meet grassland.

(iii) **Violets and butterflies**

Sea Lion is the only major site in the islands for *Viola magellanica*. The patch is north of the lodge, with sand dunes and patches of tussac (*Poa flabellata*) marking its northern boundary (Figure 2.15). It comprises, in part, broken, marshy ground, where the predominant vegetation is grass, small fern (*Blechnum penna-marina*), pig vine (*Gunnera magellanica*) and Christmas bush (*Baccharis magellanica*). This alternates with dwarf shrub heath and grass mosaic. The lodge warden knew of no other patches of *Viola* spp. on the island. The *Viola* on Sea Lion were found in small clumps, and in a range of environments such as old Magellanic penguin burrows and on the edge of marshy grassland.

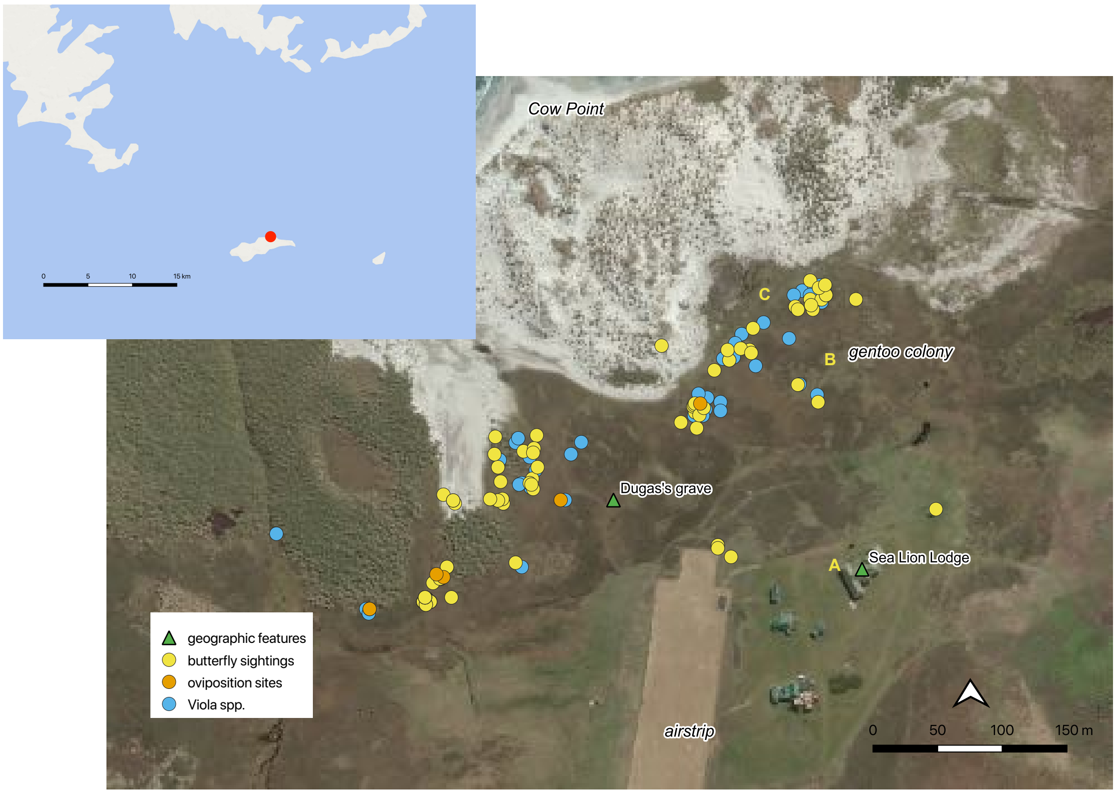

Figure 2.15 Sea Lion Island, *Viola* and butterfly site, 2016 - 2018. The site is marked in red on the smaller scale map derived from Figure 2.1. The letters A - C correspond to those in Figure 2.17. The white sand patches, particularly that to the north-west of Dugas's grave, are used by *Y. cytheris* for basking. The most westerly group of sightings were from December 2016 around a flowering Christmas bush.

One phenomenon observed, but not analysed, was the variation in observed *Viola* plants from year to year and its link to grass growth. Few *Viola* were observed in 2018, a year of strong grass growth, whereas they were relatively common in 2016 - 2017 when the grass appeared parched (Figure 2.16). While in 2018 strong grass growth was also observable in, for example, Roy Cove, extensive grazing appeared to have ensured the *Viola* were not choked out.

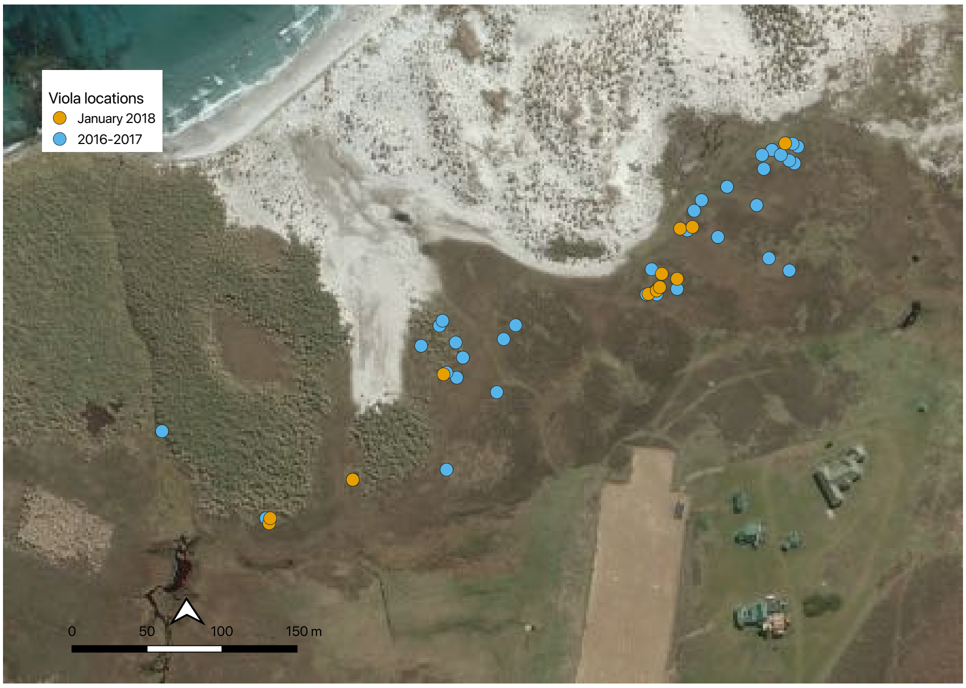

Figure 2.16 Sea Lion: comparison between *Viola* spp. found in January 2018 with those found in January and December 2016. In each case the whole site was searched. 2018 saw a reduction in *Viola* numbers and distribution, together with strong grass growth.

(iv) **Land use**

Agriculture

Sea Lion is ungrazed. Tussac is being replanted in areas where it had been overgrazed in the past.

Other

Sea Lion is a major tourist destination. Visitors primarily go for its wildlife, but also because the memorial to HMS Sheffield, lost in the 1982 conflict, is at Bull Hill. The lodge has accommodation for 20, but there are often day visitors, with regular parties of 12 visiting by helicopter from the MPA. All visitors are briefed by lodge staff to ensure they have as little impact on the environment as possible.

Figure 2.17 Sea Lion Island looking south, showing a section of *Viola magellanica* patch. The letters A - C correspond to those in Figure 2.15. "A" is the lodge, "B" is the main gentoo penguin rookery, "C" is an area of dry heath and acid grassland. *Viola* are found, in small clumps, between the grassland and the gentoo rookery, and in the dwarf shrub heath to the west of the gentoos. Photograph by the author.

(v) **Wind**

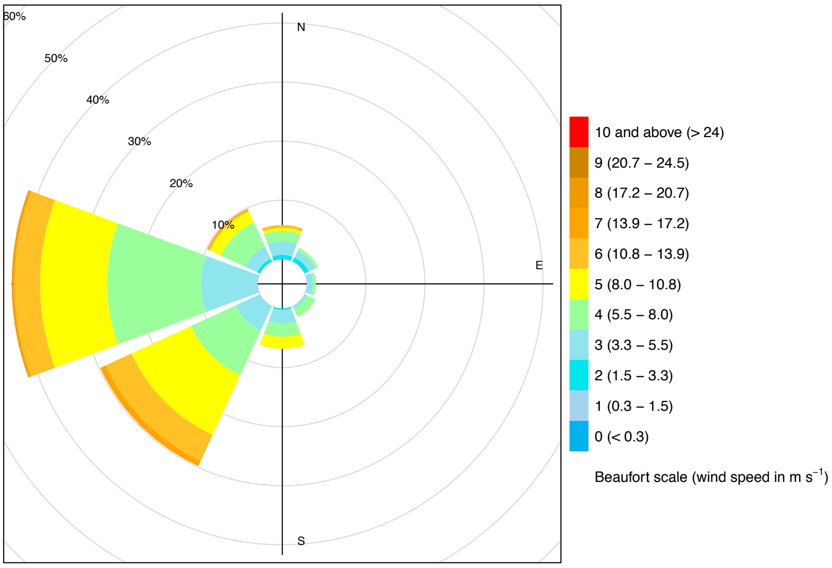

Figure 2.18 Wind rose for Sea Lion Island, November - February, 2013 to 2018. The mainly westerly prevailing wind averaged 7.4 ms^{-1}, from which the main oviposition sites had only partial shelter from tussac.

Sea Lion has its own weather station (WMO ID 88897), although its coverage is not comprehensive, and the records show down periods. Data for five summers (November to February), from 2013 to 2018 (Figure 2.18), and with records missing for 305 out of a possible 596 days, showed the wind predominantly from the west and south-west, with a mean wind speed of 7.4 ms^{-1}. The flat nature of the butterfly patch, with *Poa flabellata* only occasionally providing shelter, meant that most oviposition sites were exposed.

2.4 Rejected study sites

2.4.1 Bodie Creek

(i) **Overview**

Bodie Creek (51.86° S, 59.01° W) is part of Goose Green land, the Falklands' biggest farm, at 152,000 ha, with 77,000 sheep and 200 cattle. It is centred on Bodie Creek bridge (Figure 2.21) which, although it is the world's most southerly suspension bridge, remains a somewhat niche tourist attraction. The site is a mixture of dwarf shrub heath, coastal heathland and neutral to acid grassland. It has a few narrow valleys, mainly comprising marshy grassland (Figure 2.19). Goose Green itself is readily accessible by road from Stanley, and has its own landing strip.

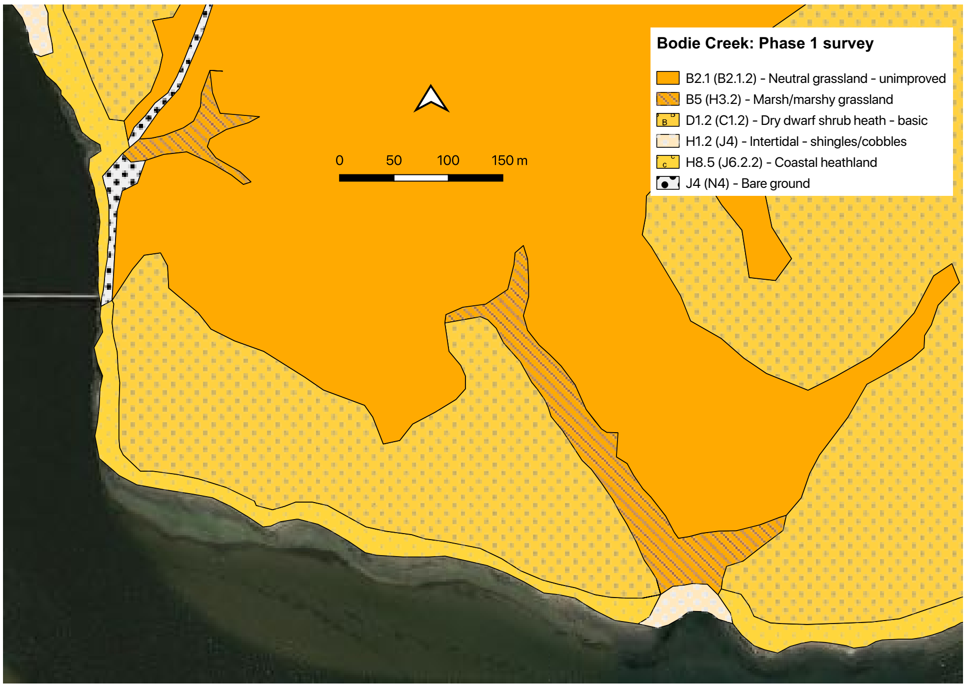

Figure 2.19 Bodie Creek Phase 1 survey, December 2016. The main *Viola* sites are where dwarf shrub heath or coastal heath meet grassland.

(ii) Violets and butterflies

There are small patches of *Viola*, mainly on the intersections between grassland and dwarf shrub heath (Figure 2.20). Although butterflies had been found on the dwarf shrub heath in previous years, and had been on occasions abundant, two exploratory visits in 2017 and 2018 failed to find evidence of butterflies or oviposition sites.

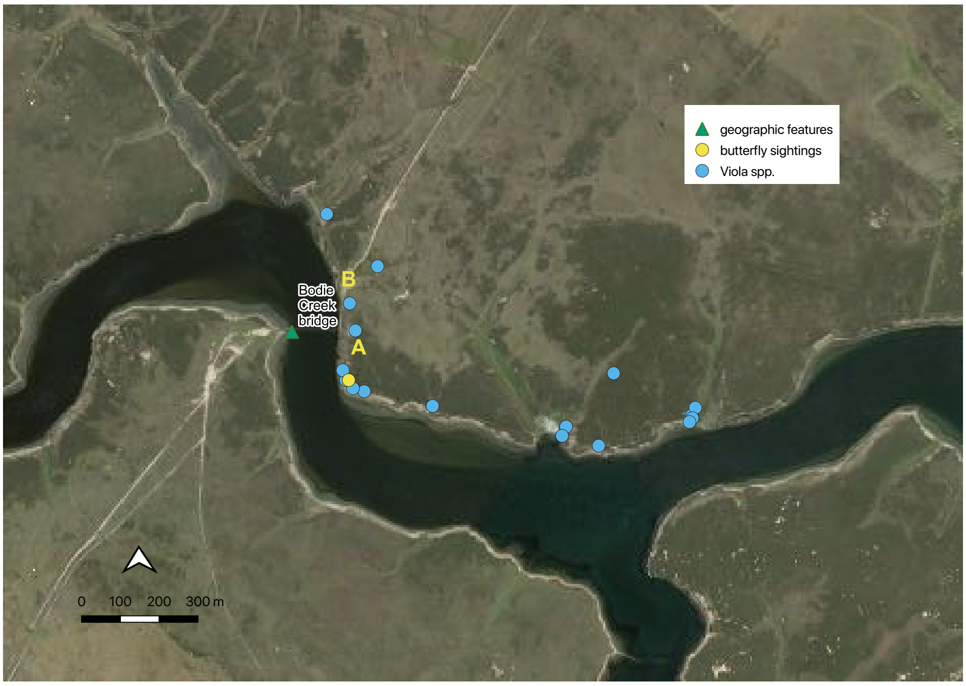

Figure 2.20 Bodie Creek *Viola* sites, December 2016. The plants were in small clumps, widely scattered. The butterfly sighting at A is a casual observation from 2013.

Figure 2.21 Bodie Creek showing the suspension bridge. The letters A and B correspond to those in Figure 2.20. Butterflies had been found in previous years on the heathland, marked "A". Photograph by the author.

2.4.2 Darwin

(i) **Overview**

Darwin (51.81° S, 58.96° W) was established in the mid 19th century and became the centre of the Falkland Island Company's operations outside Stanley. The farm is part of Goose Green lands. Like Goose Green it is a popular tourist destination because of its role in the 1982 conflict. The lodge, Darwin House, sleeps 12, and it attracts day visitors from MPA and Stanley, both easily accessible by road.

The land is mainly unimproved acid grassland interspersed with coastal heathland, with small areas of dwarf shrub heath (Figure 2.22). The settlement has a large green, of semi-improved neutral grassland, and has shelter provided by gorse hedges.

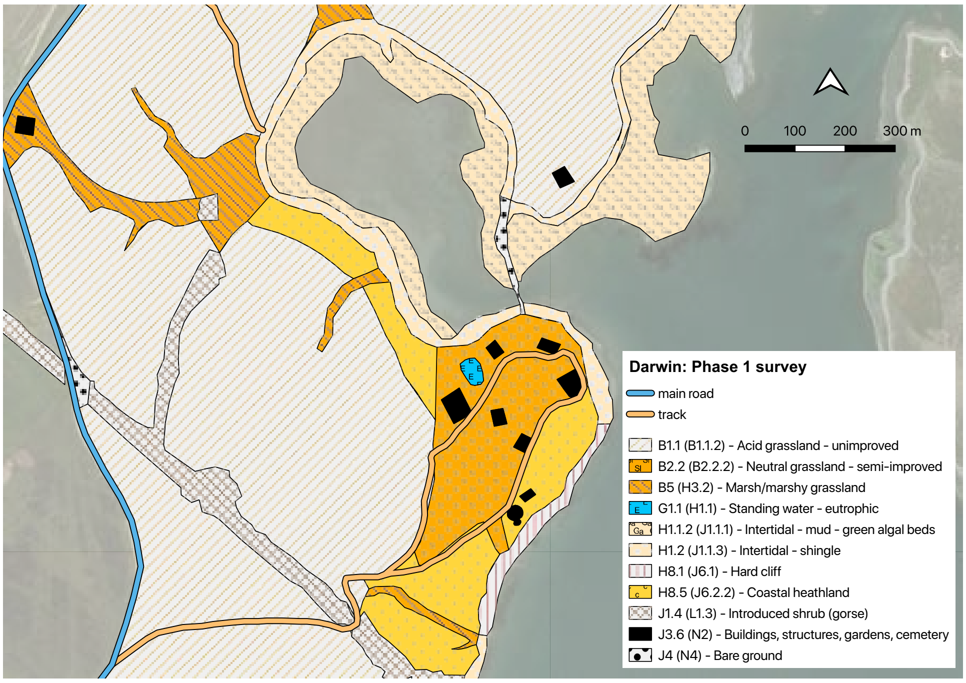

Figure 2.22 Darwin, Phase 1 survey, December 2016. The greater part of the site is acid grassland, which contains patches of dwarf shrub heath, though not in large enough areas to be mapped separately.

(ii) **Violets and butterflies**

Viola are patchily distributed around the settlement, although there is a prominent 200 m stretch of plants on the edge of the track leading to the main road. Butterflies had been found "fairly commonly" around the settlement in 1908-1909 (Vallentin, in Boyson 1924), and specimens are preserved in the collections of BMNH and OUMNH. Four days of searching in December 2016, however, only uncovered three oviposition sites and a single butterfly.

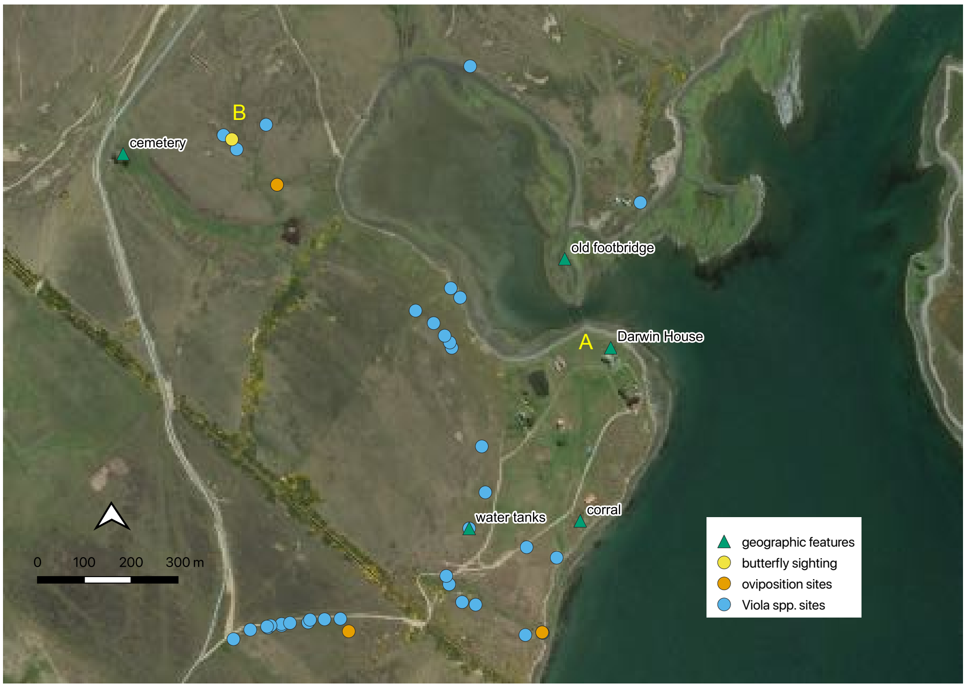

Figure 2.23 Darwin *Viola* and butterfly sites, December 2016. The largest patch of violets is seen along the south side of the track at the bottom of the picture.

Figure 2.24 Darwin looking towards the settlement from the butterfly site. The letters A (Darwin House) and B (butterfly sighting) correspond to those in Figure 2.23. Photograph by the author.

2.4.3 Hill Cove

(i) **Overview**

Hill Cove (51.49º S, 60.08º W) is a north-facing settlement, backed to the south-west by French Peaks, rising to 275 m, and to the south-east by Mt Adam, rising to 700 m. The major landowner is Peaks Farm, which controls 8600 ha, grazing 5500 sheep and 200 cattle. It contains the only forest on the Falklands (Figure 2.25) There is a small settlement, although some of the houses are only occupied seasonally, and the resident population is only in single figures. Hill Cove is accessible by road from Port Howard, and has its own airstrip.

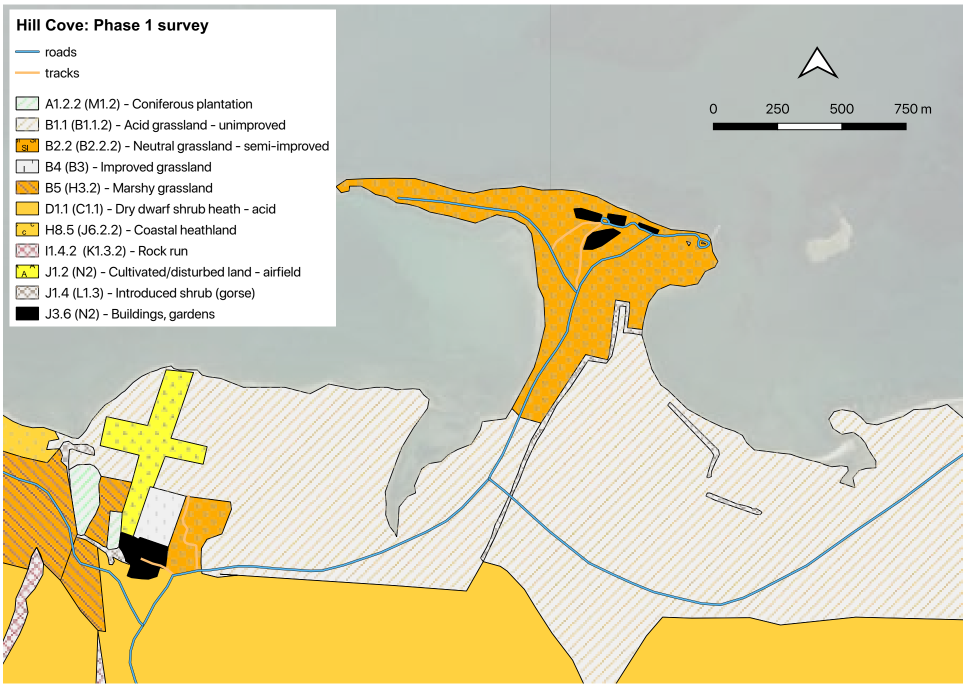

Figure 2.25 Hill Cove, Phase 1 survey, January 2016. The greater part of the site is acid grassland, with semi-improved neutral grassland greens around the main settlement. The dwarf shrub heath is in small patches within the grassland.

(ii) **Violets and butterflies**

An area west of the settlement was explored in January 2016. A solitary patch of *Viola* was found near Peaks Farm distributed throughout a rock run of approximately 80 m by 22 m. The previous landowners, in a subsequent meeting, said this was the only patch that they were aware of. The rock run, facing NNE, was predominantly diddle dee, Christmas bush, grass, dandelion (*Taraxacum* agg.) and daisy.

Figure 2.26 Hill Cove *Viola* sites, January 2016. The rock run site is to the west of Peaks Farm. The letters A and B at Deep Pass correspond to those in Figure 2.27. Aside from the rock run, *Viola* were only found around the coast.

A further 10 km of coastline was walked to the east of the settlement, and slopes were investigated to 200 m. Two further patches were found, at Fox Ridge and Deep Pass (Figure 2.26). One small group of plants was found on a point east of Peaks Farm. Two oviposition locations were found on single plants on small promontories.

There is a historical record in Cobb's diary for 1 February 1910 of a "red butterfly" at Port Howard, although it is not clear whether this was *Y.c. cytheris* or one of the painted ladies (*Cynthia* spp.) which occasionally appear in the Falklands. The landowners had seen butterflies in their garden, but knew of no other sites, nor of any sites of *Viola* spp.

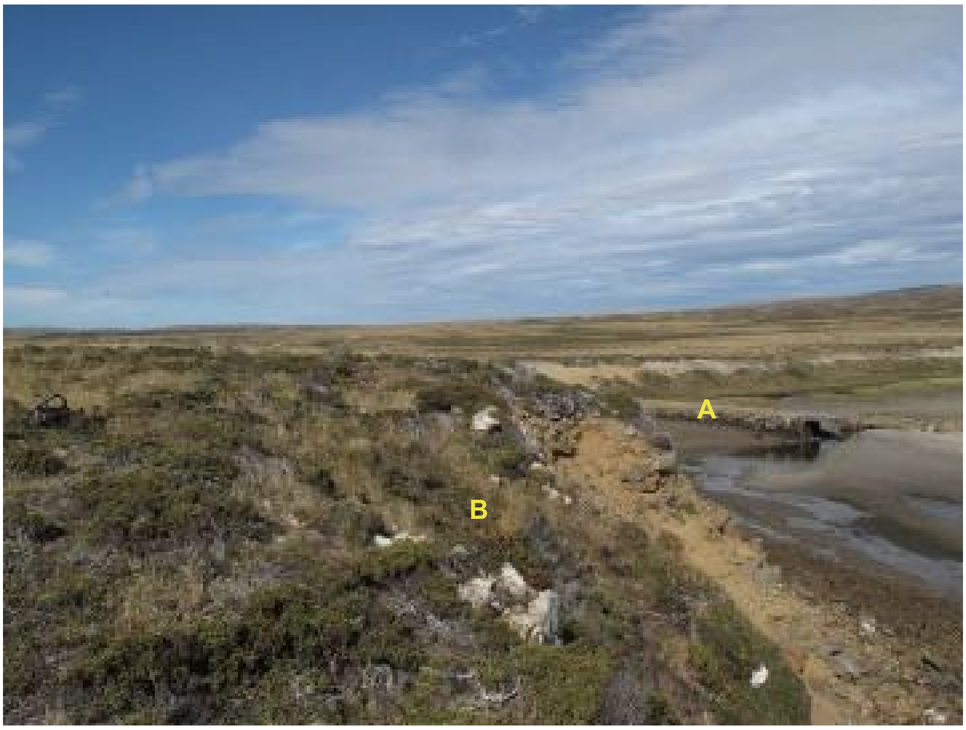

Figure 2.27 Hill Cove, Deep Pass. The letters A and B correspond to those in Figure 2.26. The *Viola* plants are amongst the dwarf shrub heath outcrops marked "B". Photograph by the author.

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Chapter 3: Population dynamics and behavioural patterns

3.1 Introduction

This chapter considers the population size and dynamics of *Yramea cytheris* using mark-release-recapture (MRR).

3.1.1 Population dynamics

An understanding of population structure and dynamics can help establish baselines, give a basis for recognising changes, and determine when, and in what form, interventions need to be made. It is an indispensable element in assessing and monitoring conservation status (Pollard and Yates 1993, Taron and Ries 2015, Henderson and Southwood 2016), although population trends in insects are marked by considerable annual variation which makes overall assessment of conservation difficult (Strien et al. 1997, van Swaay et al. 2011, Fox et al. 2019). This chapter draws on MRR data from the four study sites (Chapter 2) on three field visits to the Falkland Islands. It considers the size of the population at a given site; its distribution; and the longevity of individual butterflies.

Small, isolated colonies of butterflies, living on fragmented sites, and often at the edges of their ranges, have long been a focus of population studies, with fritillaries frequently providing the study species (Wahlberg, Klemetti, Selonen and Hanski 2002, Ehrlich and Hanski 2004). Species studied include *Melitaea aurelia* (Eichel and Fartmann 2008); *Melitaea cinxia* (Hanski et al. 1994, Nieminen et al. 2004, Mattila et al. 2012); *Euphydryas aurinia* (Wahlberg, Klemetti and Hanski 2002, Hula et al. 2004, Schtickzelle et al. 2005); *Euphydryas editha* (Hellmann et al. 2004) and *Boloria eunomia* (Baguette and Nève 1994). *M. cinxia*, in particular, has been at the centre of the development of metapopulation theory, which is rooted in stochastic space occupancy models, whereby a fraction of available habitats is unoccupied at any given time (Hanski and Thomas 1994, Hanski and Ovaskainen 2003, Ovaskainen and Saastamoinen 2018), although it is accepted that not all fragmented populations necessarily form metapopulations (Baguette 2004, Hanski 2004).

An understanding of the population structures of individual small patches, and their dynamics, is important in assessing the viability of populations, particularly where there is no evidence of a metapopulation structure (Osváth-Ferencz et al. 2017), where occupied sites are loosely connected through dispersal and where butterflies occupy most suitable habitat patches (Nowicki et al. 2007). As Hanski (2004) remarked, classical metapopulation models are not likely to be of great value for small patch networks.

Population size and dynamics underpin the assessment of conservation threat levels. This is important for local conservation efforts, but, perhaps more importantly, in global efforts (IUCN 2012), where population size, together with its growth (positive or negative) form the main criteria in assessing the threat of extinction. The challenges of using IUCN population criteria for small island butterfly populations have been explored by Grill et al. (2002), and for butterflies more widely by van Swaay and Warren (1999) and van Swaay et al. (2011), with the assessment of European butterflies' possible population decline measured as a trend over 25 rather than a ten years, and an increased focus on distribution areas (the sum of all areas within an imaginary line bounding the species population) identified as the key metrics. In the absence of any literature on the population size and dynamics of *Y. cytheris* in either Latin America or the Falklands, this chapter attempts to establish some of the basics on which future work can build.

3.1.2 Behaviour

The most obvious aspect of butterfly behaviour is a commonplace: as an ectothermic organism, the butterfly is less active in cold, windy or overcast conditions, hence the weather condition protocols in most monitoring schemes (Pollard and Yates 1993, Samways et al. 2009). On the days on which they appear, butterfly activity patterns are largely driven by strategies for mating, or, for mated females, oviposition (Dennis and Shreeve 1988).

Hannam et al. (2018) considered, through laboratory experiments, daily patterns of eclosion in the pipevine swallowtail (*Battus philenor*) and their effect on mating strategies. They investigated whether males were on the wing earlier, or even eclosed earlier, than females, ready to mate as soon as receptive females either eclosed or emerged from vegetation. Their results were inconclusive, and Sencio and Rutowski (2019) found no such pattern in an analysis of six nymphalid butterflies, including the fritillary *Euphydryas chalcedona.* As field observation of *Y. cytheris* suggested, however, some evidence of a preponderance of males early in the day, emergence, and, to the extent possible, eclosion, were investigated through MRR.

While female butterflies have a predominantly searching pattern, whether seeking nectar or oviposition locations, males generally have one of two strategies in seeking a mate, perching or patrolling (Shreeve 1987, Berwaerts et al. 2002, Dudley 2002). The relationship between the two male strategies and wing shape morphology are considered in Chapter 6. The mating process, with whichever strategy, has been shown to influence male behaviour over the course of a day (Konvička et al. 2002, Slamova et al. 2011, Vlašánek et al. 2018) with activities such as patrolling or perching, interaction and mating itself early in the day, and, primary activity done, recovery activity later. This was investigated for *Y. cytheris*, with female behaviour also considered, to assess whether mating and oviposition produced a comparable pattern.

Nectaring patterns were also investigated. In other species these show a range of behaviours from not nectaring at all through to collecting large quantities of pollen and nectar throughout the day (Odendaal et al. 1985).

3.1.3 Research questions

RQ1: to what extent is it possible to estimate the size of populations of *Y. cytheris* at the four Falkland study sites?
RQ2: to what extent do movement patterns of *Y. cytheris* at the study sites, both within and between patches, suggest the existence of metapopulation structures?
RQ3: to what extent do males and females differ in key population and behavioural metrics?

3.2 Materials and methods

3.2.1 Study sites

Population studies were carried out at the four Falkland study sites, Bleaker Island, the Frying Pan, Roy Cove and Sea Lion Island (Chapter 2), Investigation focused on individual habitat patches, distinct topographical locations containing a population of butterflies, such as an area of dwarf shrub heath or a rock run, either comprising a site in its entirety, or within a site (Table 3.1, Appendix Table A.1) Although both Bleaker and Sea Lion (2.3.1 and 2.3.4) contained areas of distinct topographical and vegetation features, adults in both cases moved freely over the whole study site. It was therefore decided to address each as a single patch.

Table 3.1 Study sites and habitat patches: sizes and distances between patches. Patches at Frying Pan and Roy Cove have a linear rather than a network relationship, therefore distances between any two patches can be calculated by adding distances between pairings together. Patch names in lower case are purely descriptive, with no formal geographic status.

			distance between patches	
site	patch	area (ha)	pairing	(m)
Bleaker	Bleaker (BL)	0.63	-	-
Frying Pan	south cliffs (SC)	0.18	-	
	fence line (FL)	0.42	SC - FL	220
	river mouth (RM)	0.08	FL - RM	1000
Roy Cove	upper meadow (UM)	0.80	-	
	bluebottle rocks (BB)	0.79	UM - BB	300
	lower meadow (LM)	0.28	BB - LM	280
	windmill hill (WH)	0.06	LM - WH	230
	rock run (RR)	0.52	WH - RR	100
Sea Lion	Sea Lion (SL)	7.00	-	-

3.2.2 Data collection

Mark-release-recapture (MRR) was used to estimate population size, imago life span and mobility, and to investigate different behaviour patterns between the sexes. Populations were sampled over three austral summers, 2015-2016, 2016-2017 and 2017-2018, although MRR data were not gathered during the first summer, which was focused on finding suitable populations for analysis.

Y. cytheris has a flying season from November to February, with no evidence of bi- or multi-voltinism. Resource constraints meant it was not possible to sample through an entire season. This made accurate assessment of population size difficult, as data only allowed estimates for the capture period at each site.

The rapidly changing daily weather conditions in the Falkland Islands made it difficult to set a daily timetable for MRR, as there were few ideal capture days of warmth, uninterrupted sunshine and minimal wind. MRR was carried out on all days which had some clear sky, no matter how strong the wind, although eight out of a possible 57 MRR days were lost to persistent rain. During such periods, other fieldwork was undertaken, during which no butterflies were sighted.

Each of the patches at the Frying Pan and Roy Cove was searched for butterflies for a minimum continuous period of 40 minutes of each day, with a start time of 0900 (although captures before this were included in data). If no butterfly was seen during that period, the next patch was examined. The order of search between patches was changed each day. Sea Lion and Bleaker, treated as single patches, were searched for two hours a day. On-site adjustments were made until it appeared that all butterflies that were likely to be caught had been caught. This indicator was a succession of within-day recaptures, with no new butterflies. Searches included a buffer zone of 100m from the main patches.

Butterflies were captured with a 40cm net on a 50cm handle, both black to minimise flash. Each butterfly was marked immediately after capture and then gently released at the place of capture. To ensure a unique ID, sequential letters were marked on the underside of a hind wing using a Sharpie fine point permanent marker in one of four colours. All markings were clear and legible during subsequent recaptures. The butterfly's number was recorded, together with its capture coordinates, taken from a Garmin GPSMAP 64S GPS Meter (nominal accuracy ± 3 m). Records were also taken of the time of capture, sex, condition on a scale of A (fresh) to D (very worn), and activity when captured. Data were recorded from repeat captures on subsequent days, but not from repeat captures on the same day.

Any butterflies needed for DNA or morphometric work were only killed on the last day of MRR to avoid distortion in the data analysis.

3.2.3 Data Analysis

(i) **Population size and dynamics**

An open population was assumed. This would allow for birth (or, in the case of adult insects, eclosion), death and migration, as well as population change over the duration of the sampling period.

Population parameters were assessed through the Jolly-Seber method (Jolly 1965, Seber 1965) which was designed for use with open populations and has been widely adopted for butterfly population analyses (e.g. Samways and Lu 2007, Wilson and Roy 2009, Sielezniew and Nowicki 2017, Sielezniew et al. 2019). It enables the estimation of population size (N), the total number of butterflies present in the study population over the study period; apparent survival probability (φ); the probability of capture during a given sampling period (p); and the probability of entry into the population between two given sampling periods (*pent*). The survival estimator (φ) cannot distinguish between death and emigration without further information, therefore the term "apparent survival", which encompasses recorded residence time, is used here (Lebreton et al. 1992, Pollock and Alpizar-Jara 2005).

Assumptions of equality of survival and catchability were tested to ensure the Jolly-Seber method was valid (Begon 1983, Henderson and Southwood 2016). The largest data set, Bleaker BL11 (Table 3.2) was used to test both assumptions. The test statistics for Manly's (1971) test ($g = -0.362$, where inequality of survival would be significant at $g > 1.64$) and Cormack's (1966) test ($z = -0.002$, where inequality of catchability would be significant at $z > 1.64$) enabled both assumptions to be made

The Bleaker BL11 data set (Table 3.2) was also used to select an appropriate model within the Jolly-Seber method based on Akaike's information criteria, corrected for small sample size (AIC_c) (Burnham and Anderson 2003). All calculations were carried out in RMark (Laake 2013). Candidate models were ranked according to their AIC_c values. The model selected was POPAN (Schwarz and Arnason 2009, Laake 2013) $\varphi(\sim 1)$ $p(\sim 1)$ *pent*(~1) $N(\sim 1)$, with an AIC_c value of 258.4. The other candidates, Link-Barker, Pradel-recruitment and Pradel-λ, each had much higher AIC_c values of 502.3, with a fifth candidate, Burnham JS showing no convergence of the parameter estimates.

The sexes were pooled to maximise the numbers available for estimating population size for each site study visit. Study visits were pooled to maximise the numbers available for estimating any variation between the sexes in φ, p and $pent$.

(ii) **Population distribution**

Heat maps were drawn up for each site, and for patches within sites, to identify areas of population concentration. The GPS coordinates of all captures and recaptures were mapped in QGIS 3.4 (QGIS Development Team 2018). The kernel density estimation feature was then used to produce a heat map, using a buffer of 10m around each capture to allow a pursuit distance between sighting and capture.

(iii) **Mobility**

Distances between captures and recaptures were calculated using the spherical rule of cosines and were visualised in QGIS 3.4. To assess whether these were correlated with patch area, the areas of sites, and patches within sites, were calculated using the field calculator tool in QGIS, based on vegetation data from Phase 1 mapping (Chapter 2), with patches in such cases containing *Viola* spp. Distances flown by females and males were compared to establish whether mobility patterns differed between the sexes.

(iv) **Imago longevity**

Field visits, ranging from between one and eight days, did not give enough time to provide a clear picture of the time spent by *Y. cytheris* as an imago. Estimates were, however, made based on (i) capture history, (ii) apparent survival probability, derived from capture history using the Jolly-Seber method, and (iii) measurement of the rate of decline of recaptured butterflies' condition.

Capture histories of more than five days duration were used to note the average number of days between first and last captures. The condition of butterflies at each capture were recorded as: A = pristine, B=some fading of colour, C = as B, but with wing damage, D = faded and battered. To assess the decline in condition over time, the condition of each recaptured butterfly, initially captured in condition A, was plotted.

(v) **Assessment of daily emergence patterns, activity and nectaring**

To assess daily emergence patterns, time of first capture during the day was noted for all butterflies. Captures were allocated to one-hour periods, and numbers were compared; comparisons were also made between the first hour of MRR, 0900 - 0959, and the last four, 1300 - 1659. To consider whether there was evidence for daily eclosion, first capture times of butterflies with wing condition A, as a proxy for newly emerged butterflies, were compared. In all cases butterfly activity immediately before capture was noted. The categories used were basking (lying with wings flat, on stone, sand or bare earth); searching (apparently aimless movement, with pauses, across the patch); interacting (pursuing, or being pursued by, another butterfly, either in courtship or conflict); mating; nectaring; and resting (pausing, usually on foliage, with no apparent motive). No butterflies were caught, and only one was seen, ovipositing. Two glosses might be added to the terminology. First, while other activities, such as nectaring and resting, contained elements of basking, and the butterfly might hold its wings flat while doing both, "basking" is here used of a very specific activity where the butterfly rests, seemingly motionless, on rocks or bare ground. Second, despite males and females having different requirements, field observation failed to distinguish between male and female flight patterns. While patrolling for males, and showing availability, or seeking to oviposit, for females might be expected to appear distinct, the flight patterns of the two sexes could not be distinguished. The term "searching" is used for all such activity.

3.3 Results

3.3.1 Data sets

Over the three seasons a total of 403 butterflies were captured at the four study sites of Bleaker, Frying Pan, Roy Cove and Sea Lion over 49 days (Appendix Table A.1). 51 captures took place during exploratory visits in January 2016 and were excluded from population analyses. For the two seasons (2016 - 2017, 2017 - 2018) in which mark-release-recapture (MRR) took place, a total of 270 (130 female, 140 male) individuals were captured; there were 82 (44 female and 38 male) recaptures, involving 49 individuals. The maximum number of recaptures on different days at any site was three for both females and males. A separate data set was drawn up for the different habitat patches of Frying Pan and Roy Cove (Table 3.3). The capture rate varied considerably both between sites and within sites on different days (Table 3.4)

Table 3.2 Summary of *Y. cytheris* captures at Falkland Island sites. These include captures and recaptures, over three seasons.

		days		all captures			marked		recaptures	
visit	date	visit	MRR	total	F	M	F	M	F	M
SL01	Jan 16	1	1	6	4	2	4	2	0	0
BL01	Jan 16	4	2	31	23	8	23	8	0	0
RC01	Jan 16	1	1	12	7	5	7	5	0	0
FP01	Jan-Feb 16	5	3	2	2	0	2	0	0	0
SL11*	Dec 16	4	4	78	33	45	27	37	3	7
BL11*	Dec 16	5	4	131	56	75	43	56	12	17
FP11	Dec 16	1	1	5	0	5	0	5	0	0
RC11*	Jan 17	7	6	44	35	9	23	8	7	1
RC12	Jan 17	1	1	7	1	6	1	6	0	0
BL12	Jan 17	2	2	25	17	8	12	7	5	1
RC13	Jan 17	5	3	10	9	1	9	1	0	0
FP12	Feb 17	1	1	2	2	0	2	0	0	0
RC21*	Jan 18	8	7	14	6	8	6	5	0	1
SL21	Jan 18	2	2	0	0	0	0	0	0	0
BL21*	Jan 18	7	7	23	9	14	4	8	2	3
FP21*	Jan-Feb 18	6	4	13	6	7	4	7	2	0
Total		60	49	403	210	193	166	155	31	30

Visits are coded by site: BL = Bleaker, FP = Frying Pan, RC = Roy Cove and SL = Sea Lion. The two digits following refer to a combination of the season (0 = 2015-2016, 1 = 2016-2017 and 2 = 2017-2018) and the site visit within that season (1-3). All captures = total of butterflies netted, including repeat captures on subsequent days. Visit = the duration, in days, of a site visit; MRR = the number of days within the visit that MRR was undertaken. Marked = the number of individuals marked for the first time in a visit; recaptures = the number of individuals recaptured in total, irrespective of the number of times they were recaptured. F and M refer to female and male. Visits marked * were long enough for analysis of population using the Jolly-Seber method and also produced recaptures.

Table 3.3 Summary of *Y. cytheris* captures at Frying Pan and Roy Cove patches. These include captures and recaptures, over three seasons.

		days		all captures			marked		recaptures	
visit	date	visit	MRR	total	F	M	F	M	F	M
LM01	Jan 16	1	1	9	4	5	4	5	0	0
RR01	Jan 16	1	1	1	1	0	1	0	0	0
WH01	Jan 16	1	1	2	2	0	2	0	0	0
SC01	Jan 16	5	1	2	0	2	0	2	0	0
WH11	Jan 17	1	1	1	1	0	1	0	0	0
BB11	Jan 17	7	3	5	2	3	2	3	0	0
LM11	Jan 17	7	5	33	27	6	10	5	7	1
RR11	Jan 17	7	2	5	4	1	4	1	0	0
UM12	Jan 17	1	1	7	1	6	1	6	0	0
BB13	Jan 17	5	1	3	3	0	3	0	0	0
WH13	Jan 17	5	1	1	0	1	0	1	0	0
LM13	Jan 17	5	3	5	5	0	5	0	0	0
RR13	Jan 17	5	1	1	1	0	1	0	0	0
FL12	Feb 17	1	1	2	2	0	2	0	0	0
WH21	Jan 18	8	1	1	1	0	1	0	0	0
LM21	Jan 18	8	2	2	2	0	2	0	0	0
UM21	Jan 18	8	2	4	2	2	2	2	0	0
BB21	Jan 18	8	4	7	1	6	1	2	0	1
FL21	Feb 18	6	3	6	5	1	2	1	1	0
RM21	Feb 18	1	1	6	1	5	1	5	0	0
Total				103	65	38	45	33	8	2

Visits are coded by patch. Frying Pan patches are: FL = fence line, RM = river mouth and SC = south cliff; Roy Cove sites are: BB = bluebottle rocks, LM = lower meadow, RR = rock run, UM = upper meadow, WH = windmill hill. The two digits following refer to a combination of the season (0 = 2015-2016, 1 = 2016-2017 and 2 = 2017-2018) and the site visit within that season (1-3). Visit = the duration, in days, of a site visit; MRR = the number of days within the visit that MRR was undertaken. These have not been totalled as they overlap. Marked = the number of individuals marked for the first time in a visit; recaptures = the number of individuals recaptured, irrespective of the number of times they were recaptured. F and M refer to female and male.

Table 3.4 Mean *Y. cytheris* daily capture rate at the four study sites over three study visits.

visit	Bleaker	Frying Pan	Roy Cove	Sea Lion
Jan 2016	15.50 ±7.78 (2)	0.67 ±1.15 (3)	12.00 ±NA (1)	6.00 ±NA (1)
Dec 2016	32.75 ±9.71 (4)	-	-	19.50 ±8.50 (4)
Jan 2017a	12.50 ±0.71 (2)	2.00 ±NA (1)	7.29 ±3.50 (7)	-
Jan 2017b	-	-	3.33 ±2.31 (3)	-
Jan 2018	3.29 ±2.06 (7)	3.25 ±2.87 (4)	2.00 ±1.41 (7)	0.00 ±0.00 (2)
Overall	14.00 ±13.60 (15)	2.12 ±2.36 (8)	4.83 ±3.85 (18)	12.00 ±11.3 (7)

Jan 2017 was split into a and b to incorporate two separate visits to Roy Cove. Figures represent mean number of individuals ±*SD*, with the number of days on which butterflies were pursued shown in brackets.

3.3.2 Population size

There was a wide range in estimated population sizes between and within sites (Table 3.5). Bleaker, for example, had both the highest estimated numbers (213.1±31.7; 95% CI [165.8, 293.8]) in December 2016 and the second lowest (14.4±2.5; 95% CI [12.4, 24.7]) in January 2018.

Table 3.5 Jolly-Seber analysis of *Y. cytheris* MRR data for Falkland study sites. Visits of four days or more, containing recaptures, were included. Females and males were pooled.

					n			φ		*p*		*pent*	
visit	date	days	mark	rec	*n*	*SE*	95% CI	φ	*SE*	*p*	*SE*	*pent*	*SE*
BL11	Dec 16	5	99	25	213.1	31.7	165.8, 293.8	0.84	0.11	0.19	0.05	0.06	0.06
SL11	Dec 16	4	64	8	139.0	59.6	83.0, 359.8	0.49	0.26	0.34	0.25	0.18	0.07
RC11	Jan 17	7	31	8	53.0	11.0	39.7, 86.5	0.66	0.09	0.35	0.10	0.07	0.02
BL21	Jan 18	7	12	5	14.4	2.5	12.4, 24.7	0.69	0.10	0.58	0.13	0.04	0.26
FP21	Jan 18	6	11	1	17.5	9.6	11.8, 64.5	0.63	0.24	0.42	0.35	0.13	0.03
RC21	Jan 18	8	11	1	21.7	9.9	13.3, 61.1	0.56	0.14	0.32	0.17	0.04	0.02

days = duration of visit in days; mark = number of individuals marked; recap = total number of recaptures; *n*= estimated size of population over study visit; φ = apparent survival probability; *p* = probability of capture during sampling period; *pent* = probability of entry into the population between sampling periods.

The variation in population estimates reflected the wide range in apparent survival probability φ, probability of capture over the marking period *p* and probability of entry into the population between sampling periods *pent*.

Capture histories by sex (Table 3.6) showed φ to be higher for females, 0.78 compared with 0.68 for males, while *pent* was higher for males, 0.14 compared with 0.13 for females. There was little difference in *p.*

Table 3.6 Estimated means of probability of survival (φ), capture (*p*) and entry into the population (*pent*) of female and male *Y. cytheris.* Data are taken from the field visits recorded in Table 3.5

		φ		*p*		*pent*	
	ch	φ	*SE* [95% CI]	*p*	*SE* [95% CI]	*pent*	*SE* [95% CI]
all	228	0.73	0.05 [0.61, 0.83]	0.24	0.04 [0.17, 0.32]	0.14	<0.01 [0.13, 0.14]
female	107	0.78	0.08 [0.60, 0.90]	0.24	0.05 [0.15, 0.36]	0.13	<0.01 [0.12, 0.14]
male	121	0.68	0.07 [0.52, 0.81]	0.25	0.06 [0.15, 0.37]	0.14	<0.01 [0.14, 0.14]

ch= number of capture histories; φ = apparent survival probability; *p* = probability of capture during sampling period; *pent* = probability of entry into the population between sampling periods.

Capture histories by site (Table 3.7) showed Bleaker and Roy Cove to be comparable for φ (0.75 and 0.74 respectively) and *p* (0.26 and 0.23), though with a contrast in *pent* (0.14 and 0.07). Sea Lion showed a different pattern with a low φ (0.49), and a high *pent* (0.18). Both Sea Lion and Frying Pan showed less robust data sets, with large confidence intervals for φ and *p.*

Table 3.7 Estimated means of probability of survival (φ), capture (*p*) and entry into the population (*pent*) of *Y. cytheris* at each site, combining data from all visits in Table 3.5, with sexes pooled.

		φ		*p*		*pent*	
site	ch	φ	*SE* [95% CI]	*p*	*SE* [95% CI]	*pent*	*SE* [95% CI]
all	228	0.74	0.05 [0.61, 0.83]	0.24	0.04 [0.174, 0.325]	0.14	<0.01 [0.13, 0.14]
BL	111	0.75	0.07 [0.59, 0.86]	0.26	0.05 [0.173, 0.378]	0.14	<0.01 [0.14, 0.14]
FP	11	0.63	0.24 [0.18, 0.93]	0.42	0.35 [0.041, 0.926]	0.13	0.03 [0.08, 0.20]
RC	42	0.74	0.08 [0.57, 0.86]	0.23	0.07 [0.121, 0.384]	0.07	0.02 [0.04, 0.11]
SL	64	0.49	0.26 [0.11, 0.88]	0.34	0.25 [0.056, 0.818]	0.18	0.07 [0.08, 0.36]

ch= number of capture histories; φ = apparent survival probability; *p* = probability of capture during sampling period; *pent* = probability of entry into the population between sampling periods.

3.3.3 Population distribution and mobility

Population distribution at all sites showed tight clustering within patches (Figures 3.1, 3.2, 3.3, 3.4) with hot spots reflecting habitat features which, in turn, might impact on different activities. Thus, at Bleaker, a hot spot was a large patch of flowering groundsel, *Senecio vulgaris* (Figure 3.1), and at Sea Lion, a flowering Christmas bush, *Baccharis magellanica*, (Figure 3.4), in December 2017, both providing sources of nectar.

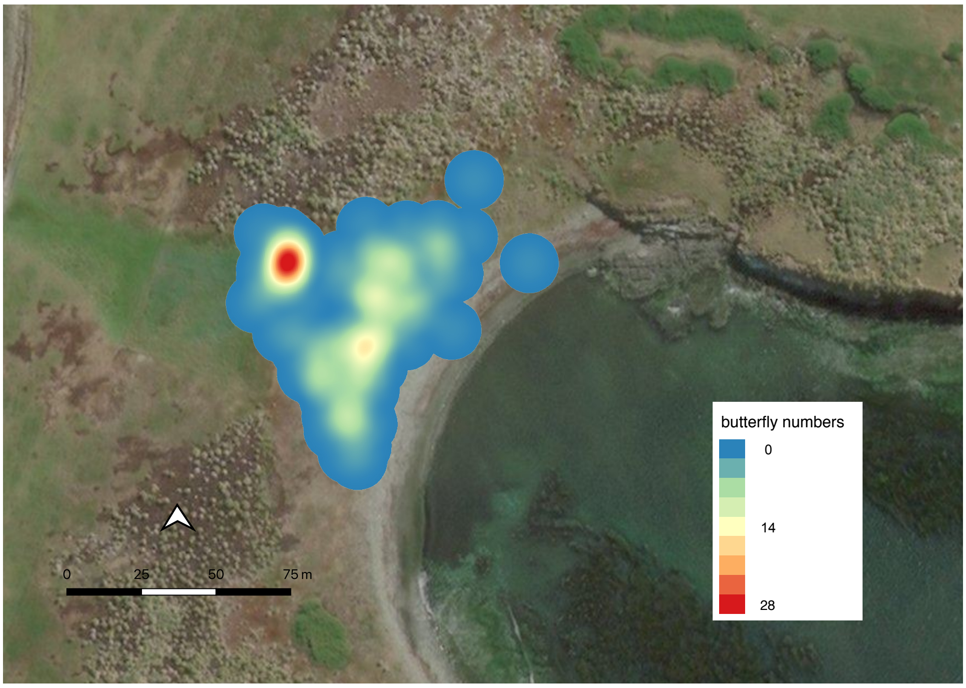

Figure 3.1 Population distribution of *Y. cytheris* at Bleaker, showing all captures 2016-2018. A 10m buffer is marked around each individual. The principal population focus was a patch of groundsel, *Senecio vulgaris*, shown here as a red patch.

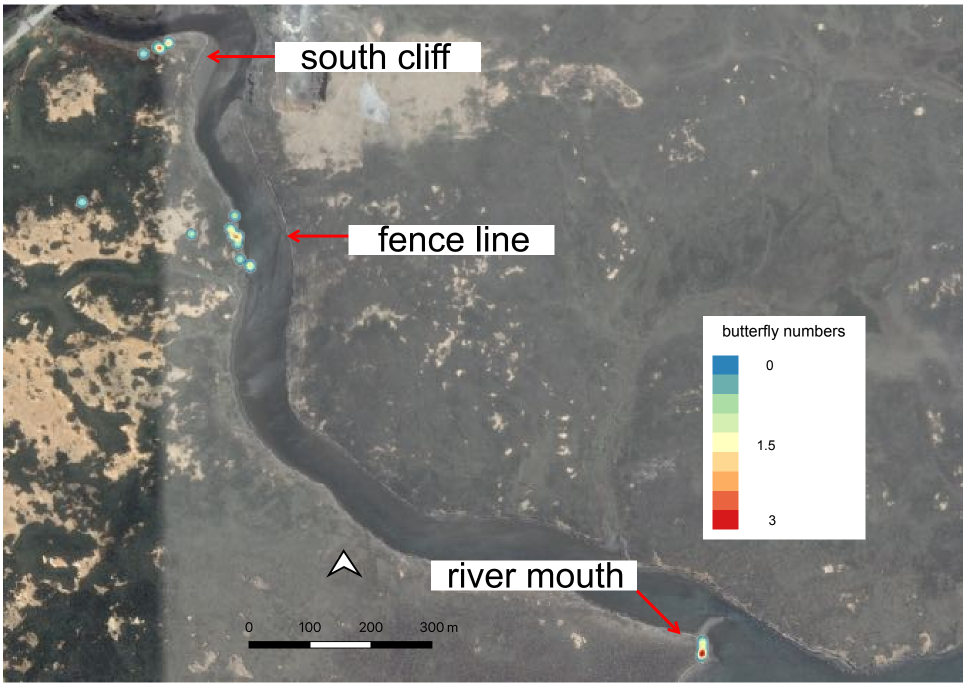

Figure 3.2 Population distribution of *Y. cytheris* at the Frying Pan in the three main patches (the south cliff, the fence line and the river mouth) showing all captures 2016-2018. A 10m buffer is marked around each individual.

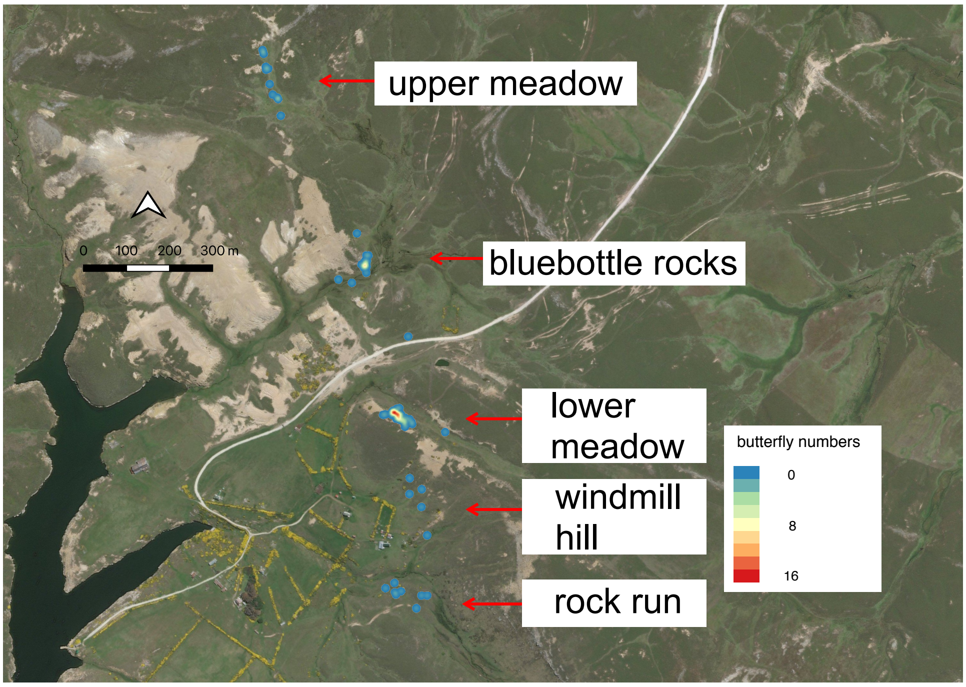

Figure 3.3 Population distribution of *Y. cytheris* at Roy Cove in the five main patches (the upper meadow, bluebottle rocks, the lower meadow, windmill hill and the rock run) showing all captures 2016-2018. A 10m buffer is marked around each individual.

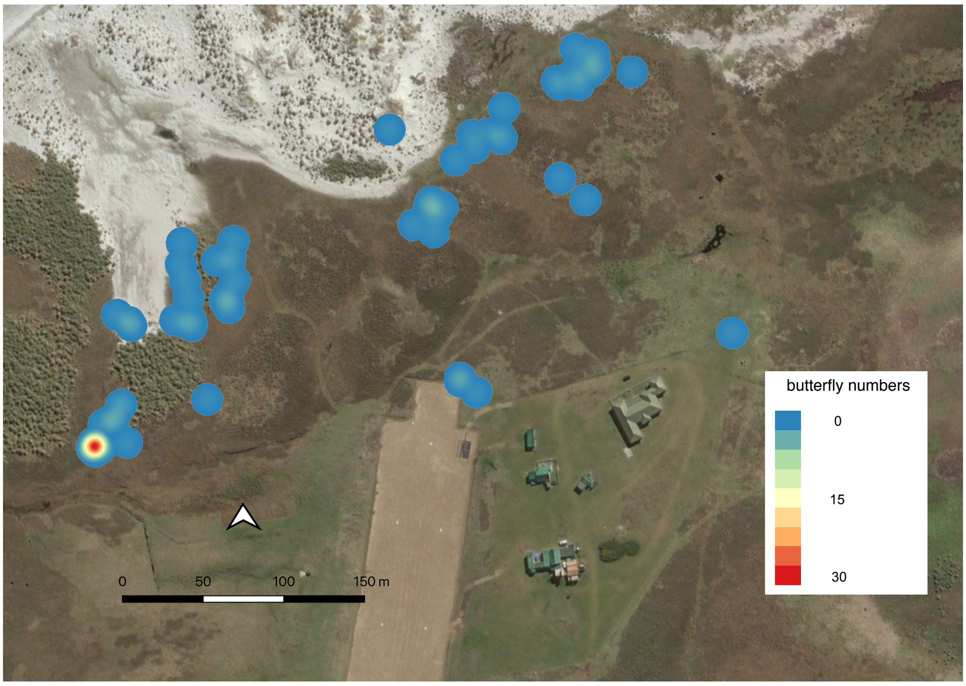

Figure 3.4 Population distribution of *Y. cytheris* at Sea Lion, showing all captures 2016-2018. A 10m buffer is marked around each individual. The principal population focus was a flowering Christmas bush, *Baccharis magellanica*, in December 2016, shown here as a red patch.

A concentration of butterflies on the leeward side of a large gorse bush, *Ulex europaeus* (Figure 3.5) at the lower meadow of Roy Cove suggested that shelter, to aid mating and oviposition, could also provide a focal point.

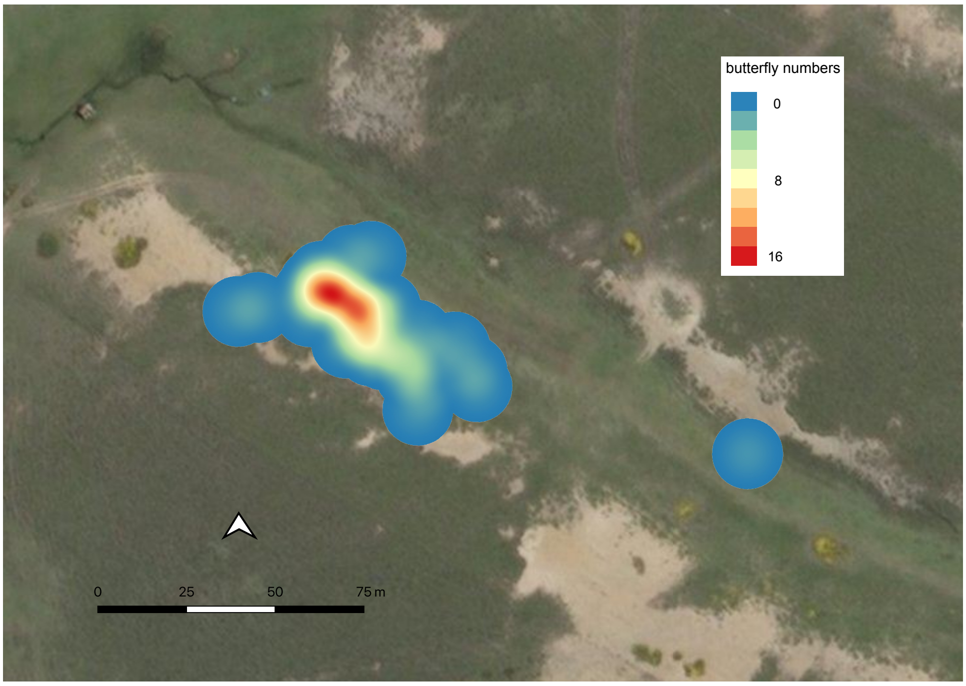

Figure 3.5 Population distribution of *Y. cytheris* at the lower meadow, Roy Cove, showing all captures 2016-2018. A 10m buffer is marked around each individual. The principal population focus was an area in the lee of a large gorse bush, *Ulex europaeus*, shown here as a red patch.

There were 76 movements between capture and recapture recorded. All of these were within sites or within individual patches within sites. No movements were recorded between patches, or between sites. The sites or patches showing the most repeat captures were Bleaker (Figure 3.6), Sea Lion (Figure 3.7) and the lower meadow at Roy Cove (Figure 3.8) The mean distance flown between captures was 26.6m (*SD* = 21.3; 95% CI 21.7, 31.4), with the maximum distance 119m and minimum 3.4m (Table 3.8, Figure 3.9). There was no evidence to suggest female flight distances differed significantly from male

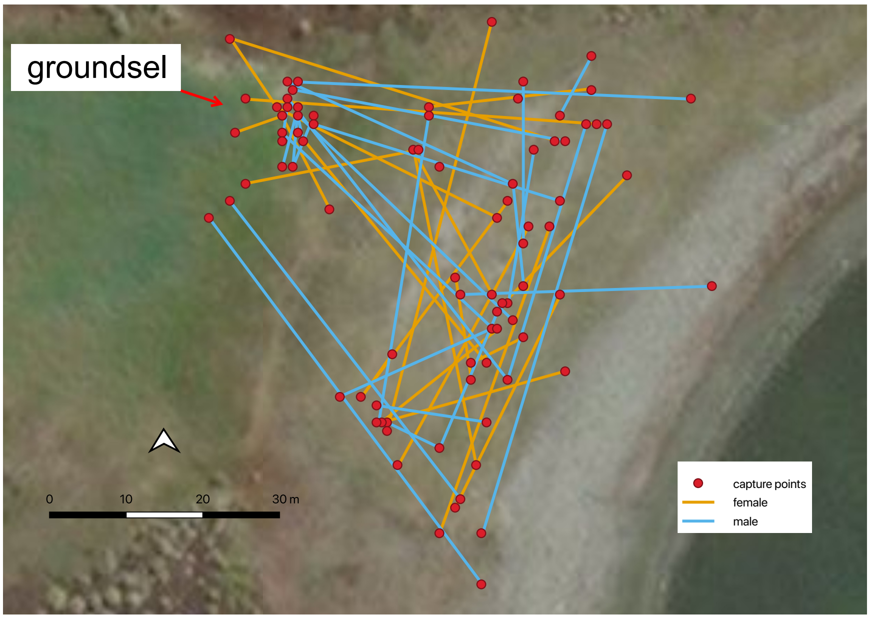

Figure 3.6 Flight patterns of *Y. cytheris* at Bleaker, showing that both sexes ranged widely across the patch, although with frequent movements to and from the groundsel flowers.

Figure 3.7 Flight patterns of female and male *Y. cytheris* between capture and recapture at Sea Lion, 2016-2018, showing the attraction of a flowering Christmas bush, *Baccharis magellanica*, in December 2016. Five of the recaptures were of butterflies caught at the bush on successive days.

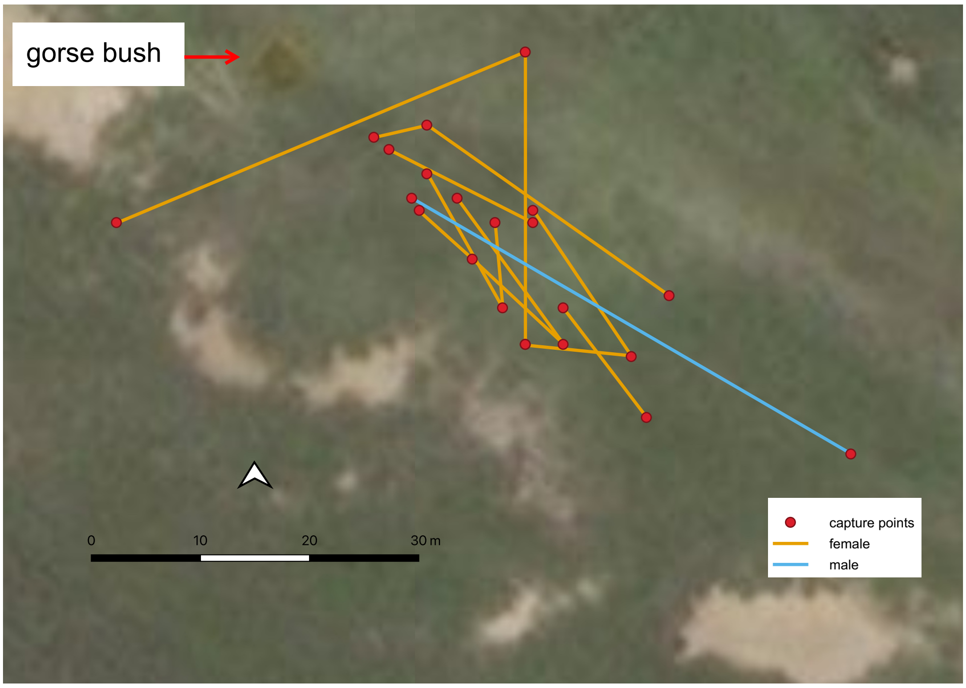

Figure 3.8 Flight patterns of female and male *Y. cytheris* between capture and recapture at the lower meadow, Roy Cove, 2016-2018, showing that butterflies movement was focused on a 30m x 20m area in the lee of a gorse bush, *Ulex europaeus.*

Table 3.8 Distances flown between captures within patches.

			flight distance (m)			
site	patch	area (ha)	*n*	*M*	*SD*	95% CI
BL	BL	0.63	48	26.64	15.56	22.26, 31.01
FP	FL	0.42	2	45.24	10.48	48.87, 139.36
	RM	0.08	-	-	-	-
	SC	0.18	-	-	-	-
RC	BB	0.79	3	7.97	3.15	0.15, 15.79
	LM	0.28	13	17.90	13.38	9.81, 25.99
	RR	0.52	-	-	-	-
	UM	0.80	-	-	-	-
	WH	0.06	-	-	-	-
SL	SL	7.00	10	33.72	43.88	2.33, 65.10

Key: sites: BL = Bleaker, FP = Frying Pan, RC = Roy Cove, SL = Sea Lion; patches: FL = fence line, RM = river mouth, SC = south cliffs, BB = bluebottle rocks, LM = lower meadow, RR = rock run, UM = upper meadow, WH = windmill hill. Patches at which there were no recaptures have data recorded as "-".

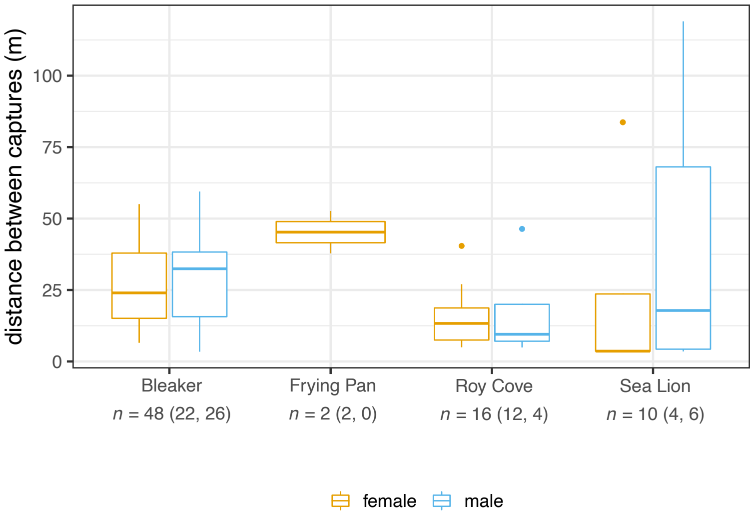

Figure 3.9 Distances flown between captures, by site and sex

Only two patches at Roy Cove (bluebottle rocks, lower meadow) and one at the Frying Pan (fence line) had repeat captures, which made meaningful comparisons difficult (Table 3.3). No strong correlation was, however, found between distances flown between captures and patch size (Spearman: $\rho = 0.420$, $p = 0.227$), and the two patches with the most repeat captures, Bleaker and the lower meadow at Roy Cove, were the fourth and sixth largest respectively.

Both sexes flew extensively over their patches, whether patrolling (males) or searching (males and females) with a focus on particular areas: flowering groundsel, *Senecio vulgaris*, at Bleaker (Figure 3.1); a flowering Christmas bush, *Baccharis magellanica*, at Sea Lion (Figure 3.7) and the shelter of a large gorse bush, *Ulex europaeus*, at the lower meadow, Roy Cove (Figure 3.8).

The only butterflies found away from the main patches were on Bleaker. One, a male of wing condition 3, was in the settlement, 300m from the main patch; the other, a female of wing condition 3, on the track 850m southwest of the main patch. While both were old adults, the sample size was inadequate to support any hypothesis of the age at which butterflies might move from patches.

3.3.4 Imago longevity

Analysis over a five-day period, initial capture followed by four days of potential recapture, showed a mean apparent survival (φ) period of 2.93 days (*SD* = 1.62; 95%

CI [2.33, 3.53]) from first capture. There was a significant difference between female (*M* = 3.53, *SD* = 1.55) and male (*M* = 2.33, *SD* = 1.49) survival over the five days *F* (1, 28) = 4.65, *p* = 0.040, 95% CI [-2.34, -0.06], *d* = 0.79. Inclusive of first and last days, this gave a mean age for females of 4.53 days, and for males 3.33 days. The maximum period between first and last capture was six days, inclusive of first and last days, in the case of females and seven in the case of males.

Jolly-Seber analysis (Table 3.6) showed female survival rates (φ) to be higher than male. Data from the sample population used to estimate five-day longevity predicted that the more rapid decline in male numbers would lead to females taking over the larger initial number of males by day four (Figure 3.10a). The application of survival rates to initial populations of each sex predicted that 10% of females would survive until day 11, whereas 10% of males would only survive to day 7 (Figure 3.10b). The estimated mean life spans produced by this analysis, over a 20-day period, were 4.73 days, 95%CI [2.61, 7.41] for females and 3.35 days, 95%CI [2.16, 5.34] days for males.

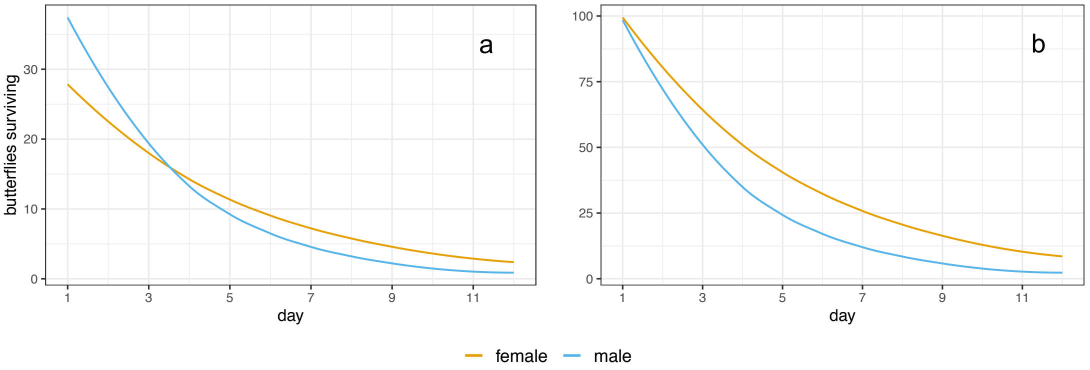

Figure 3.10 Predicted numbers of *Y. cytheris* on each day after initial capture, by sex The starting numbers on day 1 (a) were female, *n* = 28 and male, *n* = 38, representing the numbers of butterflies with potentially three further days of capture ahead of them. Each previous day's figure is multiplied by the mean apparent survival rate (φ) for the sex, derived from the Jolly-Seber analysis of MRR data (Table 3.5). (b) compares predicted losses to the population from a starting point of 100 butterflies for each sex.

Plotting butterfly wing condition against day of capture (Figure 3.11) showed a daily decline in condition, measured by the number of categories moved, significantly less in females (*M* = 0.42, *SD* = 0.38) than in males (*M* = 1.05, *SD* = 0.24) over the study period, with a large effect size *F* (1, 37) = 9.05, *p* = 0.005, 95% CI [-0.21, -1.07], *d* = 0.99.

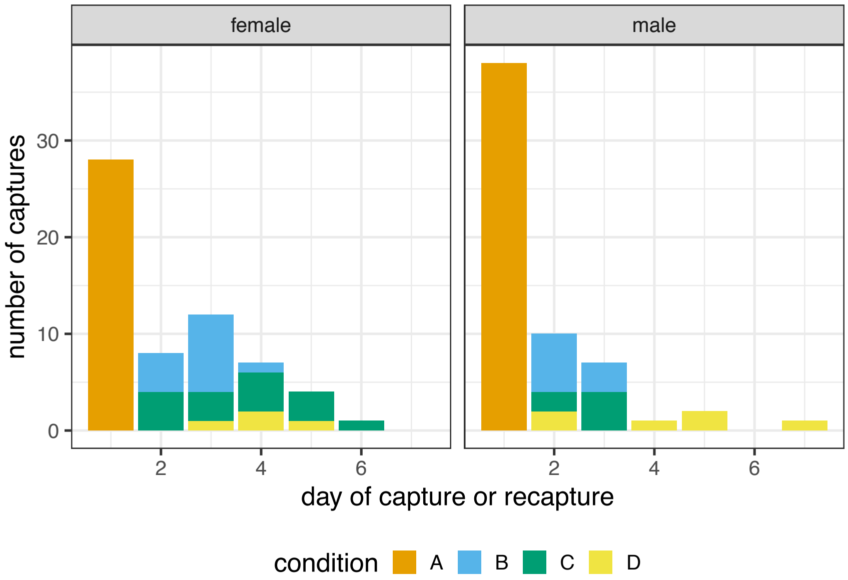

Figure 3.11 Wing condition of *Y. cytheris* on recapture. Key to condition: A = pristine, B = some fading of colour, C = as B, but with wing damage, D = faded and battered. Day 1 includes only butterflies in pristine condition with at least three further days of possible capture ahead of them. Captures on days 2 – 7 are of butterflies originally captured on day1. Repeat captures on the same day are not recorded.

3.3.5 Sex ratio and emergence patterns

Captures were effectively balanced in terms of sex: of the 403 captures, 210 (52.1%) were female and 193 (47.9%) male (Table 3.2); of the 321 individuals marked, 166 (51.7%) were female, and 155 (48.3%) male. More males than females were caught in the first hour of MRR, 0900 - 0959, and fewer in the last four, 1300 - 1659 ($\chi^2 = 7.93$, *df* = 1, *p* = 0.005) (Figure 3.12).

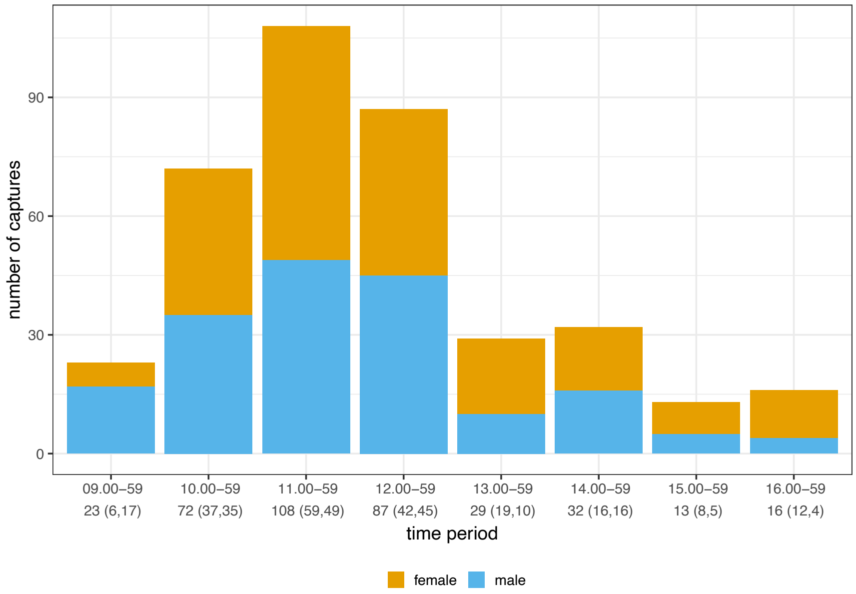

Figure 3.12 Daily first capture times by sex of *Y. cytheris* over all study visits.

First captures of butterflies with wing condition A showed more males (31) than females (20) were caught in the morning, with numbers of males (19) and females (21) nearly equal in the afternoon, although, in considering evidence for early eclosion, the difference between the sexes in the morning was not significant ($\chi^2 = 2.37$, $df = 1$, $p = 0.123$, 95% CI [0.46, 0.74]) (Figure 3.13).

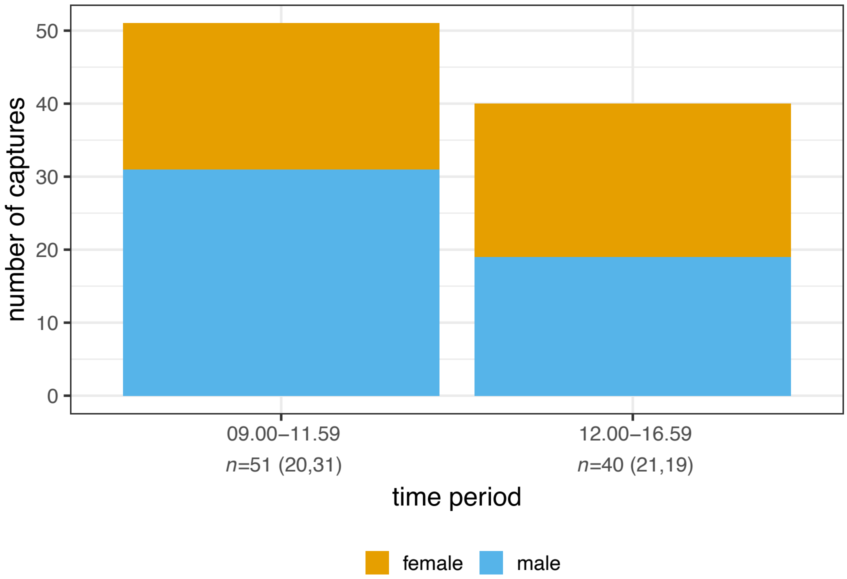

Figure 3.13 Daily first capture times by sex of pristine (wing condition A) *Y. cytheris* over all study visits.

3.3.6 Activity at the time of capture

The main pattern of activity for both sexes was a seemingly aimless, restless movement across the patch, characterised in this chapter as searching (Figure 3.14). There was no evidence of male perching as a prelude to launching into mating or other interaction. Activity generally declined in the afternoon, with captures falling off after 1300. More males (6) than females (17) were caught before 1000, and more females (12) than males (4) after 1600.

There was an overall difference in behaviour pattern for the combined sexes in the afternoon compared with the morning ($\chi^2 = 13.88$, $df = 5$, $p = 0.016$), although the difference for each sex was not significant (females: $\chi^2 = 6.64$, $df = 5$, $p = 0.249$, males: $\chi^2 = 9.58$, $df = 5$, $p = 0.088$). Nor was the difference overall in behaviour pattern of the sexes significant ($\chi^2 = 7.75$, $df = 5$, $p = 0.170$), although the overall tendency was for females to pursue much the same course of behaviour over the day, but for the males to change. Nectaring, with wings open, for both sexes was more prevalent in the morning, while basking on bare surfaces was more prevalent in the afternoon. Male interacting was commoner in the morning than the afternoon.

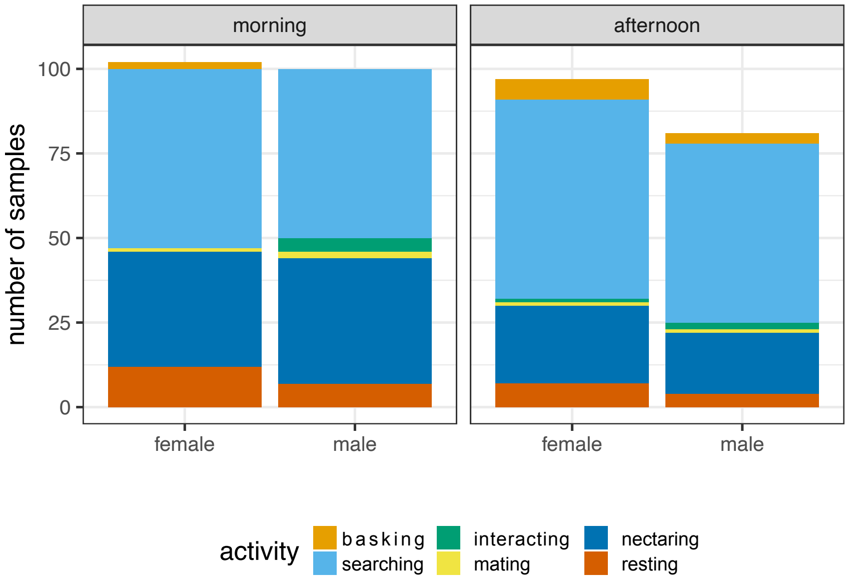

Figure 3.14 Activity by *Y. cytheris* at the time of capture, by sex and time of day. Key: morning = up to 1159, afternoon = 1200 onwards; basking = lying with wings flat, on stone, sand or bare earth; searching = seemingly aimless movement, with pauses for nectaring across the patch; interacting = pursuing, or being pursued by, another butterfly, either in courtship or conflict; mating and nectaring are self-explanatory; resting = pausing, usually on foliage, with no apparent motive. No butterflies were caught, and only one was seen, ovipositing.

3.3.7 Nectaring preference

Y. cytheris was observed nectaring on seven species of plant, apparently indiscriminately (Figure 3.15). Choice of nectaring plant appeared to be determined by what was flowering at the time, with the dominance of Christmas bush *Baccharis magellanica* on Sea Lion, where a large patch was in flower in December 2016, and of groundsel, *Senecio vulgaris*, which was in flower in December and January on Bleaker (Figure 3.1).

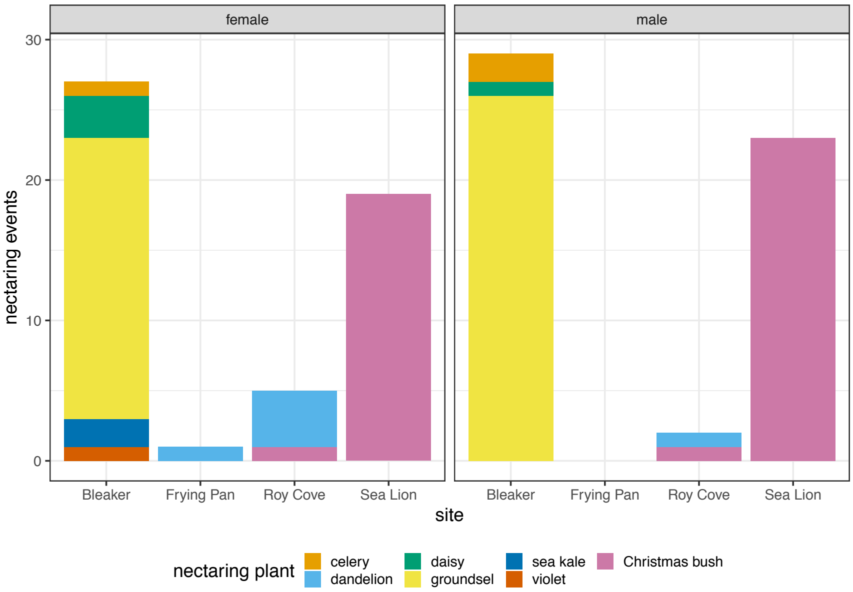

Figure 3.15 Plants used by *Y. cytheris* for nectaring at the time of capture. Key: celery = wild celery, *Apium australe*; dandelion = *Taraxacum* agg; daisy = *Bellis perennis*; groundsel = *Senecio vulgaris*; sea kale = *Senecio candicans*; violet = *Viola* spp.; Christmas bush = *Baccharis magellanica.*

3.4 . Discussion

3.4.1 Population size and structure

(i) **Findings**

There were no records of *Y. cytheris* moving between habitat patches, although two butterflies were found at 350 m and 850 m from the nearest patch. All flights measured were within the boundaries of a patch, with a mean distance flown between captures of 26 m.

Y. cytheris spent little time as an adult, with an apparent survival (φ) mean of 4.5 days for females and 3.3 days for males. Evidence from declining wing condition supported these time scales.

While MRR was able to provide data for some metrics, such as survival, replenishment rates, and capture probabilities, there was inadequate evidence to estimate overall population size, other than to provide estimates of orders of magnitude at certain time periods.

(ii) **Discussion**

The linkage between sedentary behaviour in butterflies and niche breadth has been extensively explored (Hanski 1982, Shreeve 1995, Gaston et al. 1997, Komonen et al. 2004) and linked to wider studies of dispersal (Stevens et al. 2010). Larval host plant specificity and narrowness of habitat were shown to be two key indicators. A further factor was position at the end of a species range, where Komonen et al. (2004) concluded that, having found a suitable patch, with the prospect of few others within range, a butterfly was likely to remain in it. On this basis, *Y. cytheris,* dependant on *Viola* spp., living in small patches, and at the edge of its range, would be readily predicted to be sedentary.

The extent to which *Y. cytheris* is sedentary remains unclear. Sekar (2012), investigating predictors of mobility, found that flight period duration, the length of time during which adults could be observed on the wing, was an important predictor, second only to wingspan (considered in Chapter 6). *Y. cytheris*, with a recorded 121 days (Boyson 1924) has a long flight period compared with other butterflies: Bubová et al. (2016), for example, found flying seasons in European butterflies varied from 16.5 days

(*Pseudophilotes bavius*) to 76 days (*Coenonympha pamphilus*). In the Falklands, with constant winds and heavy gusting, this maximises the opportunities for the butterfly to be blown distances beyond its habitual flying range, although further investigation is necessary to determine the extent of that range. On available evidence it might best be described as sedentary, though stochastically mobile.

No gap within the flying season was found to suggest *Y. cytheris* was bivoltine, and Välimäki et al. (2008) showed that butterflies were more likely to be univoltine at higher latitudes.

A meta-analysis of adult longevity amongst European butterflies (Bubová et al. 2016) showed considerable variation between species. Mean life spans ranged from 2.4 days (*Maculinea alcon*) to 15 days (*Cupido minimus*). By comparison, *Y. cytheris*, with apparent survival rates (φ) of 4.5 days for females and 3.3 days for males, with maxima of 6 and 7 days respectively, appeared to be short-lived. This unexpected combination of a short adult lifespan and a long flying period requires further investigation.

While it proved possible to estimate the key metrics in population analysis, apparent survival and replenishment rates, together with capture probabilities, attempts to measure population could only provide estimates of orders of magnitude over certain time periods. The conclusion reached was essentially that of Osváth-Ferencz et al. (2017): that estimates of daily population size were "unreliable (or impossible or meaningless)" when sampling did not cover the whole flight period, or when either sampling frequency or the number of daily captures and recaptures was low.

The difference in apparent survival between the sexes also requires further investigation. As overall capture figures showed a balance between the sexes, and there were no records of immigration into habitat patches, the *pent* for males, 0.141 compared with 0.135 for females, suggests that a higher number of males eclosed each day than females.

The absence of any repeat captures demonstrating movement between patches at Roy Cove and the Frying Pan give no grounds for regarding population structures as networks between patches, and no evidence for metapopulation structures on sites. The capture of two butterflies away from the patch Bleaker showed some mobility, but the question of population structure at the local level remains largely unsolved. As the patches on Roy Cove are only 200-300m apart, it would be plausible for there to be movement between them.

A comparison was made with the *Boloria* cluster of butterflies, the nearest relatives to *Yramea* (Simonsen 2005), to identify any commonalities in population structure. The picture was mixed. There were no species matching *Y. cytheris's* recorded 121 days of flight period (Boyson 1924). *B. napaea* was the nearest, recorded at 68 days in the Alps, although only 26 in the Arctic (Ehl, Holzhauer et al. 2019); *B. pales* was at 63 days (Ehl, Böhm et al. 2019); while Bubová et al. (2016) recorded maximum flight periods for *B. eunomia* at 45 days, *B euphrosyne* at 32 days, and *B aquilonaris* at 21 days. Nor was there any evidence that *Y. cytheris* in the Falkland Islands shared the biennialism recorded in some *Boloria* fritillaries. As the examples came from butterflies found at high elevations or latitudes, such as *B. acrocnema*, at 3500m, though only 40ºN (Britten and Riley 1994); *B. chariclea*, above 60ºN (McFarland 2003), and *B. alberta* (Pinel 1995), at 2500m, 51ºN, biennialism would not be predicted from low elevations at 51ºS.

Ehl, Böhm et al. (2019) considered apparent survival (φ) of *B. pales*, which, at 3.3 days for females, and 4.5 for males, was as low as *Y.cytheris*, although the variation between the sexes differed, with *Y. cytheris* at 4.5 days for females and 3.3 days for males. Bubová et al. (2016) recorded φ of other *Boloria* fritillaries at between 4.3 days (*B. aquilonaris*) and 9 days (*B. euphrosyne*), with a maximum of 11.5 days (*B. eunomia*).

Boloria mobility is generally low. An analysis by Komonen et al. (2004) of butterflies in Finland, using a mobility index of 0-10, from least to most mobile, only scored *B. euphrosyne* (7.5) and *B. selene* (6.5) above 5.0, with the ten other species listed scoring between 1.8 and 3.7. Their approach was based on an expert survey, therefore it is not possible to compare *Y. cytheris* directly with the other species. It appears, however, consistent with their results.

Further work is needed to understand mobility, as it is difficult for a single investigator, running a variety of studies within tight time frames at a number of sites, to produce sufficient data. Next steps might involve more investigators spending longer at individual sites, especially those containing numbers of patches such as Roy Cove and the Frying Pan, tracking individuals and spending time on the terrain between patches. Subsequent work might also include finding more sites with distinct and separate patches of *Viola* and continuing MRR activity there. Next Generation Sequencing of DNA should then be considered as a way of identifying relationships between patches (Ekblom and Galindo 2011, Klepsatel and Flatt 2011) and, in particular, between sites, as an assessment of the overall population distribution in the Falklands is possible at this stage. This would lead on to examining the Falkland populations within the context of the overall population of *Y. cytheris*, by undertaking similar studies in Latin America.

3.4.2 Behavioural patterns

(i) **Findings**

There was some evidence of males emerging earlier in the day than females, with the greater proportion of males caught in the morning than in the afternoon. The main pattern of activity for both sexes was a seemingly aimless, restless movement across the patch, described here as searching.

Patterns of behaviour for both sexes changed over the course of a day, with nectaring more common in the morning and basking in the afternoon. Male interaction, whether courting or chasing away competition, was more prevalent in the morning. Nectaring appeared indiscriminate, with *Y. cytheris* observed feeding on seven species of plant. As the flight records showed, large flowering patches exerting a strong pull on population distribution.

(ii) **Discussion**

Sea Lion provided an illustration. In December 2016, when a single *Baccharis magellanica* patch was flowering, 64 individuals were marked over four days, with a population estimated, through Jolly-Seber, at 139, 95% CI [83.0, 359.8]. On two other visits, however, in January 2016 and January 2018, by which time *B. magellanica* had ceased to flower, the total catches were 6 (over one day) and 0 (over two days).

The Sea Lion example does, however, point to other hypotheses. Analysis of nectaring suggested that *Y. cytheris* was not particularly discriminating. Furthermore, Thomas et al. (2011) in a meta-analysis of factors which might underpin evidence-based conservation, observe that nectar resources were not a driver in dynamic population change. Yet the flowering of *B. magellanica* on Sea Lion, and of *Senecio vulgaris* on Bleaker coincided with the appearance of large numbers of butterflies drawn in from all over their patches. Possible explanations include (i) there are large populations throughout the season on both, but they spend most of their time concealed; (ii) populations peak around the time of a major flowering; (iii) the butterfly peaks in December, and only small populations are found thereafter.

Further investigation is needed to understand the dynamics. Ideally, sites should be identified that appear to have large populations, and that can be monitored from November to February. If specific roosting areas can be identified, these might form focal points. Sites should be visited weekly, or ideally, given the imago life span, twice a week and undergo MRR. Particular note should be taken of nectaring plant flowering seasons. As part of this process a system of Pollard Walks (Pollard 1977, Pollard and Yates 1993), with robust steps to minimise bias (Harker and Shreeve 2008) should be considered to produce and monitor changes in population indices.

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Chapter 4: Habitat requirements

4.1 Introduction

Effective conservation planning for species with complex life cycles requires an understanding of how different life stages respond to the environment at different scales (Taboada et al. 2013, Curtis and Isaac 2015, Samways 2019). These may be considered at two levels: that of the individual butterfly, and that of populations. For an individual butterfly, the scale for oviposition is at the level of the leaf; for larval feeding, at the level of the plant, or group of plants; and, for pupation, at the level of the plant, or the location of attachment or concealment. Adult flight, including the activities of nectaring, basking, mating, interacting and oviposition, can cover levels from a small patch up to a landscape, and, in the case of migratory butterflies, beyond (Turlure et al. 2010, Evans et al. 2020, Kral-O'Brien et al. 2020). Population level takes a step beyond the flight of an individual, and considers the distribution of the butterfly, a function of its mobility and suitable habitat. The levels used in this chapter for *Yramea cytheris* are set out in Box 1.

Each stage of life has its own habitat, the specific resources, encompassing topography, climate and vegetation, on which it depends. While there is a debate about the meaning of habitat and its semantic network (Guthery and Strickland 2015), the advice of Morrison and Mathewson (2015) has been followed here: to spell out the intended meaning early on and then religiously apply it. In this chapter, the butterfly's habitat is understood as the resources it utilises, and the way in which those resources interact with each other, at a given scale (Dennis et al. 2003, Dennis and Hardy 2018).

This chapter investigates the habitat requirements of *Yramea cytheris* by contrasting the biotic and abiotic aspects of locations where eggs had been laid, and those where they had not. It assesses the threats to those requirements, considering the wider population of the species both in the Falklands and South America, and suggests ways in which they might be addressed.

Box 1: Scales at which habitat data are analysed

Geographical: the complete geographical range of the species or subspecies.

Landscape: a major geographical area. Here Latin America or the Falkland Islands.

Site: a defined geographical area within the landscape, such as a settlement or island. Here generally one of the four study sites, Bleaker, Frying Pan, Roy Cove or Sea Lion.

Patch: a distinct topographical location within a site, such as meadow, dwarf shrub heath, rock run or edge within a site.

Location: a single *Viola* plant, or small group of *Viola* plants, within a patch which are potential larval hosts. A fundamental scale in this chapter, as contrasts between non-oviposition or oviposition are generally at this level.

Plant: a single plant within a location, *Viola* spp. unless otherwise stated.

Leaf: a single leaf on a plant

4.1.1 Habitat factors at a range of scales

(i) **The geographical and landscape scales: the impact of climate change**

Yramea cytheris has a wide distribution in South America, from metropolitan Santiago in Chile to Tierra del Fuego (Chapter 1), a north-south spread of 1500 km. It is found at elevations from sea level to 890 m (Bariloche, Chile), at sites with mean December maximum temperatures from 15º C (Falkland Islands, Tierra del Fuego) to 29º C (Santiago, Chile). This suggests that it is, as a species, tolerant of a range of abiotic circumstances.

In the Falklands, *Y. cytheris* is a species at the edge of its range, in a region, the sub-Antarctic, which is particularly sensitive to climate change (Pendlebury and Barnes-Keoghan 2007, Terauds et al. 2012, Chown and Convey 2016). Regional climate models have predicted a 1.8°C (± 0.34 *SD*) rise in the Falklands' mean annual maximum temperature by the 2080s compared with the period 1961-90 (Jones et al. 2013). An increase in air temperature at its present oviposition sites may not only have a direct effect on the butterfly itself, but also an indirect one through its impact on the butterfly's larval host plants, with potential for changes in distribution and physiology (Becklin et al. 2016). Maritime warming threatens a change in the Antarctic Circumpolar Current (ACC), a rise in sea level and an increase in storms (Pendlebury and Barnes-Keoghan 2007), potentially threatening the existence of those oviposition sites which are close to the shoreline. A greater, though remoter, risk in a change in the ACC is its impact on the unstable north slope of the Burdwood Bank 150 km to the south of the islands, and the resultant threat of a tsunami (Regnauld et al. 2008, Nicholson et al. 2020).

An important potential response of butterflies to climate change is a range shift, towards the poles or to higher elevations (Parmesan et al. 1999, Hill et al. 2009, Chen et al. 2011, Mason et al. 2015). Parmesan (2003) observed that, with each 1º C increase in mean annual temperature, a species' range would need to shift several hundred kilometres to maintain its climatic envelope. There is limited scope for either the butterfly or its larval host plant to maintain its present climatic envelope at the landscape level in the Falklands, which barely cover 1º 30' of latitude, representing a temperature change of 0.75º C (La Sorte et al. 2014) from north to south, and have a maximum elevation of 705 m, a temperature change of 4.6º C (Ingleby 2013). The butterfly's tolerance of a wide range of temperatures in Latin America, together with evidence that the rate of retreat of the warmer, trailing edge of the climate envelope for lepidoptera is slower than the expansion of the leading, cooler edge (Chen et al. 2011) suggest that even at the landscape level there is the possibility of successful adaptation.

(ii) **The site and patch scales: the impact of changing land use**

Largely sedentary butterflies, such as *Y. cytheris* (3.4.1 (ii)), mainly spend their lives at site, and often simply at patch, level. The principal constraint at these levels is the presence or absence of the larval host plant, in the case of *Y. cytheris*, *Viola* spp. Both sites and patches are defined through separation by areas without larval host plants, such as improved grassland, acid grassland, scrub, bog or rock.

The structure of separate patches of potential or actual habitat within a site is important in considering population structures, as it underpins metapopulation theory (Hanski 1999, 2004). While the Falklands, and in particular Roy Cove, would allow further exploration of this for *Y. cytheris*, adequate resources were not available to pursue it in this thesis.

It is at the site and patch level that changing land use (1.5.2 (ii)) potentially has the greatest effect, The Falklands' heathland is a plagioclimax environment, shaped by human activity including grazing and fires, particularly in those areas which would otherwise have been covered by *Poa flabellata.* This environment, existing in its present form from the mid 19th century (Palmer 2004), is a fragile one, at threat from improved agricultural practices on the one hand and conservation measures such as suspension of grazing, removal of established invasive species, and replanting of tussac on the other (Tourangeau et al. 2019). While this man-made environment is recent, it is one which has favoured, and potentially enabled, the wide distribution of *Viola* spp., and the consequent wide distribution of *Y. cytheris,* on the islands.

Climate variables, such as temperature and wind speed, are generally first analysed at the site level, using data drawn from WMO weather stations. This is helpful when looking at wider scale changes, such as range shift, where the data contribute to the identification of a pattern (Warren et al. 2001). WMO data is less helpful in making finer scale comparisons, particularly as weather stations are positioned in such a way as to avoid precisely those characteristics, such as shade and shelter, which influence the microclimate, a key determinant of habitat selection (Frenne and Verheyen 2016, Montejo-Kovacevich et al. 2020). Comparing data within the same topographic area, but for a range of habitats, Suggitt et al. (2011) recorded differences amongst them of more than 5°C in monthly maxima and minima, and of 10°C in thermal range, comparable, as they observed, with the level of warming expected for extreme future climate change scenarios.

(iii) **The location scale and microhabitat scales**

For butterflies which are largely sedentary, such as *Y. cytheris* (3.4.1 (ii)), two other responses to climate change are possible, at least when the threat of stochastic inundation is not imminent: a phenological adjustment to eclosion (Macgregor et al. 2019) and changing microhabitat to maintain the same microclimate (Parmesan et al. 1999, Davies et al. 2006, Bennett et al. 2015, Singer 2017). There are inadequate data to consider possible phenological change in *Y. cytheris* (1.4.3). Microhabitat adjustment, however, offers additional scope, beyond latitude and elevation shift, to mitigate potentially harmful temperature changes. The variability between actual and potential microhabitats (Weiss et al. 1988, Bennie et al. 2008, Lawson et al. 2014) affords the possibility of finding suitable conditions nearby when one habitat becomes unsuitable. Bennie et al. (2008), for example, noted a mean annual temperature difference between adjacent north and south facing slopes in British calcareous grassland of 2.5-3º C. In the Falklands, the difference between sheltered and exposed sites in a mean wind speed of 8 ms^{-1} would be 3.3º C in summer (Osczevski and Bluestein 2005). Either of these would be equivalent to a shift of 5º in latitude or 500m in elevation.

This chapter aims to identify the microhabitat preferences of *Y.c. cytheris*, about which virtually nothing has been recorded (1.4.3). The main area of focus is its preferred microclimate, particularly its temperature preferences and factors, such as wind and microtopography (e.g. shelter, and heat retaining features, such as bare earth rocks) which contribute to it. This is important in conservation planning. Microclimates are particularly important at range limits (Thomas et al. 1999), and ensuring that a heterogeneous environment exists at site level which provides microclimate options is potentially important in ensuring a species survival in the context of environmental stochacity (Gillingham 2010, Oliver et al. 2010, Bennett et al. 2015).

(iv) **Factors in the selection of oviposition and larval host plants**

Literature searches showed, as with microclimates, a considerable knowledge gap about *Y. cytheris's* selection of locations for oviposition and larval feeding. Like the majority of the Argynnini tribe of fritillaries, *Y. cytheris* appears to be a specialist on Violaceae as larval host plants (Simonsen 2006), which it also uses for oviposition. There is no evidence of its ovipositing on nearby vegetation or litter (1.4.3), as can be the case with related *Boloria* species (Brakefield et al. 1992).

But even within a single species of host plant, butterflies have been observed to show preference for a particular size (Thomas et al. 1991, Randle 2009) or chemical composition (Myers 1985, Dicke 2000, Vickerman and de Boer 2002).

Selection is likely to be more critical if the butterfly lays its eggs in clutches. Singer (2004) observed that the clutch-laying *Euphydryas editha* spent several minutes searching for the right leaf, whereas species that laid eggs singly might only investigate a leaf for a few seconds and then move on to the next. *Y. cytheris* lays its eggs singly, again a feature of the Argynnini; of the 13 members of the tribe recorded in Germany, for example, (Fartmann and Hermann 2006), only *Boloria eunomia* laid its eggs in groups.

Stefanescu et al. (2006) suggested that chlorophyll content was a factor in oviposition location selection, observing that *Euphydryas aurinia* generally laid eggs on leaves with the highest chlorophyll content, although they found no difference in chlorophyll levels between between non-oviposition and oviposition plants.

Plant size and chlorophyll were tested at both plant and leaf level. The conservation implications of any strong preference would be similar to those for microhabitat selection: a range of options would need to be made available at any given site to mitigate environmental stochacity. A key question is, as Jaumann (2017) put it in connection with the preferences of *Pieris rapae*, just how choosy the butterflies are.

4.1.2 Research Questions

RQ1: what are the key factors in *Y. cytheris's* selection of oviposition sites?
RQ2: which factors provide the best-fitting model for predicting an oviposition site?

4.2 Materials and methods

4.2.1 Selection of study sites, patches and locations.

The selection of study sites is discussed in Chapter 2. The one common factor in all patches containing oviposition sites was the presence of *Viola* spp*.* During the initial field visit other plants, together with leaf litter around *Viola* spp., were searched for eggs, particularly the *Acaena magellanica* on Bleaker, given the reference to *Acaena* as a host genus in Latin America (Shapiro 1992), but no oviposition was found anywhere other than on *Viola* plants. Analysis of ground cover was therefore based only on those locations with *Viola* plants, whether non-oviposition or oviposition.

The study sites were divided into patches, corresponding to the differing topographic areas identified in the Phase 1 Analysis (Chapter 2). Each patch was first searched for *Viola* spp. Patches on which *Viola* were found were subsequently searched for oviposition locations following a decision-making flow chart (Figure 4.1).

All oviposition locations found in a patch were marked. Five *Viola* plants were then selected on each patch for comparison with plants where oviposition had taken place. This was done by estimating, by a combination of eye and pacing, the size of a patch and the number of paces required between markers to make five stopping points in a W-shaped walk (JNCC 2009, Franklin et al. 2018). If the stopping point was within a metre of the edge of a patch, an additional two steps were taken to ensure a similarity of habitat in each case. The nearest *Viola* plant to the front foot was then marked.

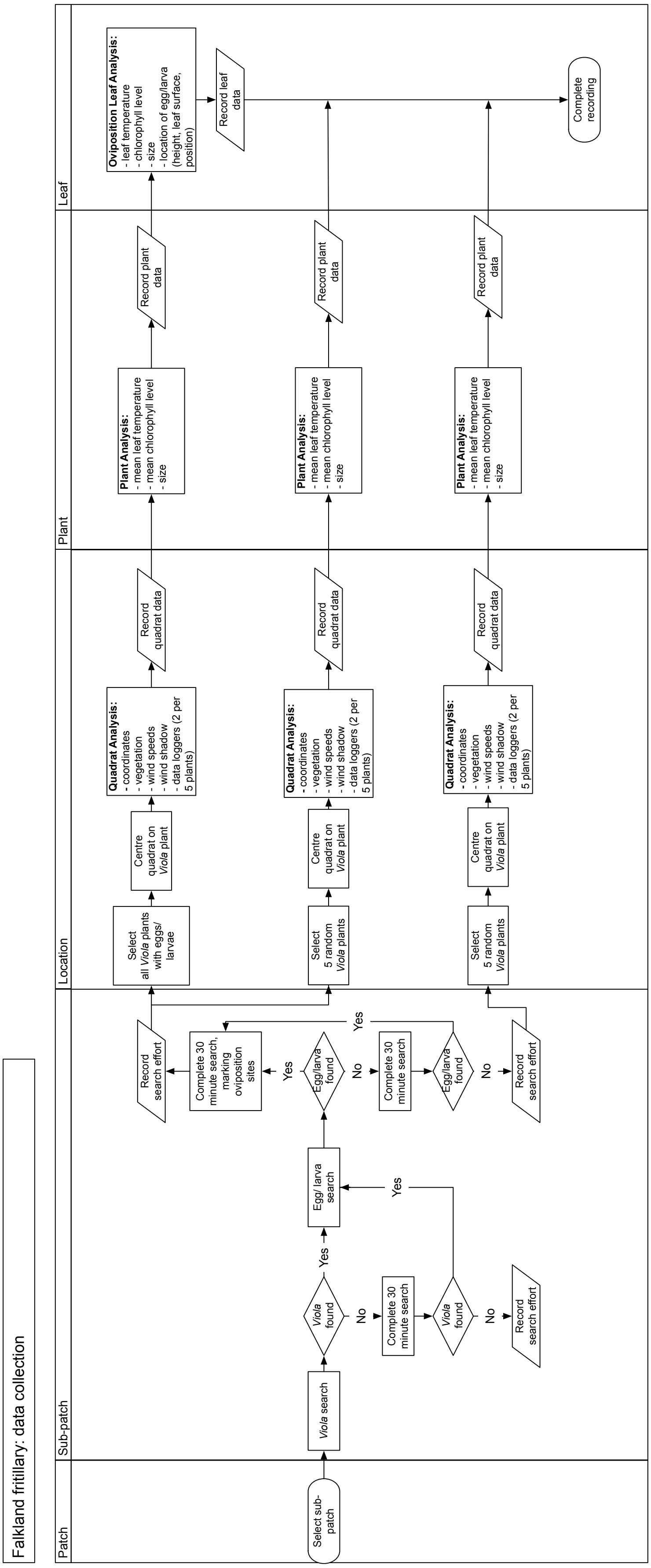

Figure 4.1 Decision-making flow chart for data collection at the study sites.

All oviposition locations found in a patch were marked. Five *Viola* plants were then selected on each patch for comparison with plants where oviposition had taken place. This was done by estimating, by a combination of eye and pacing, the size of a patch and the number of paces required between markers to make five stopping points in a W-shaped walk (JNCC 2009, Franklin et al. 2018). If the stopping point was within a metre of the edge of a patch, an additional two steps were taken to ensure a similarity of habitat in each case. The nearest *Viola* plant to the front foot was then marked.

Oviposition locations were marked with red flags or plant markers, non-oviposition locations with yellow. Flags were used on the first field visit, but on the following visits they were replaced by plant markers to counter strong winds and the attentions of striated caracara (*Phalcoboenus australis*), both of which tended to tear flags apart (Figure 4.2).

Figure 4.2 Challenges to field work in the Falklands. A striated caracara (*Phalcoboenus australis*) on Sea Lion starts to demolish the flags marking oviposition locations. The following season small plant markers rather than flags were used, albeit with only slightly greater success. Photograph by the author.

Subsequent analysis of each location was based on a 50cm quadrat (50cm x 50cm) centred on the *Viola* plant selected. Data for all variables, other than temperature, were collected for each quadrat. The limited number of data loggers available for temperature measurement meant that, at each patch, two were deployed for every five non-oviposition locations, and a maximum of two for the oviposition locations. In each case they were allocated at random by drawing numbered plant markers from a pocket.

A 10cm quadrat (10cm x 10cm) around the selected *Viola* was also used for analysis of ground cover and vegetation, to determine whether there were any key characteristics of the immediate surroundings of an oviposition plant.

4.2.2 Approaches to quantifying topography

(i) **Elevation, slope and aspect: direct measurements**

Elevation, slope and aspect were coded as ordinal categorical variables (Table 4.1, Table 4.2). Elevation and slope were split into five categories, and aspect into eight octants, to determine whether a particular level in a variable, or combination of variables, was preferred for oviposition. The distribution of *Viola*, comparing non-oviposition and oviposition sites, was then analysed to determine whether there was a preference for any factor or combination of factors.

Elevation

Elevation measurements were taken at each potential or actual oviposition location with a Garmin GPSMAP 64S GPS Meter. The manufacturer gave a vertical accuracy of 15-38m with auto-calibration enabled. Tests against known datum points in the field gave an accuracy of ± 5m. Elevations recorded as negative when visibly at, or just above, high tide level, were regarded as being at 1m. The elevation of each location was allocated to one of five thirty-metre levels (Table 4.1).

Slope

A quadrat was held with one of its edges aligned with the slope. The angle of the slope was calculated using the application Angle Meter (Phagdeechat, N., n.d.) on an iPhone held along the upper edge of the quadrat. There was no published accuracy for Angle Meter, but tests with a protractor and spirit level, which would otherwise have been used in the field, showed a correspondence of ± 1°. The slope of each location was allocated to one of five ten-degree levels (Table 4.1).

Table 4.1 *Viola* locations: elevation and slope categories. Each location is assigned to one of the five levels for elevation and slope respectively. These are then used to help determine whether *Y. c. cytheris* favours particular topographic combinations in selecting an oviposition location.

level	elevation (m)	slope (º)
1	0 - 29	0 - 9
2	30 - 59	10 - 19
3	60 - 89	20 - 29
4	90 - 119	30 - 39
5	120 - 149	40 - 49

Aspect

A hand-held compass, a Silva Expedition 4 (Silva Sweden AB, Bromma, Stockholm) calibrated to true north was used to assign the downward direction of a given slope to the nearest cardinal or intercardinal point.

Aspect was considered as two factors (

Table 4.2), wind shade and direct radiation, each allocated to an octant numbered clockwise from 1 to 8. For wind shade, octant 1 was the direction facing the prevailing wind, ascertained from wind roses drawn up for each site from meteorological station data (Chapter 2). This was west for Bleaker, Frying Pan and Sea Lion, and south west for Roy Cove. Individual octants were analysed as, although, for example, octants 2 and 8 might appear to afford the same level of wind shade, being either side of 1, there was not necessary any symmetry, as can be seen in the wind roses of Frying Pan (Figure 2.9) and Sea Lion (Figure 2.18). For direct radiation octant 1 was north, the position of the sun at noon.

Table 4.2 Location-specific wind shade and direct radiation variables. Categories were numbered clockwise by compass point. The direction facing the prevailing wind, which varied by site, was categorised as 1, as was the maximum exposure to direct radiation (marked dir. radn.), facing north (sun position at midday).

	wind shade				dir. radn.
orientation	Bleaker	Frying Pan	Roy Cove	Sea Lion	all
N	3	3	4	3	1
NE	4	4	5	4	2
E	5	5	6	5	3
SE	6	6	7	6	4
S	7	7	8	7	5
SW	8	8	1	8	6
W	1	1	2	1	7
NW	2	2	3	2	8

(ii) **Wind shade and openness: hemispherical photography**

Hemispherical or fish-eye photography has been used extensively in plant ecology, particularly in forestry, for measuring the amount of direct sunlight, or indirect light, falling on a particular location (Anderson 1964, Newton 2007, Fournier and Hall 2017). As it does this by measuring the percentage of an image in which light is blocked, the method is extended here to measure the percentage of the image which blocks the prevailing wind.

Images were recorded with a Nikon D90 digital single lens reflex camera (Nikon Corporation, Tokyo, Japan) and a Sigma 4.5mm f 2.8 circular fisheye lens (Sigma Corporation, Kanagawa, Japan). The back of the camera was placed on the ground and aligned horizontally with plastic wedges using a two-directional bubble level mounted in the accessory shoe, with the front element of the lens 14 cm above the ground. This was selected as the minimum distance possible with the equipment. A distance of 3cm above ground for the front lens would have been preferable, representing oviposition height. Attempts to achieve this with an iPhone 6 (Apple Inc, Cupertino, CA, USA) and a 180º fish eye lens (QTOP 3-in-1, Ningbo, China) were unsuccessful, as the lens (i) failed to produce a circular image, which proved irremediable in post-processing, and (ii) only recorded 165º.

Images were imported into GLA 2.0 (Frazer et al. 1999). Magnetic correction of 02º 47" W (2018) was applied during registration, using data for Stanley from the British Geological Survey website (http://www.geomag.bgs.ac.uk/operations/falklands.html), to produce an image aligned to true north. Projection was set as Lambert's Equal Area, for which the lens was designed. Latitude and longitude were entered as parameters (although longitude is not taken into account in GLA's calculations), as was elevation.

The colour image was converted to greyscale. The grey scale image was further reduced to either black or white pixels, with the density of grey scale in the image which returned a black pixel adjusted by eye. All adjustments were made by the author to maximise consistency. The resulting image was split into 16 azimuth bins, the dividing lines radiating to the 16 points of the wind compass. Each azimuth bin was split into four zenith bins (Figure 4.3). All statistical output from GLA reflected those divisions.

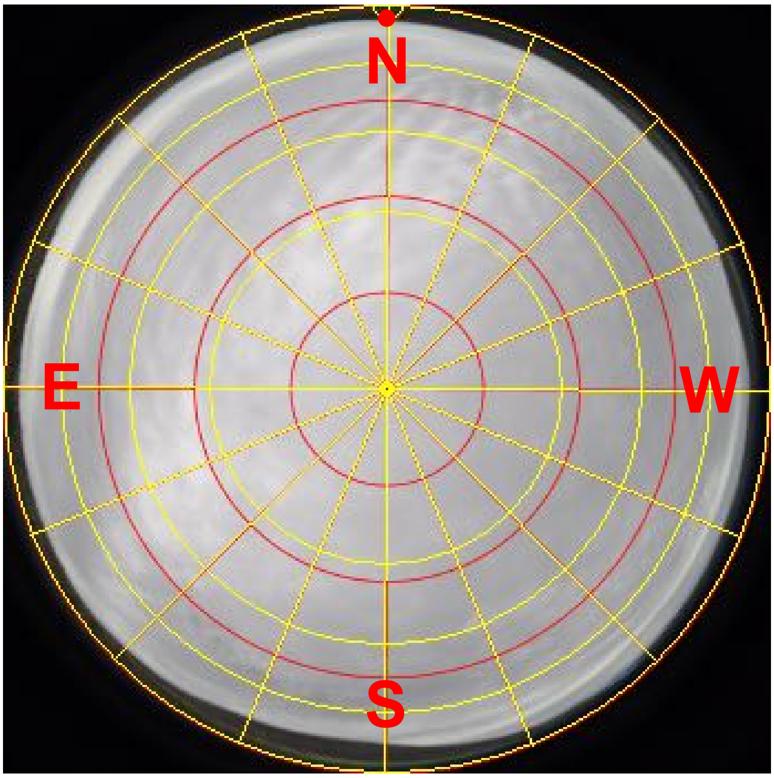

Figure 4.3 Divisions of a hemispherical photograph used for gap light analysis. The yellow lines show the sixteen azimuth divisions. The yellow circles show the four zenith divisions using equal-area projection, the red show equal angle division of the sky. Note that images taken with hemispherical lenses show east to the left of north. Photograph by the author.

To assess wind shade, a quadrant, comprising four azimuth bins, was selected, centred on the prevailing wind direction of each site (Chapter 2). Each of the azimuth bins was split into four zenith bins, and the percentage of open sky in each bin calculated through a count of the white pixels. From this the overall percentage of the quadrant not open, and therefore representing wind shelter, was derived. A similar approach was adopted to ascertain openness, the percentage of direct and indirect light received by a location, in this case through a count of white pixels in all bins.

(iii) **Direct solar radiation: hemispherical photography**

The maximum amount of direct sunlight received by a location was calculated for the butterfly's peak flying season of 1 November to 28 February. Derived from the hemispherical photographs used to calculate wind shelter, the calculation is based on the sun track for a given period at a given latitude (Figure 4.4). It is expressed as the mean, in moles per square metre per day ($mol \cdot m^{-2} \cdot d^{-1}$), of direct radiation, unobscured by topography or vegetation, falling on the location each day over the November-February flying season.

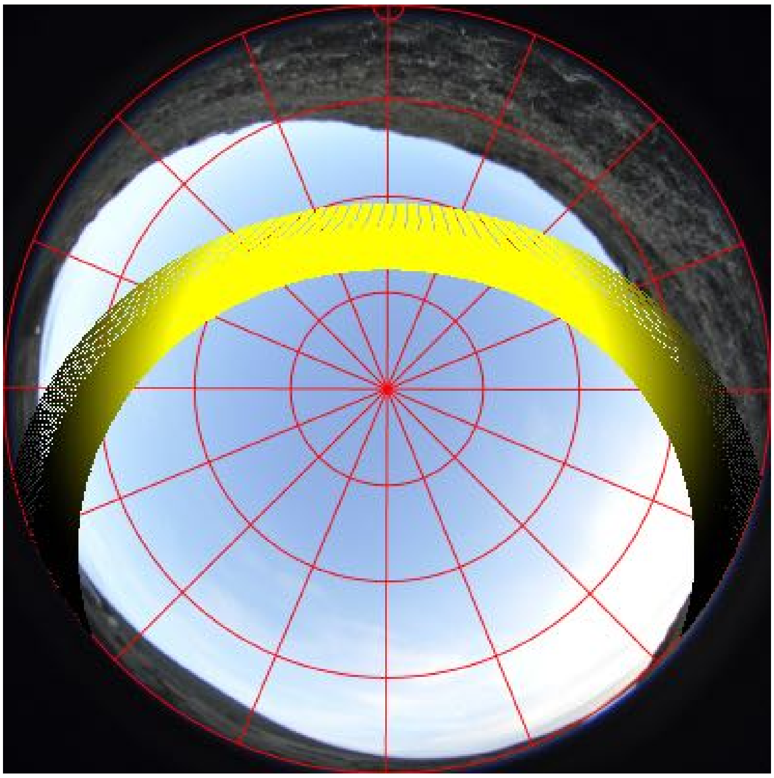

Figure 4.4 Hemispherical photograph showing a sun path overlay for November to February. True north is at the top of the image. This allows the calculation of direct radiation (expressed in mol·m^{-2}·d^{-1}) received at the location of the camera's focal plane. Photograph by the author.

4.2.3 Climate analysis

(i) **Wind speed at site and location level**

Wind speeds were measured with a hand-held Kestrel 4500 weather station. All measurements were taken in wind speeds greater than 5 ms^{-1}, when the wind was blowing from the quadrant of the prevailing wind.

A reading (“site measurement”) was taken at each site on each day that location measurements were taken. This was from a high central point with no topographic obstruction of the wind, with the meter held at approximately 2m above ground level. Further readings were then taken at each location at three levels: 1.5m ("ceiling"), representing the observed flying ceiling for *Yramea cytheris*; 30cm ("flight"), the observed height of a butterfly patrolling or searching; and 3cm ("oviposition"), representing a nominal oviposition height. In all cases the Kestrel was held until the measurement of average wind speed stabilised.

To enable comparisons between sites, location wind speeds at each of the three levels were converted to a proportion of their respective site wind speeds. Thus, for example, it was possible to determine whether the mean wind speed at flight level at site A was a higher proportion of the mean site measurement than that at site B, suggesting that the butterflies at site A were more exposed.

To enable visualisation of those percentages in terms of actual wind speeds, an illustrative transformation was applied:

- a mean overall noon wind speed was calculated for the flying period, November-February, 2013-2018. This was based on combined data from the four Falkland Island meteorological stations nearest to the study sites (Bleaker, Mount Byron, Mount Pleasant, Sea Lion). This was 8.11 ms^{-1}.
- the calculated proportions from each site at each level were then applied to this overall mean speed. Thus a measurement of wind speed at flight level at site A was transformed to show what it would have been had the site measurement been 8.11 ms^{-1}, assuming that wind speed at ceiling, flight and oviposition levels responded in a linear way to that at site level.

A weakness in this approach is the assumption of linearity in the impact at lower elevations of changing site wind speeds. In the absence, however, of a method which can be deployed easily across a large and remote terrain to produce simultaneous anemometric readings at microhabitat levels, it at least enables a framework for making microclimate comparisons to be constructed.

(ii) **Temperature at site and location level**

HOBO UA-002 data loggers (Onset Computer Corporation, Bourne, MA, USA) were used to record temperatures at 15 min intervals. Loggers were placed for a minimum of 24 hrs at each site, and measurements recorded at three time scales: over 24hrs (96 data points); over the butterflies' peak observed flying period, 1000-1600 (24 data points); and at night, 2200-0400 (24 data points).

Two loggers were placed in each patch, randomly located at non-oviposition sites. A HOBO logger was placed at each oviposition site (up to a maximum of two) in the patch. The loggers were all placed in foliage, usually dwarf shrub heath, to protect them from direct sunlight but to afford some ventilation (Gillingham 2010). While this method could produce inconsistencies, particularly temperature spikes, compared with a system of screened loggers (Bramer et al. 2018), it was relatively straightforward to identify affected loggers by their data output, and to remove them from analyses. This affected four loggers (two Bleaker, one Roy Cove, one Sea Lion), their daytime temperatures (> 35º C) suggesting direct exposure to the sun. In addition, two loggers were lost at Roy Cove, possibly as a result of wildlife interventions.

As with wind speeds, temperature data were transformed to enable the use of a larger data set, and comparison between sites:

- data from each site were merged to provide overall mean temperatures for that site at three time scales;
- the mean temperature from each logger for each of those periods was expressed as a proportion of the site temperature;
- data from all sites were merged to provide overall mean landscape (Box 1) temperatures at three time scales. These were: 24 hr, 14.93º C; day, 21.35º C; and night, 9.55º C;
- in comparing sites, the site temperature proportion for each logger was applied to the mean landscape temperature for the appropriate time scale.

The weakness, noted at 4.2.3 (i), in assuming linearity, also applies to temperatures.

4.2.4 Ground cover and vegetation

(i) **Viola distribution**

Distribution of *Viola* spp. at each site was investigated through counts of individual plants at the patch level. Patches (*n* = 27) were identified across the four study sites, principally those investigated for oviposition locations on the basis of Phase 1 habitat analyses (Chapter 2). Thirty 50 cm quadrats were placed on each patch through a W-shaped walk (4.2.1).

(ii) **Ground cover**

Ground cover was assessed through 50cm quadrats, placed at the locations identified in 4.2.1, centred upon the selected *Viola.* Analysis focused on six categories: *Viola* spp., bare ground, litter, grass, dwarf shrub heath and other vegetation (principally forbs). Given the relatively low extent of layering at all the sites, percentage cover was summed to 100%, with no account taken of, for example, earth or litter under vegetation. The same categories were also analysed at 10 cm quadrat level, to determine whether the immediate surroundings of an oviposition plant differed significantly from a non-oviposition one. For both the 50 cm and 10 cm quadrats vegetation was identified to species level to enable the "other vegetation" category to be expanded upon where necessary.

Differences in ground cover between quadrats were evaluated through a non-parametric multivariate analysis of similarities (ANOSIM) (Clarke 1993). A similarity percentages analysis (SIMPER) was used to determine which sort of cover contributed most to the average between-group Bray-Curtis dissimilarity. The analyses were carried out in the package *vegan* in R (Oksanen et al. 2019).

(iii) **Vegetation height**

Maximum vegetation height for each quadrat was recorded by a single drop disc measurement, in which a light plastic disc was lowered until it touched the top of the highest plant. This was effectively the sward stick method advocated by Stewart et al. (2001). The aim was to identify and quantify taller vegetation in locations which mostly comprised dwarf shrub heath, bare ground and grass, each of which was recorded separately.

(iv) **Bare patches**

Bare patches within 30cm and 60cm of quadrat edge

Bare patches can help provide warm microhabitats, as well as areas for basking, of particular value for ectotherms (Randle 2009, Taboada et al. 2013, Dennis et al. 2003, 2006, 2007). The number of patches larger than 10cm in diameter, whether sand, earth or stone, within distances of 30cm and 60cm from the edge of each quadrat, were recorded.

Basking locations

A distinctive feature of Bleaker and the Frying Pan was the large quantity of flat stones by the side of the estuary and on the raised beach respectively. In the case of Bleaker there was also a large patch of vegetation litter by the side of groundsel plants used for nectaring. At both locations long term basking by butterflies (up to five minutes) was observed. On the other locations, one butterfly was observed basking on sand at Sea Lion, but none were observed doing so at Roy Cove.

A series of measurements was taken to determine the difference between the ambient air temperature at 150cm above the features, and the surfaces of the features themselves. All temperatures were taken with a Testo 810 infra-red thermometer (Testo SE & Co. KGaA, Lenzkirch, Germany), shaded by the body.

4.2.5 Plant and leaf data

(i) **Plant size**

Numbers of individual *Viola* plants were recorded in each 50cm quadrat, whether used for oviposition or not. Each plant was checked for eggs or larvae. It was then assigned to one of four categories: (1) small, compact, one or two leaves of <20 mm; (2) erect, three to five leaves of <35 mm; (3) larger, more erect, more than five leaves <40 mm; (4) large, vigorous, sprawling, some leaves >40 mm (Randle 2009).

(ii) **Chlorophyll content of plants and leaves**

Chlorophyll content was measured on the 2018 field visit with an Opti-Sciences CCM-300 meter (Opti-Sciences, Inc., Hudson, NH03051, USA), designed for use on difficult and small samples. On the previous visit a CCM-300 meter by the same company was used, but the large sensor size produced highly variable results on small leaves. The CCM-300 measures fluorescence ratios, comparing the ratio of fluorescence emission at 735 nm and 700 nm. This ratio shows a linear response to chlorophyll content in the range from 41 to 675 mg m^{-2}, thus allowing the CCM-300 to provide, in effect, a direct read-out of chlorophyll content (Gitelson et al. 1999).

The mean of ten readings was taken from each leaf, using the CCM-300's inbuilt calculator together with its two SD outlier removal protocol. Measurements taken were: (i) single leaf, where oviposition had taken place or a larva was found; (ii) the mean of three leaves, excluding the oviposition/larva leaf. These were selected by dividing the overall number of leaves on the plant by three, and taking the first from each group. Where the number of leaves ≤ 4, all leaves other than the oviposition/larva leaf were measured; (iii) as (ii), but for a non-oviposition larva plant, measuring all leaves where the number ≤ 3.

Data exploration of *Viola* began at site level, as the Sea Lion population was of *V. magellanica*, whereas other sites were of *V. maculata*. It was therefore necessary to assess whether their chlorophyll levels varied to an extent that they could not be regarded as a single statistical population.

(iii) **Plant and leaf temperatures**

Plant and leaf temperatures were measured to determine whether butterflies selected plants for oviposition that were significantly warmer, or colder, than other plants, and whether they selected leaves for oviposition that were significantly warmer, or colder, than other leaves on the same plant.

As plant temperatures were measured in varying air temperatures, comparisons were made, at plant level, by calculating a ratio between plant and air temperature for each plant. The ratios were then compared through *t*-tests. At leaf level, as the oviposition leaf temperature was measured in the same time frame as the temperatures of surrounding leaves, a paired *t*-test was used to compare the oviposition leaf with the mean of the other leaves.

Leaves were selected for temperature measurement in the same way as they were for chlorophyll analysis (4.2.5 (ii)). All temperatures were taken with a Testo 810 infra-red thermometer (Pryke et al. 2012). Readings were taken with the plant shaded from direct sunlight. Air temperature, also in the shade, was measured, using the Testo's air/temperature [*sic*] function. It was taken at 80cm above the ground, to minimise the impact of heat radiated from rocks or bare patches.

(iv) **Characteristics of oviposition at leaf level**

The numbers of eggs and larvae per leaf and per plant, together with their positions, were recorded.

4.2.6 Exploration, through multivariate analysis, of possible habitat models for Roy Cove

The extent to which *Y. cytheris's* preferred habitat could be modelled was approached through a combination of mixed linear effects models and multi-model inference. There were inadequate data across all the analyses to produce a model for all sites, particularly as HOBO data loggers and the CCM-300 chlorophyll meter were only used on the final field visit in 2018. This approach was therefore restricted to the largest data set, of 17 locations, at Roy Cove, and should be regarded as exploratory.

Factors showing levels of variation $p < 0.1$ between non-oviposition and oviposition locations in the univariate tests (4.2.2 to 4.2.5), particularly those with large effect size, were considered for inclusion in the candidate models. This approach bridged classical hypothesis testing and information-theoretics (Harrison et al. 2018), avoiding the pitfalls of data-dredging (Ellison 1999, Anderson et al. 2000, Anderson and Burnham 2002). Particular attention was paid to collinearity, as the nexus of topography, climate and vegetation could potentially show correlations which would damage the model. In obvious cases, the analysis of topography through both direct observation and gap light analysis, and the three different time periods for measuring temperature, only one such metric was used in a model at a time.

A set of competing linear models was drawn up. An information-theoretic approach was adopted to find the model with the greatest explanatory power using *MuMIn* (Bartoń 2019). Selection was based on Akaike's information criteria, corrected for small sample size (AIC_c) (Burnham and Anderson 2003), with those with $\Delta AIC_c < 2$ considered robust (Burnham and Anderson 2004).

4.2.7 Statistical analysis software where not otherwise listed

The majority of calculations were done in *R* (R Core Team 2018), with *t*-tests carried out in the included package *stats*. The *stats* package was used for χ^2 tests; as sample sizes were small the χ^2 *p*-value was re-evaluated a 2000 replicate Monte Carlo test (Hope 1968) included in the package. The *tidyverse* package, in particular *ggplot2* and *dplyer* (Wickham 2016, 2017), was used for visualisations. Descriptive statistics were explored in *pastecs* (Grosjean and Ibanez 2018) and *Psych* (Revelle 2018), which was also used to test for correlation. Effect size was calculated using *lsr* (Navarro 2015) and *effsize* (Torchiano 2017). All mapping was done in *QGIS 3.4* (QGIS Development Team 2018).

4.3 Results

4.3.1 Approaches to quantifying topography

(i) **Elevation, slope and aspect: direct measurements**

Elevation

Elevation at the study sites ranged from sea level to 262 m at Roy Cove (Cook Hill). The highest oviposition location, at Roy Cove, was 86 m, and the highest non-oviposition *Viola*, also at Roy Cove, was at 127 m. The majority of oviposition locations (89.9%) were in levels 1 and 2 (0 - 59 m), with only 10 oviposition locations (10.1%) in level 3 (60 - 89 m), and none in levels 4 or 5 (> 90 m) (Figure 4.5). A chi-square test indicated a significant association between oviposition and elevation, χ^2 (4, n = 191) = 18.9, $p < 0.001$. This p-value was confirmed by a 2000-replicate Monte Carlo test.

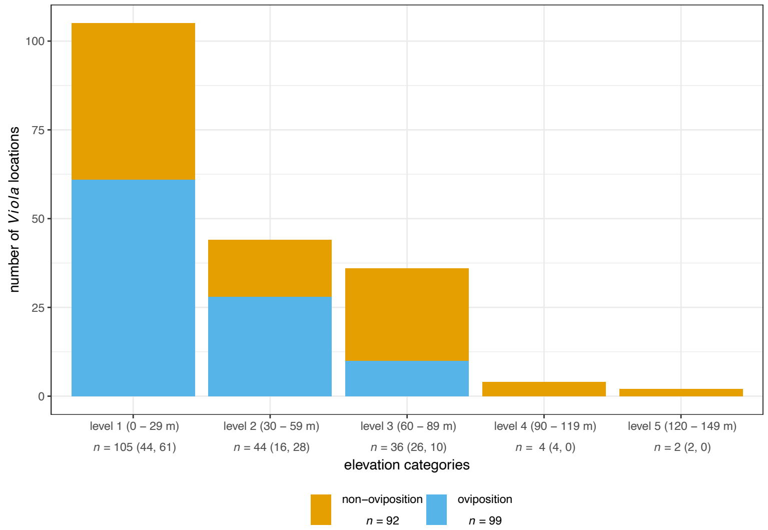

Figure 4.5 Non-oviposition and oviposition locations by elevation, divided into five 30 m levels. The *n* - numbers under each level refer to the number of samples in that level, with numbers for non-oviposition and oviposition respectively shown in brackets.

Slope

Although a chi-square test indicated a significant association between oviposition and slope, χ^2 (4, n = 191) = 12.6, $p = 0.014$ ($p = 0.009$, 2000-replicate Monte Carlo test), the pattern was mixed (Figure 4.6). The proportion of oviposition locations compared with non-oviposition locations increased over levels 2 - 4 (10 - 39º), but then fell to its lowest level at level 5 (40 - 49º). 52.9% of all *Viola* were in level 1 (0 - 29º), and 86.9% at levels 1 - 3 combined (0 - 29º).

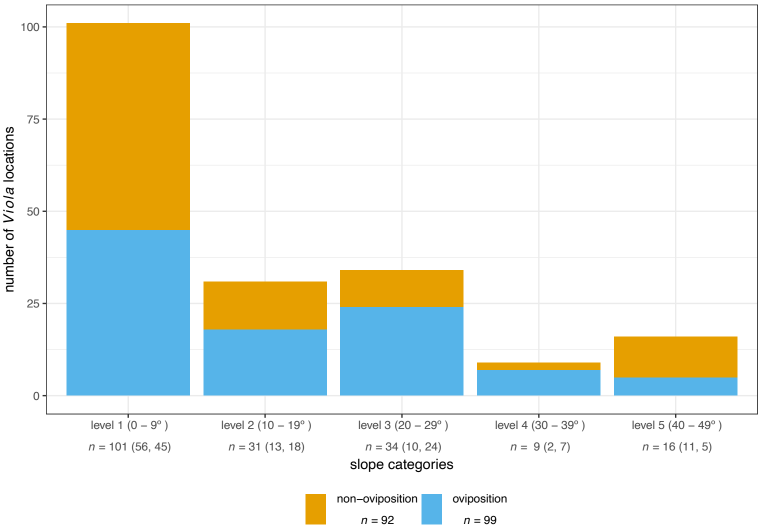

Figure 4.6 Non-oviposition and oviposition locations by slope, divided into five 10º levels. The *n* - numbers under each level refer to the number of samples in that level, with numbers for non-oviposition and oviposition respectively shown in brackets.

Aspect: wind shade

A chi-square test indicated a significant association between oviposition and wind direction, χ^2 (7, *n* = 191) = 27.1, *p* < 0.001. This *p*-value was confirmed by a 2000-replicate Monte Carlo test. 55.5% of oviposition locations were in the quadrant facing away from the prevailing wind, with only 7.1% in the windward quadrant (Figure 4.7).The pattern was less clear for non-oviposition locations at 45.6% and 31.5% respectively. The predominant aspect for *Viola* was facing octant 4 (north for Roy Cove, north-east for the other sites) This was shared by 33.5% of all *Viola* (32.6% of non-oviposition, 34.3% of oviposition). Octant 4 was one octant north of leeward; octant 6, one octant south of leeward, by contrast, showed no oviposition sites. There was, however, a strong influence of site on the distribution caused by Bleaker (Figure 4.7), where most locations faced octant 4.

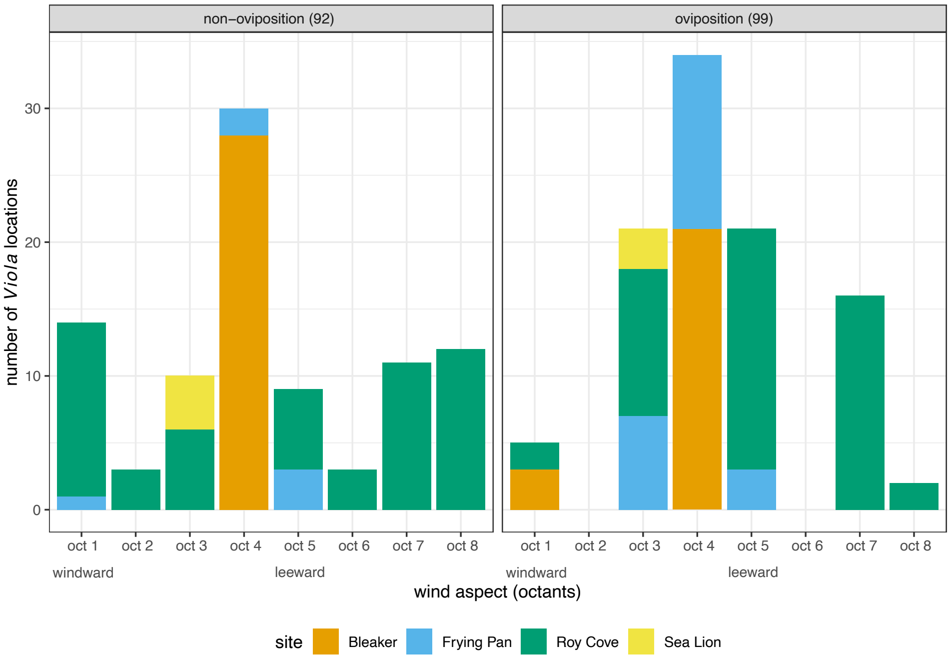

Figure 4.7 Distribution of *Viola* by orientation of location towards the prevailing wind, and, within that, by site.

Interaction between slope and wind shade

There was a significant association between oviposition and the interaction between slope and wind shade: oviposition was more likely on a gentle slope facing away from the prevailing wind (Figure 4.8), χ^2 (27, n = 191) = 47.6, p = 0.008. A 2000-replicate Monte Carlo test gave p = 0.001. The predominant combination of octant 4 and slope level 1 again reflects the distribution at Bleaker (Figure 4.7).

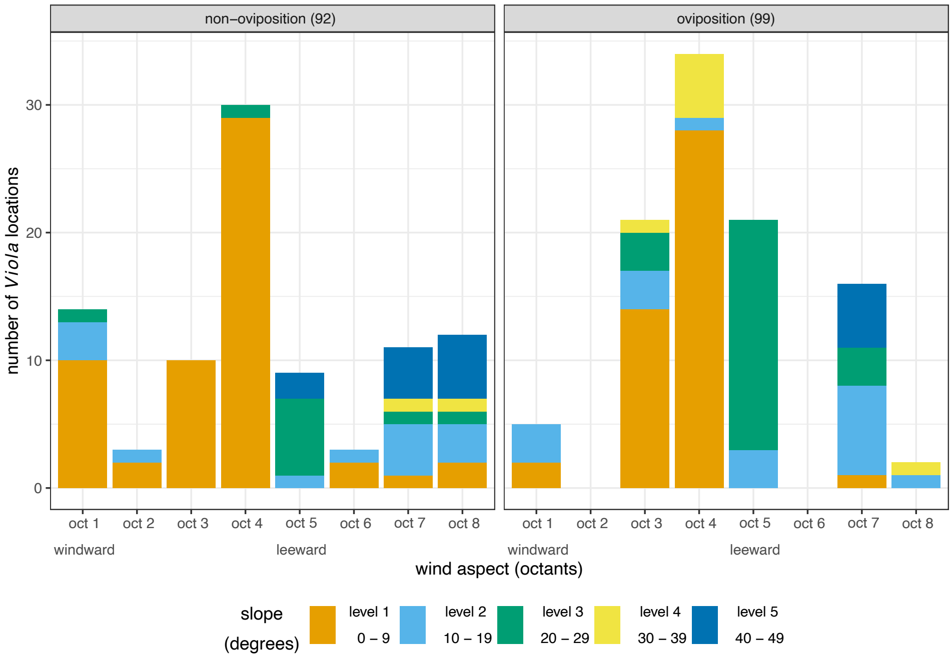

Figure 4.8 Distribution of *Viola* by orientation of location towards the prevailing wind, and, within that, by slope. The wind rose was divided into octants, clockwise from octant 1, which faces windward. This was south-west at Roy Cove, and west for Bleaker, Frying Pan and Sea Lion. Octant 5, leeward facing, was therefore the most sheltered by any slope. This was north-east for Roy Cove, and east for the others.

Aspect: direct radiation

A chi-square test indicated a significant association between oviposition and direct radiation: oviposition was more likely on a gentle slope facing the mid-day sun, χ^2 (4, $n = 191$) = 24.0, $p = 0.001$ ($p < 0.001$, 2000-replicate Monte Carlo test). The predominant aspect for *Viola* (46.1%) was north-east (Figure 4.9)This was particularly so for oviposition sites, 52.5%, compared with 39.1% for non-oviposition sites.

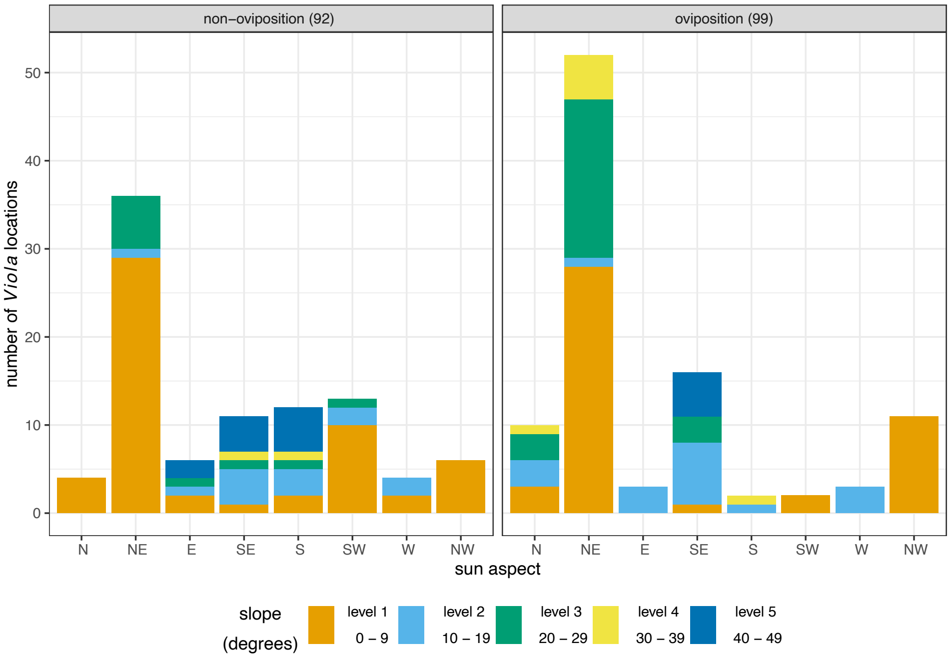

Figure 4.9 Distribution of *Viola* by orientation of location towards the midday sun and, within that, by slope.

Interaction between slope and direct radiation

There was a significant association between oviposition and the interaction between slope and direct radiation (Figure 4.9), χ^2 (26, *n* = 191) = 46.8, *p* = 0.008. A 2000-replicate Monte Carlo test gave *p* = 0.001.

(ii) **Wind shade and openness: hemispherical photography**

Gap light analysis showed that non-oviposition locations had a significantly lower percentage, analysed by pixel count, of wind shade (*M* = 19.39, *SD* = 10.75) than oviposition locations (*M* = 29.18, *SD* = 18.81), *df* = 83, *t* = -2.94, *p* = 0.004, 95% CI [-16.42, -3.16], with a medium effect size, *d* = 0.63. There was a difference in openness, albeit less significant, between non-oviposition locations (*M* = 80.46, *SD* = 6.56) and oviposition locations (*M* = 77.22, *SD* = 8.79), *df* = 83, *t* = 1.92, *p* = 0.058, 95% CI [-0.12, 6.58], *d* = 0.42.

Wind shade at site level

At site level, the pattern of wind shade was mixed (Figure 4.10), with all sites other than Sea Lion showing greater wind shade for oviposition sites than non-oviposition sites, although in no case was the difference significant (Appendix Table B.1).

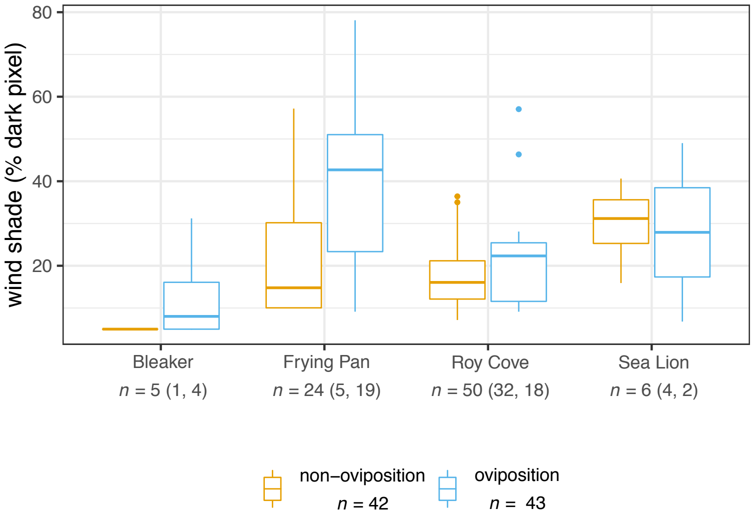

Figure 4.10 Wind shade, measured through gap light analysis, at the four study sites. For each location, represented by a circular image, set to show dark or light pixels, the four azimuth bins were selected which were centred on the direction from which the prevailing wind blew. This varied by site. The lower the percentage of dark pixels, the more the location was exposed to the prevailing wind.

Openness at site level

At site level, the pattern of openness was consistent (Figure 4.11) with all sites showing greater openness for non-oviposition locations than oviposition locations. In no case was the difference significant (Appendix Table B.2).

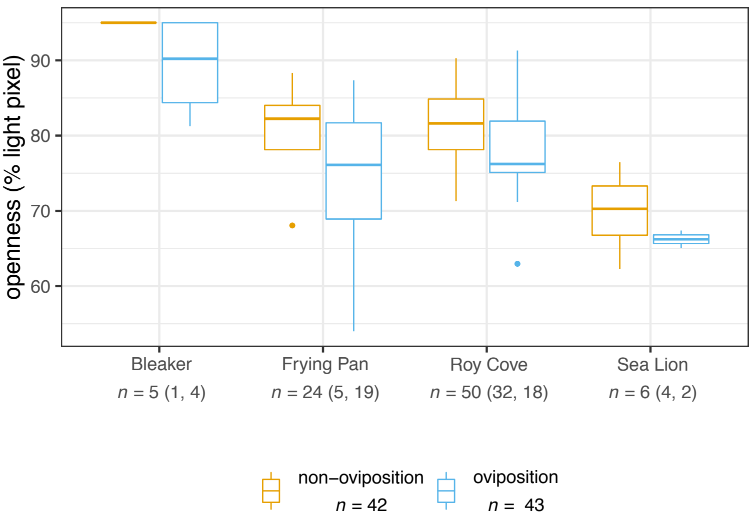

Figure 4.11 Openness, measured through gap light analysis, at the four study sites. For each location, represented by a circular image, set to show black or white pixels, the percentage of light pixels was recorded. The higher the percentage of light pixels, the more the location was open to light.

(iii) **Direct solar radiation: hemispherical photography**

Sun track analysis of hemispherical photographs showed no significant difference in mean moles per square metre per day ($mol \cdot m^{-2} \cdot d^{-1}$) of direct radiation between non-oviposition locations (*M* = 22.55, *SD* = 1.78) and oviposition locations (*M* = 22.16, *SD* = 1.6), *df* = 83, *t* = 1.06, *p* = 0.29, 95% CI [-0.34, 1.12], *d* = 0.23.

Direct radiation differed little between non-oviposition and oviposition locations (Figure 4.12), apart from in the small Sea Lion sample, and to a lesser extent Frying Pan, where vegetation obstruction occurred at either end of the sun track. In no case was the difference significant (Appendix Table B.3).

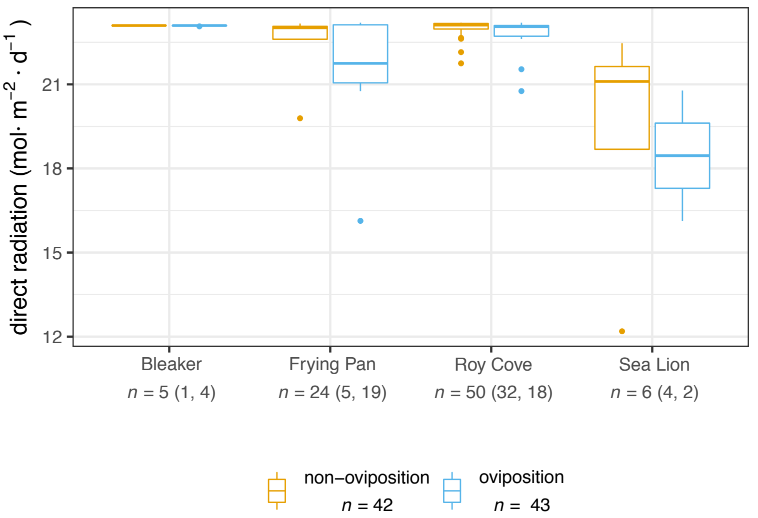

Figure 4.12 Direct solar radiation, based on a sun track analysis of hemispherical photography. The circular image produced for each location, set to show black or white pixels, had an overlay added to it representing the daily track of the sun over the November to February flying season of *Y.c.Cytheris.* The daily percentage of light pixels falling within the track was recorded, and the mean calculated, to represent the mean number of moles per square metre per day falling on the location.

4.3.2 Climate

(i) **Landscape, site and location wind speeds**

Wind speeds, transformed on the basis of a landscape mean of 8.11 ms^{-1}, showed that, across all sites, wind at non-oviposition locations was stronger than that at oviposition locations at all three measured levels above ground (Figure 4.13). A series of *t*-tests (Appendix Table B.4) showed that each contrast was significant at $p < 0.01$, although effect sizes were only medium.

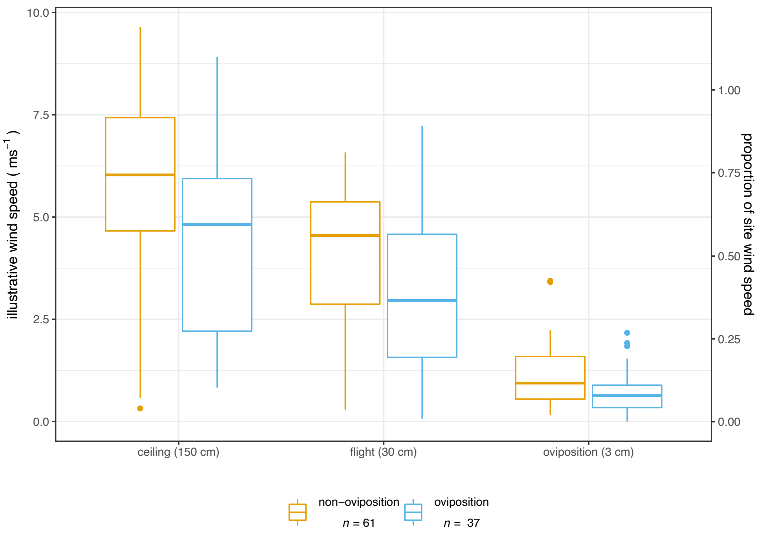

Figure 4.13 Illustrative wind speeds at oviposition and non-oviposition locations across all sites. Ceiling represented the observed highest level of butterfly flight; flight, the normal patrolling height; and oviposition, the height of egg laying. These were standardised at 150 cm, 30 cm and 3 cm respectively. Data were recorded as proportions of the mean wind speed at a site at the time when measurements were taken (right hand y axis), and, to aid visualisation, applied to a base of 8.11 ms^{-1} , the mean wind speed of the four study sites (left hand y axis). Proportions of site wind speeds > 1.00 are a product of wind funnel effect from nearby rocks at Bleaker and Roy Cove.

Data from the four study sites, transformed on the basis of a site measurement of 8.11 ms^{-1}, showed a wide range of median wind speeds, with Bleaker and Sea Lion having particularly high winds, reflecting their lack of wind shading from slopes (although Sea Lion's sample size was very small) (Figure 4.14). While at all sites the mean wind speeds at oviposition locations were lower than those at non-oviposition locations, the only significant variation was at Roy Cove at both ceiling (p = 0.044) and patrolling (p = 0.018) heights, although in each case only with medium effect size (d = 0.65 and 0.79 respectively) (Appendix Table B.5).

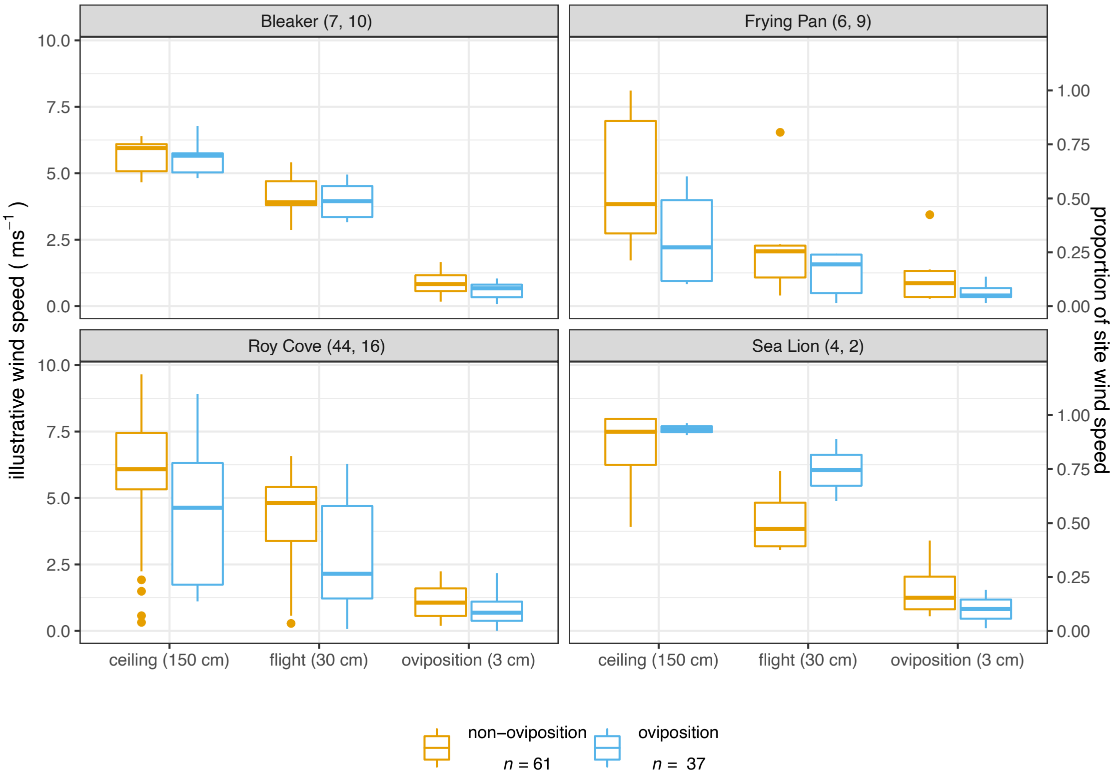

Figure 4.14 Illustrative wind speeds at oviposition and non-oviposition locations at the four study sites. Ceiling represented the observed highest level of butterfly flight; flight, the normal patrolling height; and oviposition, the height of egg laying. These were standardised at 150 cm, 30 cm and 3 cm respectively. Data were recorded as proportions of the mean wind speed at a site at the time when measurements were taken (right hand y axis), and, to aid visualisation, applied to a base of 8.11 ms^{-1} , the mean wind speed of the four study sites (left hand y axis). Proportions of site wind speeds > 1.00 are a product of wind funnel effect from nearby rocks at Bleaker and Roy Cove

(ii) **Temperatures at site and location level**

A comparison of data logger output, transformed to enable comparison between different sites at different times, indicated that non-oviposition locations were cooler than oviposition locations during daytime (1000 - 1600), although there was little difference at night (2200 - 0400). The combined 24 hr period reflected the difference between the daytime temperatures (Figure 4.15). *t*-tests showed that daytime and 24 hr contrasts were significant at $p < 0.01$, with large effect sizes (Appendix Table B.6).

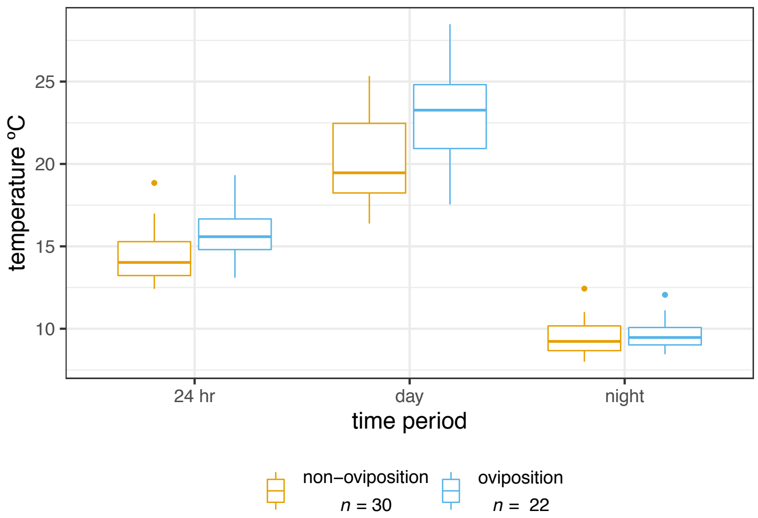

Figure 4.15 Illustrative temperatures (ºC) at potential and actual oviposition locations at three time scales: 24 hours; day (1000 - 1600); and night (2200 - 0400). Temperatures from data loggers were recorded as proportions of the mean temperature of each site. Those proportions were transformed by mean combined temperatures for 24hr (14.93), day (21.35) and night (9.55) to enable comparisons to be made between different sites at different times.

Daytime temperatures, the main indicator, only showed significant temperature differences at Roy Cove ($p = 0.005$, $d = 1.18$) (Figure 4.16). While Bleaker showed temperature differences with a large effect size, sample size was too small to demonstrate significant variation ($p = 0.182$, $d = 1.22$) (Appendix Table B.7).

Figure 4.16 Illustrative day time temperatures at potential and actual oviposition locations at the four study sites. Temperatures (ºC) were recorded at the butterflies peak flying time, 1000-1600. Temperatures from data loggers were recorded as proportions of the mean temperature of each site. Those proportions were transformed by the mean day time temperature of all sites, 21.35º, to enable comparisons between different sites at different times.

4.3.3 Ground cover and vegetation

(i) **Viola distribution**

Falklands-wide and site level

Analysis of 810 quadrats, covering all study sites, placed randomly over 27 patches where *Viola* had been seen, showed a mean *Viola* % cover of 1.94, *SD* = 5.8. The *SD* is explained in part by the unusually high concentration of *Viola* on Bleaker, *M* = 9.5, *SD* = 11.74. Concentration at sites other than Bleaker was *M* = 0.7, *SD* = 2.31.

A series of *t*-tests showed a greater concentration of individual *Viola* plants in "edge" patches, strips 2 m wide between vegetation stands such as dwarf shrub heath or tussac and grass (*M* = 3.2*, SD* = 6.0) than in patches within a feature, such as pasture or dwarf shrub heath (*M* = 1.65, *SD* = 5.72), *t*(808) = 2.96, *p* = 0.003, 95% CI [0.52, 2.57], although with a small effect size, *d* = 0.27. This variation was highly significant at Roy Cove, although with only a small effect size (*p* < 001, *d* = 0.48) (Table 4.3)

Table 4.3 Distribution of individual *Viola* plants at the study sites. Patches within the main matrix were compared with "edge" patches between stands of vegetation, principally dwarf shrub heath, and grass.

site	topog	*n*	*M*	*SD*	*df*	*t*	*p*-value	95% CI	*d*
Bleaker	edge	1	9.90	8.63	118	0.46	0.649	-3.78, 6.05	0.09
	matrix	3	8.97	12.63					
Frying Pan	edge	0	NA	NA	NA	NA	NA	NA	NA
	matrix	4	0.05	0.22					
Roy Cove	edge	3	2.03	4.01	418	4.04	**<0.001**	0.64, 1.84	0.48
	matrix	11	0.79	2.03					
Sea Lion	edge	1	0.00	0.00	148	-0.69	0.491	-1.13, 0.54	0.14
	matrix	4		8.73					

Significance at *p* < 0.05 is shown in bold; topog = topography of site.

Overall, *Viola* were unevenly distributed. This was observable both at patch level (Figure 4.17) and at quadrat level, where there were no *Viola* in 68.5% of the quadrats analysed.

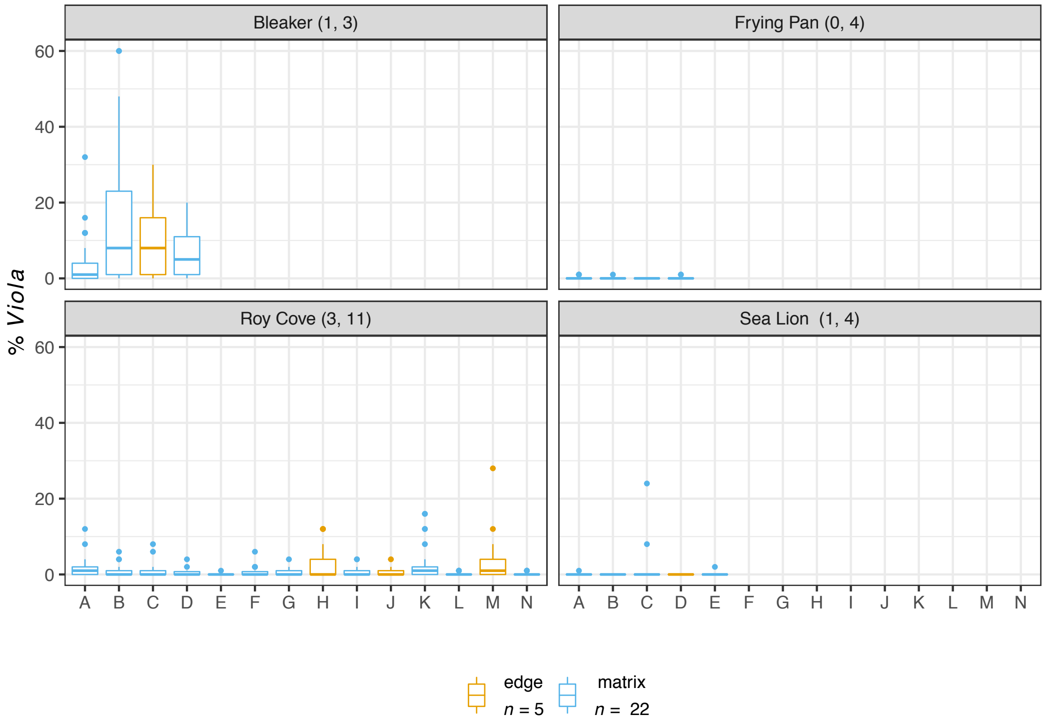

Figure 4.17 Distribution of *Viola spp.* at the study sites. Each bar, designated by a capital letter, represents a patch. 27 patches in total were investigated, each of which had shown *Viola.* 30 x 50cm quadrats were randomly placed in each patch and the percentage of *Viola* in each quadrat was noted. Key: edge = 2m wide patches immediately adjacent to a feature, such as tussac, rock runs or dwarf shrub heath; matrix = patches within homogeneous matrices, such as dwarf shrub heath or grazed meadow. The patch on Bleaker with the highest percentage was, uniquely, in an area comprising large clumps of *Viola.*

(ii) **Ground cover**

Analysis across all sites

Quadrat-level analysis across all four study sites showed that there was a significant contrast between non-oviposition and oviposition locations for four out of six types of ground cover (Figure 4.18). Non-oviposition sites had higher percentage coverage of dwarf shrub heath ($p < 0.001$, $d = 0.57$), and of vegetation (other than *Viola* and grass) ($p = 0.007$, $d = 0.39$); oviposition locations had more bare ground ($p < 0.001$, $d = 0.65$), and litter ($p < 0.001$, $d = 0.68$) (Appendix Table B.8).

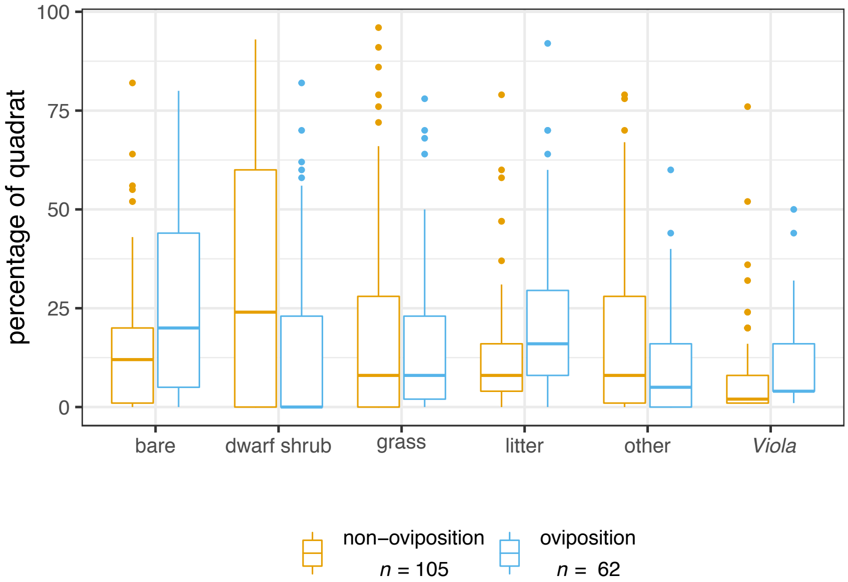

Figure 4.18 Ground cover structures of *Viola* locations across all four study sites. Non-oviposition and oviposition locations were compared by analysing the percentages of six categories of ground cover, each quadrat summing to 100%.

Analysis of similarities (ANOSIM) across all sites.

Analysis of similarities (ANOSIM) between vegetation and other ground cover in, respectively, non-oviposition and oviposition locations, using the binomial index (where the null hypothesis is that the two categories of location are equal) showed no significant differences at either the 50cm (p = 0.294) or 10cm (p = 0.246) quadrat level, following 9999 permutations. R-values were close to 0 at, respectively, 0.01 and 0.015, with R on a scale of 0 to 1, where a value of 1 indicates complete dissimilarity between the groups (Clarke 1993).

Despite the lack of significant differences, post-hoc testing was undertaken, following Wilcox (1987) and Howell (2012). A Bray-Curtis dissimilarity test (SIMPER) was applied to the quadrat data to test for significant contributions to whatever differences there were in ground cover (Table 4.4).

Table 4.4 Similarity in ground cover between non-oviposition and oviposition locations at (a) 50cm and (b) 10cm quadrat level. The contribution of each type of ground cover to the average between-group Bray-Curtis dissimilarity (SIMPER) was calculated through pairwise comparisons between quadrats, measuring the percentage cover in each case.

a

factor	average	*SD*	*ratio*	non-ov	ov	cumul %	*p*-value
dwarf shrub heath	0.164	0.144	1.14	31.54	17.15	26.6	0.539
grass	0.126	0.118	1.07	22.21	19.50	47.1	0.954
bare ground	0.121	0.103	1.17	15.95	26.06	66.7	**0.022**
litter	0.078	0.073	1.06	11.25	16.24	79.4	0.115
other vegetation	0.074	0.894	0.89	11.37	10.59	91.5	0.720
Viola spp.	0.052	0.815	0.81	7.68	10.47	100.0	0.250

b

factor	average	*sd*	*ratio*	non-ov	ov	cumul %	*p*-value
dwarf shrub heath	0.131	0.138	0.95	22.96	12.21	22.4	0.923
litter	0.116	0.100	1.16	18.29	19.29	42.2	0.630
Viola spp.	0.114	0.108	1.06	29.48	40.59	61.7	**0.027**
grass	0.085	0.102	0.84	14.16	7.94	76.2	0.968
bare ground	0.085	0.093	0.91	8.23	14.12	90.7	0.061
other vegetation	0.055	0.055	0.59	6.87	5.83	100.0	0.620

Quadrat coverage: non-oviposition locations, *n* = 56; oviposition locations, *n* = 34. Pairwise comparisons based on 9999 permutations. Significance at $p < 0.05$ is shown in bold. key: factor = type of ground cover, ordered by size of contribution, largest to smallest; average = factor contribution to average between-group dissimilarity; *SD* = standard deviation of that contribution; ratio = average to *SD* ratio; non-ov, ov = average abundances (%) of each factor in non-oviposition and oviposition locations respectively; cumul % = cumulative contribution of each factor, based on average. *p*-value is for comparison of averages of non-oviposition and non-oviposition locations for each type of ground cover.

At 50 cm quadrat level, the only significant factor ($p = 0.022$), contributing 19.6% to the dissimilarity, was the greater amount of bare ground at oviposition locations compared with non-oviposition locations. The next most significant factor, although at $p = 0.115$ (12.7%) was litter, again with the greater amount at oviposition locations

At 10 cm quadrat level, there was significantly greater coverage of *Viola* at oviposition locations ($p = 0.027$), contributing 19.5%, and, again, a greater percentage of bare ground, although at $p = 0.061$ and 14.5%. Litter did not appear to be an important factor overall ($p = 0.63$), although it provided 19.8% of the structural difference between locations.

The overall picture, with a number of factors showing little significant variation between non-oviposition and oviposition locations, while contributing high percentages in the Bray-Curtis dissimilarity tests, showed a lack of homogeneity in location structure, with bare ground the one potentially significant factor.

Lack of homogeneity at site level

Both 50cm and 10 cm quadrats showed lack of homogeneity of structure between the sites (Figure 4.19). There was a clear difference between sites where 50 cm quadrats usually included dwarf shrub heath, Frying Pan and Roy Cove, and those which did not, Bleaker and Sea Lion. Bleaker's main vegetation was "other", principally wild celery *Apium australe* (Table 4.6), while Sea Lion's was grass. Grass on Sea Lion was discussed at 2.3.4. While in some cases it provided shelter, it also appeared to choke out *Viola* growth, as a comparison between the already limited *Viola* distribution of 2015-2017 and the much less dense distribution of 2017-2018, when there was strong grass growth, showed (Figure 1.16). Similar distributions of ground cover were seen in 10 cm quadrats, although *Viola* provided a greater percentage cover, unsurprisingly as a 10cm quadrat centred on a *Viola* plant was likely to be at least 25% *Viola.*

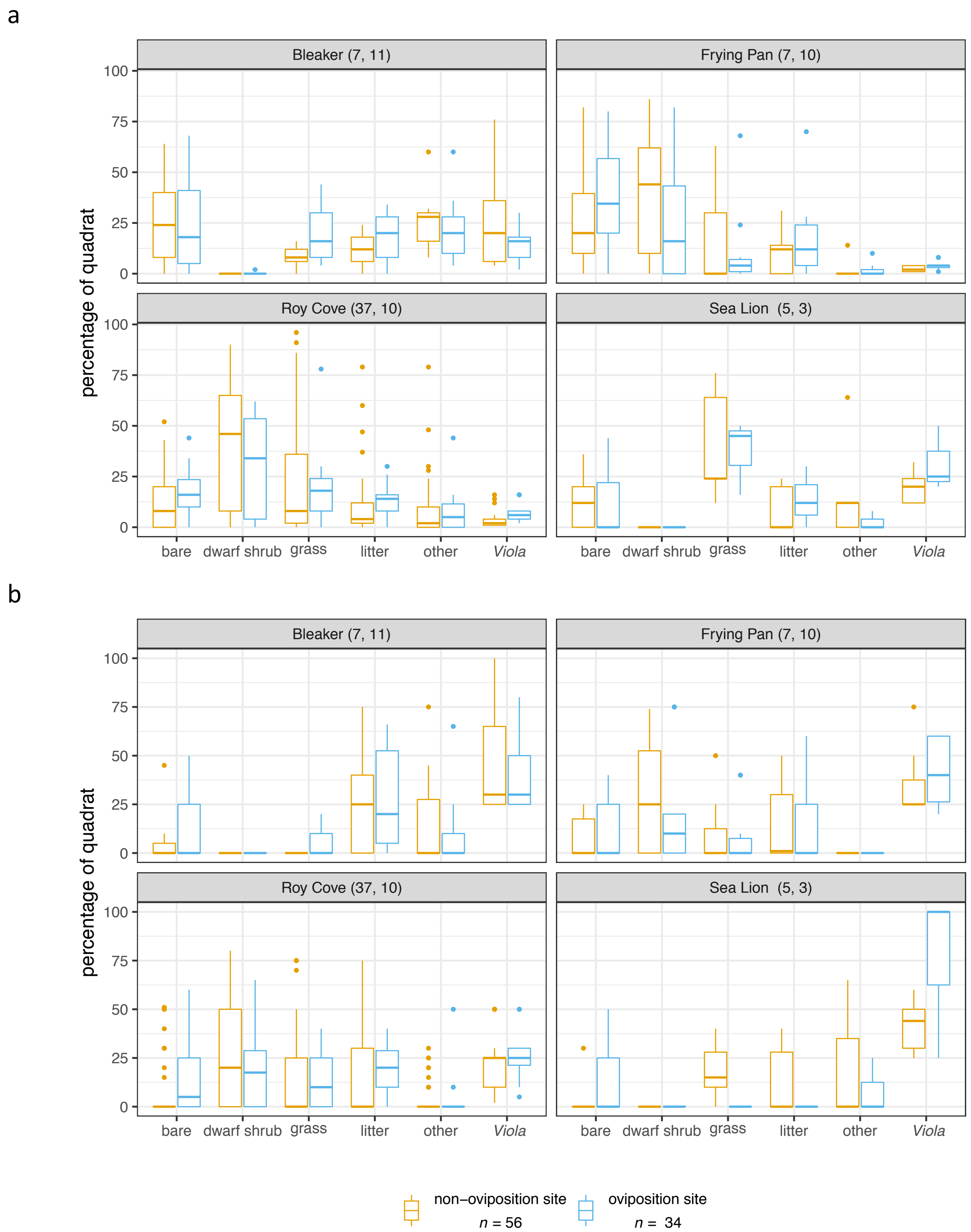

Figure 4.19 Differing vegetation structures of *Viola* locations in the four study sites in (a) 50cm and (b) 10 cm quadrats. Site names are followed, in brackets, by *n* for, respectively, non-oviposition and oviposition locations. The *Viola* under analysis was centred. Percentages for each quadrat sum to 100.

Comparison between ground cover profiles at Bleaker and Roy Cove

Quadrat data at Bleaker and Roy Cove were analysed further, given their very different ground cover and vegetation profiles at 50cm (Figure 4.19), to investigate if there were common themes underlying differences between non-oviposition and oviposition sites.

ANOSIMs of Bleaker and Roy Cove quadrats respectively showed significant contrasts between non-oviposition and oviposition locations: Bleaker at $p = 0.015$, $R = 0.15$; Roy Cove at $p = 0.014$, $R = 0.05$, each following 9999 permutations. Bray-Curtis dissimilarity tests (SIMPER) were used to investigate factors contributing to those differences (Table 4.5).

At Bleaker (Table 4.5 a), "other vegetation" (more at non-oviposition than oviposition, $p = 0.004$), contributed 29.7% to the dissimilarity while litter (more at oviposition than non-oviposition, $p = 0.001$), contributed 15.3%. At Roy Cove (Table 4.5 b), litter (more at oviposition than non-oviposition, $p < 0.001$), contributed 23.7% to the dissimilarity while *Viola maculata* (more at oviposition than non-oviposition, $p = 0.002$), contributed 4.4%.

Table 4.5 Comparison in ground cover between non-oviposition and oviposition locations at (a) Bleaker and (b) Roy Cove at 50cm quadrat level. The contribution of each type of ground cover to the average between-group Bray-Curtis dissimilarity (SIMPER) was calculated through pairwise comparisons between quadrats, measuring the percentage cover in each case.

a

factor	average	*SD*	*ratio*	non-ov	ov	cumul %	*p*-value
other vegetation	0.126	0.085	1.49	42.92	22.75	29.7	**0.004**
bare ground	0.108	0.084	1.28	20.92	27.87	55.0	0.116
litter	0.065	0.051	1.27	9.23	17.25	70.3	**0.001**
Viola maculata	0.065	0.053	1.23	12.42	18.25	85.5	0.215
grass	0.061	0.054	1.14	14.50	13.75	99.8	0.611
dwarf shrub heath	<0.001	0.002	0.26	0.00	0.12	100.0	0.377

b

factor	average	*SD*	*ratio*	non-ov	ov	cumul %	*p*-value
dwarf shrub heath	0.155	0.111	1.39	41.33	35.74	28.4	0.648
litter	0.129	0.119	1.09	13.02	29.67	52.1	**<0.001**
grass	0.116	0.123	0.95	21.73	11.81	73.5	0.644
other vegetation	0.064	0.076	0.85	11.07	6.88	85.3	0.916
bare ground	0.056	0.054	1.03	9.24	9.77	95.6	0.469
Viola maculata	0.024	0.024	0.98	3.62	6.12	100.0	**0.002**

Quadrat coverage: Bleaker: non-oviposition locations, $n = 26$; oviposition locations, $n = 16$; Roy Cove: non-oviposition locations, $n = 55$; oviposition locations, $n = 43$. Pairwise comparisons based on 9999 permutations. Significance at $p < 0.05$ is shown in bold. key: factor = type of ground cover, ordered by size of contribution, largest to smallest; average = factor contribution to average between-group dissimilarity; *SD* = standard deviation of that contribution; ratio = average to *sd* ratio; non-ov, ov = average abundances (%) of each factor in non-oviposition and oviposition locations respectively; cumul % = cumulative contribution of each factor, based on average. *p*-value is for comparison of averages of non-oviposition and non-oviposition locations for each type of ground cover. Percentages for each quadrat sum to 100.

The "other vegetation" category at Bleaker was then expanded to species level in a SIMPER (Table 4.6). This was inconclusive, with only sheep's sorrel, *Rumex acetosella* significant, at *p* = 0.023, but with a contribution of only 0.1%.

Table 4.6 Ground cover, with vegetation at the species level, at Bleaker at 50cm quadrat level. Bray-Curtis dissimilarity (SIMPER) was calculated through pairwise comparisons between quadrats, measuring the percentage cover for each factor.

factor	average	*SD*	*ratio*	non-ov	ov	cumul %	*p*-value
bare ground	0.107	0.084	1.28	20.92	27.87	21.3	0.118
Apium australe	0.089	0.069	1.29	21.23	18.37	38.8	0.750
litter	0.065	0.051	1.28	9.23	17.25	51.7	**0.002**
Viola maculata	0.065	0.053	1.23	12.42	18.25	64.5	0.209
grass	0.061	0.054	1.14	14.50	13.75	76.6	0.613
moss	0.054	0.071	0.76	10.81	0.75	87.3	0.612
Acaena magellanica	0.030	0.053	0.57	5.77	0.75	93.3	0.956
Bellis perennis	0.024	0.029	0.82	4.77	1.00	98.0	0.712
Rumex acetosella	0.009	0.027	0.35	0.00	1.87	99.9	**0.023**
Empetrum rubrum	<0.001	0.002	0.26	0.00	0.12	100.0	0.152

Quadrat coverage: Bleaker: non-oviposition locations, *n* = 26; oviposition locations, *n* = 16; Roy Cove: non-oviposition locations, *n* = 55; oviposition locations, *n* = 43. Pairwise comparisons based on 9999 permutations. Significance at *p* < 0.05 is shown in bold. key: factor = type of ground cover, ordered by size of contribution, largest to smallest; average = factor contribution to average between-group dissimilarity; *SD*= standard deviation of that contribution; ratio = average to *SD* ratio; non-ov, ov = average abundances (%) of each factor in non-oviposition and oviposition locations respectively; cumul % = cumulative contribution of each factor, based on average. *p*-value is for comparison of averages of non-oviposition and non-oviposition locations for each type of ground cover.

(iii) **Vegetation height**

An exploratory plotting of quadrat vegetation height (50 cm quadrats) showed considerable variation between sites (Figure 4.20) although a *t*-test showed no significant difference between non-oviposition (*M* = 11.47, *SD* = 7.7), and oviposition (*M* = 12.95, *SD* = 9.47) locations *t*(92.15) = -1.07, *p* = 0.286, 95% CI [-4.21, 1.26], *d* = 0.16. The differences between sites are explicable by the abundance of wild celery, *Apium australe*, at Bleaker and of tussac, *Poa flabellata*, together with rank grass, at Sea Lion. All of these provide shelter at sites which otherwise do not have much shelter from the prevailing wind.

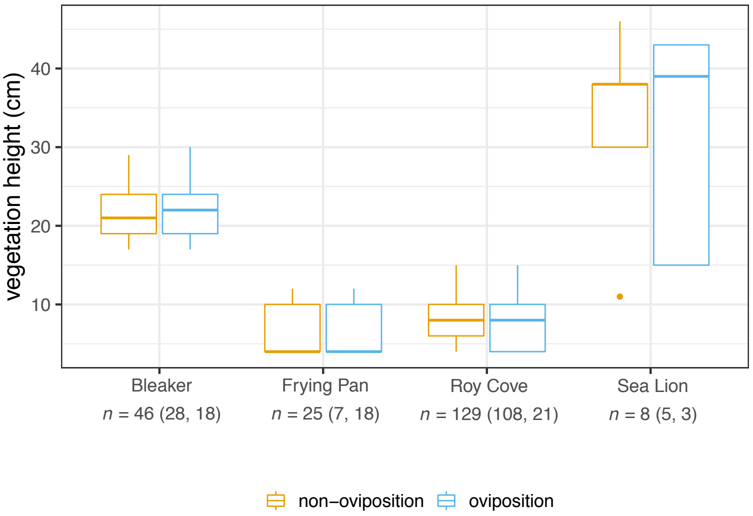

Figure 4.20 Vegetation height at the four study sites. Site names are followed, in brackets, by overall numbers of locations and by numbers for, respectively, non-oviposition and oviposition locations.

(iv) **Bare patches**

Bare patches within 30cm and 60cm of quadrat edges

There were significantly more bare patches (over 10cm diameter) at distances of 60cm and 30cm respectively from the edge of an oviposition quadrat than from the edge of a non-oviposition quadrat, although, in each case, only with a medium effect size.

Table 4.7 Numbers of bare patches >10 cm diameter within 60 cm and 30 cm of 50 cm quadrat edges. Non-oviposition and oviposition locations were compared by *t*-tests

distance	oviposition	*n*	*Mdn*	*M*	*SD*	*df*	*t*	*p*-value	95% CI	*d*
60cm	no	48	2	2.50	1.71	76	-3.25	**0.002**	-4.04, -0.49	0.76
	yes	30	4	3.77	1.61					
30cm	no	48	1	1.08	0.94	76	-3.15	**0.002**	-1.17, -0.26	0.73
	yes	30	2	1.80	1.03					

Significance at $p < 0.05$ is shown in bold. *Mdn* = median number of bare patches.

Basking locations

Paired *t*-tests showed the mean temperature (ºC) of rocks or large bare patches of ground (*M* = 26.74, *SD* = 4.67) at Bleaker and the Frying Pan was, on average, significantly higher than the ambient air temperature at the same location (*M* = 19.45, *SD* = 4.18), $t(119) = 17.08$, $p < 0.001$, 95% CI [6.44, 8.13], with a very large effect size, $d = 1.56$.

4.3.4 Plant and leaf level characteristics

(i) **Plant size**

Y. cytheris mainly selected plants of size 2 (medium sized, erect, and with three to five leaves of up to 35 mm) and size 3 (larger, more erect, more than five leaves of up to 40 mm) rather than the very small or very large (Table 4.8). A χ^2 test showed the difference to be highly significant, χ^2 (3, 1093) = 11.6, $p = 0.009$. Given the low sample size for oviposition on size 1 and size 4 plants, the *p*-value was re-evaluated with a 2000 replicate Monte Carlo test (Hope 1968), which still showed a significant difference, in this case at $p = 0.01$.

Table 4.8 Total number of individual *Viola* plants in a sample of 187 quadrats. The quadrats comprised both non-oviposition ($n = 62$) and oviposition locations ($n = 125$). *Viola* recorded as having oviposition status held eggs, not larvae.

Viola status	size 1	size 2	size 3	size 4
non-oviposition	221	590	265	17
oviposition	4	50	20	0

Key to *Viola* size: 1 - small, compact, one or two leaves of up to 20 mm; 2 - erect, three to five leaves of up to 35 mm; 3- larger, more erect, more than five leaves of up to 40 mm; 4 - large, vigorous, sprawling, leaves can be larger than 40 mm.

(ii) **Chlorophyll**

Plant level

Concerns in comparing chlorophyll levels of *Viola* spp. with and without oviposition were (i) that two species might not share the same mean levels, Sea Lion having *V. magellanica*, the other sites *V. maculata*, and (ii) chlorophyll levels even in the same species might, in any case, vary by site for autecological reasons not explored in this thesis. These concerns were explored through a one-way between-groups ANOVA in which sites differed highly significantly, with a very large effect size ($F = 14.03$, $p < 0.001$, $\eta_p^2 = 0.3$). Sea Lion plants, *V. magellanica*, had the highest chlorophyll levels ($mg \cdot m^{-2}$), *M* = 513.4, *SD* = 74.86; followed by *V. maculata* at Roy Cove, *M* = 450.1, *SD* = 77.51; Bleaker, *M* = 376.8, *SD* = 63.24; and Frying Pan, *M* = 372.8, *SD* = 67.24.

A further test on the same data set compared the chlorophyll levels of plants at non-oviposition locations, those with eggs and those with larvae. This was to determine whether, as for other analyses, plants with larvae and eggs could be aggregated as oviposition locations, despite the damage larvae did to their host plants (Figure 4.21).

A one-way between-groups ANOVA did not show a significant difference between categories ($F = 2.58$, $p = 0.08$, $\eta_p^2 = 0.04$). There was, nevertheless, a wide variation between sites, with plants with eggs having the highest chlorophyll levels (mg·m-2), $M = 459.74$, SD = 67.36, followed by those without oviposition, $M = 427.11$, $SD = 90$; and finally those with larvae $M = 398.98$, $SD = 95.39$.

Figure 4.21 Larval feeding pattern. The larva eats the leaf tissue from each side, giving the leaf a diagnostic filleted appearance. This has an impact on chlorophyll levels; in extreme cases it can lead to the destruction of the host plant. Photograph by the author.

The outcome of the tests suggested that aggregation was unsafe in both cases, even accepting the $p = 0.08$ for the eggs, larvae and non-oviposition comparison, producing a wide spread of averages when the two were combined (Figure 4.22). Oviposition locations were therefore represented only by plants with eggs.

Analysis of the largest site data set ($n = 53$), Roy Cove, suggested that a chlorophyll analysis merited further exploration, even if the null hypothesis could not be rejected overall. Chlorophyll level at Roy Cove non-oviposition locations ($n = 40$, $M = 435.3$, $SD = 77.2$) was significantly lower than at locations with eggs ($n = 13$, $M = 495.5$, $SD = 60.6$), $t(51) = -2.6$, $p = 0.013$, 95% CI [-107.42, -12.98], with a large effect size, $d = 0.82$. Differences at Bleaker and Frying Pan were not significant, $t(19) = -1.63$, $p = 0.12$ and $t(11) = -1.51$, $p = 0.159$.

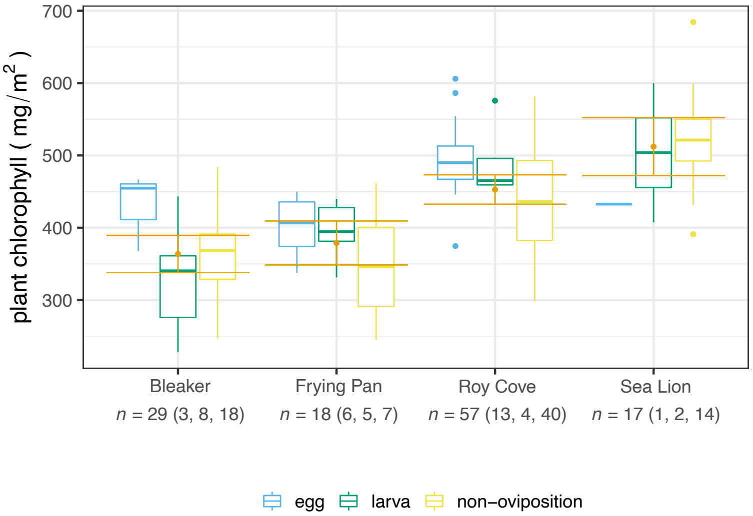

Figure 4.22 Levels of chlorophyll in plants showing *Y. cytheris* eggs, larva and non-oviposition at the four study sites. 95% confidence intervals of the mean for each site are shown in orange. Numbers in brackets refer to plants with eggs, larvae and no oviposition respectively. Plants on Sea Lion were *Viola magellanica*, those on other sites *Viola maculata.*

Leaf level

A paired *t*-test provided no significant evidence for *Y. cytheris's* selecting individual leaves for oviposition on the basis of chlorophyll content ($mg \cdot m^{-2}$): leaves selected for oviposition ($M = 448.87$, $SD = 90.52$); mean of random leaves from the same plant ($M = 459.74$, $SD = 67.36$), $t(22) = -1$, $p = 0.327$, 95% CI [-33.38, 11.64], $d = 0.14$.

Chlorophyll and plant size

A one-way ANOVA showed that chlorophyll level did not vary significantly with plant size ($F\ (7, 38) = 0.45$, $p = 0.638$, $\eta_p^2 = 0.009$. (Figure 4.23).

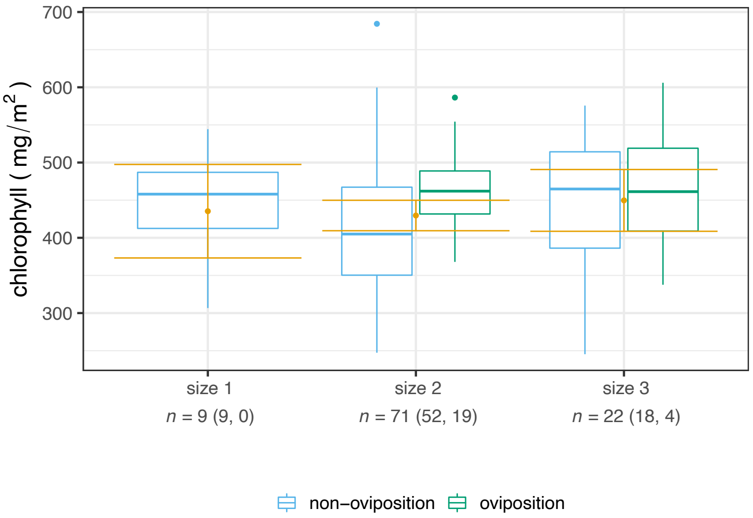

Figure 4.23 Levels of chlorophyll by size of *Viola.* Sizes are: 1 - small, compact, one or two leaves of up to 20 mm; 2 - erect, three to five leaves of up to 35 mm; 3 - larger, more erect, more than five leaves of up to 40 mm. Sizes are followed by overall numbers and, in brackets, by numbers for, respectively, non-oviposition and oviposition locations.

(iii) **Plant temperature**

A *t*-test showed that the temperature (ºC) of plants where oviposition had occurred (*M* = 15.79*, SD* = 4.53) did not differ significantly from other plants (*M* = 14.54, *SD* = 4.13), *t*(109) = 1.49, *p* = 0.139, 95% CI [-0.41, 2.91], *d* = 0.29

A further *t*-test, recognising that the plant temperature test data had been taken in varying air temperatures, showed that the ratio of plant temperature to air temperature for oviposition plants (*M* = 0.78*, SD* = 0.21) did not differ significantly from that for non-oviposition plants (*M* = 0.77, *SD* = 0.15), *t*(108) = 0.32, *p* = 0.752, 95% CI [-5.80, 8.00], *d* = 0.06

(iv) **Characteristics of oviposition at leaf level**

Leaf temperature

A paired *t*-test showed the temperature (ºC) of a leaf where oviposition had occurred (*M* = 16.17*, SD* = 4.6) was, on average, significantly higher than other leaves on the same plant (*M* = 15.62, *SD* = 4.54), *t*(38) = 2.71, *p* = 0.01, 95% CI [0.14, 0.95], but with a small effect size, *d* = 0.43.

Position on the leaf

The majority of eggs were laid on the abaxial side of the leaf, predominantly near the base or in the middle (Figure 4.24, Figure 4.25)

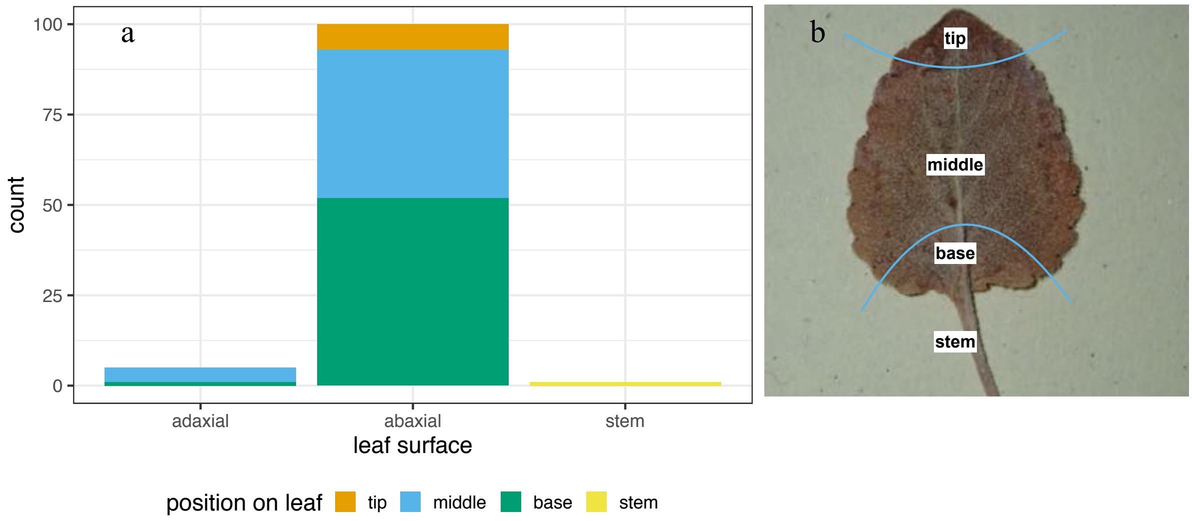

Figure 4.24 Position of *Y. cytheris* eggs on the leaf. Bar chart (a) shows distribution of samples (*n* = 104) by surface and position. Positions were attributed by eye. Base and tip form small, but distinct, sections of the leaf (b). Photograph by the author.

Figure 4.25 The commonest oviposition location - a single egg on the abaxial leaf surface near the stem. The leaf was turned over to enable the egg to be photographed. Photograph by the author.

Occupation of individual leaves and plants

The majority of eggs were laid singly on leaves, with most plants only showing oviposition on a single leaf. Larvae were similarly solitary (Table 4.9).

Table 4.9 Numbers of eggs and larvae recorded per leaf and per plant across all sites. This included data from exploratory visits to Darwin and Hill Cove. Data included one FP plant occupied by two eggs and one larva, each on a separate leaf, and one RC plant occupied by one egg and one larva on the same leaf. Excluded is a stone at Frying Pan under which were seven larvae.

		number per leaf					number per plant							
	site	1	2	3	4	5	1	2	3	4	5	6	7	8
eggs	BL	41	6	1	0	1	21	3	2	2	1	0	1	1
	DA	4	0	0	0	0	4	0	0	0	0	0	0	0
	FP	24	4	1	0	0	15	3	2	2	0	0	0	0
	HC	1	0	0	0	0	1	0	0	0	0	0	0	0
	RC	37	0	1	0	1	35	1	1	0	1	0	0	0
	SL	1	0	0	0	0	1	1	0	0	0	0	0	0
	Total	108	10	3	0	2	77	7	5	4	2	0	1	1
larvae	BL	16	1	0	0	0	9	1	0	0	0	0	0	0
	DA	0	0	0	0	0	0	0	0	0	0	0	0	0
	FP	11	1	0	0	0	11	3	1	0	0	0	0	0
	HC	1	0	0	0	0	1	0	0	0	0	0	0	0
	RC	17	0	0	0	0	17	0	0	0	0	0	0	0
	SL	2	0	0	0	0	2	0	0	0	0	0	0	0
	Total	47	2	0	0	0	40	4	1	0	0	0	0	0

Key: BL = Bleaker; DA = Darwin; FP = Frying Pan; HC = Hill Cove; RC = Roy Cove; SL = Sea Lion

4.3.5 Multivariate analysis: Roy Cove

Multivariate analysis on Roy Cove data was carried out using the key predictors identified in 4.3.1 - 4.3.4 (Table 4.10). All factors with $p < 0.05$ were included in modelling, as were the two with $p < 0.1$, openness ($p = 0.058$) and bare ground ($p = 0.061$). Although variance inflation factor testing, using the R package *car* (Fox and Weisberg 2018), showed no evidence of collinearity (all scores were below 2.5), wind shade measurements and wind speed were not used in the same model as they might expect to be related: here tests in a wider data set ($n = 68$) showed a medium correlation between the two (Pearson's r = 0.4, $p < 0.001$).

Table 4.10 Factors considered for inclusion in multivariate analysis. These are based on *p*-values and effect sizes of comparisons for each factor between non-oviposition and oviposition locations.

paragraph	category	factor	*p* <0.05	p <0.1	*d* >0.8
4.3.1(i)	topography: direct measurements	elevation	<0.001	-	note 1
		slope	<0.001	-	note 1
		wind shade (direct)	0.015	-	note 1
		direct radiation (direct)	<0.001	-	note 1
		slope/wind shade interaction	0.003	-	note 1
4.3.1(ii)	topography: GLA	wind shade (GLA)	0.004	-	n
		openness (GLA)	n	0.058	n
4.3.1(iii)		direct radiation (GLA)	n	n	n
4.3.2(i)	location wind speed	wind at 150cm	0.004	-	n
		wind at 30 cm	0.003	-	n
		wind at 3 cm	0.005	-	n
4.3.2(ii)	location temperature	24hr	0.002	-	0.93
		day	0.003	-	0.89
		night	n	n	n
4.3.3(ii)	ground cover : SIMPER 50cm	Viola spp.	n	n	note 2
		bare ground	0.022	-	note 2
		dwarf shrub heath	n	n	note 2
		grass	n	n	note 2
		litter	n	n	note 2
		other vegetation	n	n	note 2
	ground cover : SIMPER 10cm	Viola spp.	0.027	-	note 2
		bare ground	n	0.061	note 2
		dwarf shrub heath	n	n	note 2
		grass	n	n	note 2
		litter	n	n	note 2
		other vegetation	n	n	note 2
4.3.3(iii)	vegetation height	height	n	n	n
4.3.3(iv)	bare patch numbers	60cm from quadrat edge	0.002	-	n
		30cm from quadrat edge	0.002	-	n
4.3.4(i)	*Viola* spp. of a given size	50cm quadrat	0.011^{a}	-	n
		10cm quadrat	n	n	n
4.3.4(ii)	chlorophyll	plant level	0.013^{b}	n	0.82
		leaf level	n	n	n
4.3.4(iii)	plant temperature	direct comparison	n	n	n
		plant/air ratio	n	n	n
4.3.4(iv)	leaf temperature	plant level	0.010	-	n

Key: n = required level of significance or effect size not met; note1: *p*-values are for χ^2 tests, with no effect size measured; note 2 : *p*-values are for comparisons contributing to Bray-Curtis dissimilarity (SIMPER); superscript a : for *Viola* spp. of a given size, only the number of size 2 *Viola* differed significantly; superscript b: for Roy Cove only (*n* = 53)

The top four models (Table 4.11) were all robust at ΔAIC_c <2. In each of the models the daytime mean temperature of the plant location under observation was a factor: in model 3, within the confidence limits, it was the sole factor. In the first three models wind was also a factor, whether adjusted wind speed at 30cm (models 1 and 2), or in protection from the wind, as shown by GLA (model 3). The presence of patches of bare ground, with a diameter of more than 10cm, within 60cm of the quadrat was a factor in model 4. Chlorophyll content was a factor in model 5, although, at ΔAIC_c = 3.86, the model was outside the confidence limits.

Table 4.11 Candidate habitat models for Roy Cove. The five leading candidate models under AICc; the first four, where $\Delta AIC_c < 2$, can be regarded as robust.

rank	independent variables	*df*	loglik	AIC_c	ΔAIC_c	weight	*F* (2, 14)	r^2	*p*-value
1	temp, wind	4	-0.136	11.6	0.00	0.327	14.17	**0.62**	**0.0004**
2	temp, wind shade (GLA)	4	-0.136	11.7	0.05	0.320	14.11	**0.62**	**0.0004**
3	temp, wind, patches	5	0.985	13.5	1.88	0.128	10.62	**0.64**	**0.0008**
4	temp	3	-2.823	13.5	1.89	0.127	18.07	**0.52**	**0.0007**
5	temp, wind, patches, chlor	6	2.465	15.5	3.86	0.047	9.32	**0.67**	**0.0011**

Significance at $p < 0.05$ and large effect size, $r^2 > 0.26$ are shown in bold. Key to independent variables; temp = mean temperature of plant location 1000-1600hrs; wind = adjusted wind speed at 30cm above ground; wind shade (GLA) = shelter from the prevailing wind measured through gap light analysis; patches = number of patches of bare ground over 10cm diameter; chlor = chlorophyll content ($mg \cdot m^{-2}$) of plant observed. *F*-ratio, r^2 and *p*-value all refer to the underlying linear model.

4.4 Discussion

4.4.1 Summary of findings

The four study sites on the Falklands showed that *Yramea cytheris* had adapted to a variety of physical environments: the raised beach at Bleaker Island, the low cliffs at the Frying Pan, the rolling, heavily grazed pastureland of Roy Cove and the mosaic of sand, dwarf shrub heath, tussac and penguin colonies at Sea Lion Island. The one common factor was the presence of *Viola* spp.

Viola spp. are widely spread around the Falkland Islands, but no survey has been done to identify how many sites containing them also support colonies of *Yramea cytheris.* The study sites were near the coast, and mostly at a low elevation. Where there was scope for the butterfly to go higher, up to 262 m at Roy Cove, the highest oviposition location was at 86 m.

Data from the study sites showed that *Y. cytheris* preferred north-east facing patches, which had the combined benefits of being sheltered from prevailing westerly (and, at Roy Cove, south-westerly) winds and having the maximum direct exposure to the morning sun. At patch level, oviposition locations could be characterised most simply as warmer, sunnier and more sheltered from the wind than non-oviposition locations.

A preference was shown for oviposition on medium-sized *Viola,* with three to five leaves of up to 35 mm. Selected plants tended to have a higher chlorophyll level. Oviposition, usually of single eggs, was on the warmer leaves of the *Viola.*

In modelling an oviposition location, based on Roy Cove data, the most important factor was temperature, to the extent that it featured in the top four candidate models. It was, by itself, one of the models, the other factors were wind or wind shelter, and the number of bare patches of ground near to the oviposition site.

4.4.2 The importance of microhabitat

The data show that variability of topography, vegetation and ground cover was important at all scales in *Y. cytheris's* choice of an oviposition location, although all with the common effect of providing a location of the right temperature - that is to say, warmer than other potential locations - for eggs to hatch and larvae to develop. To achieve the necessary conditions, the realised niches to provide them might substantially differ (Anthes et al. 2008). In the case of *Y. cytheris* at the four, very different, study sites, the range of possible factors contributing to the temperature of an oviposition location was considerable: shelter, for example, being principally from wild celery *Apium australe* at Bleaker; cliffs at the Frying Pan; hills at Roy Cove; and Tussac at Sea Lion. But the important factors were those which led to a *Viola* plant's being sheltered from the wind, with enough bare earth to provide additional warmth, and facing the late morning sun. Microclimates, resulting from configurations of microhabitat, were key determinants in choice of oviposition and larval locations (Eilers et al. 2013, Vilbas et al. 2016, Čelik and Vreš 2018). This supports the view, derived from an evidence-based approach to butterfly conservation, that variation in the intrinsic quality of larval habitat is the most important factor determining the size and persistence of populations in locations where they occur (Dennis et al. 2006, 2007, Thomas et al. 2011).

4.4.3 The importance of the larval host plant

Y. cytheris appeared be totally dependent on *Viola* as an oviposition location and larval host plant. This raises a causality dilemma over the constraints on the butterfly's habitat requirements: whether *Y. cytheris's* perceived temperature requirements are those of the butterfly, or those of the larval host plant. If the latter, then the butterfly's choice for oviposition is most likely to be straightforward: the warmest location to speed up larval development (Anthes et al. 2008, Pennekamp et al. 2014). The attendant risk, that the location is too hot, causing desiccation of larvae, will have already been reduced by the *Viola's* habitat requirements (Figure 4.26).

Figure 4.26 When the balance between warmth and desiccation goes wrong. Both *Viola* and *Y. cytheris* larvae risk desiccation in an isolated location amongst heat-retaining rocks and bare ground at the Frying Pan. Photograph by the author.

Y. cytheris, like most other *Viola*-obligate members of the Argynnini, appears to lay only one egg on a leaf (Fartmann and Hermann 2006). Its movement over a patch, and then over a prospective oviposition plant, is likely therefore to be rapid, to maximise oviposition opportunities. As only one oviposition was seen over all three study trips, and actions either side of ovipositing were not observed, there was not enough evidence to confirm that. Given, however, that the significant selection criteria were the warmest location, the plant with the highest chlorophyll content, and then the warmest leaf, that approach would be appropriate (Singer 2004). Given that the butterfly still needs to lay a clutch of eggs, it suggests that a patch, to be chosen should have the potential for a number of suitable plants. While there was inadequate evidence to suggest density of *Viola* in a patch was a criterion, at the 10 cm quadrat level oviposition locations had a significantly larger density of *Viola*, in particular medium sized *Viola*, than non-oviposition, suggesting that butterflies were prioritising host-plant rich environments.

Larval mobility was not examined, so it was unclear to what extent larvae remained on their oviposition plant. As the oviposition pattern obviated the need for a large host plant to provide sufficient nutrition for a brood (Eichel and Fartmann 2008, Pennekamp et al.

2012), the choice of medium sized *Viola* would, unlike small *Viola*, be adequate for the early instars, and, with their leaves closer to the ground than larger plants, be in a more sheltered, and therefore warmer environment.

4.4.4 Implications for conservation

The evidence suggests that a primary target of conservation efforts should be *Viola* spp., and in particular the dwarf shrub heathland mosaic, to ensure the necessary range of potential oviposition sites is maintained. Continued light grazing, as for *Calluna* heathland in the northern hemisphere (Gillingham 2010, Taboada et al. 2013, Čelik and Vreš 2018), will remain important where the sites are in pasture rather than on cliffs or amongst stones. In places where grazing has ended, such as Sea Lion, some way of keeping rank grass levels low, particularly during wet summers, should be considered (WallisDeVries and Raemakers 2001, Evju et al. 2010).

Three further areas of study, centred on *Viola maculata*, suggest themselves. The first is to gain a greater understanding of its autecology. It is widely spread through the islands at a range of elevations (Vallentin and Cotton 1921). It is also widely distributed in Latin America, where it has been shown to have a strong tolerance of colder conditions at elevation (Seguí et al. 2018). Its tolerance of heat, however, appears untested. It would also be helpful to understand the extent to which, in the case of local extinctions, reintroductions might be successful.

A second area is to quantify the extent to which *Viola* levels are affected by differing weather patterns, either directly, or through their impact on other vegetation, when there is no grazing. An exclosure study on a sheep farm should be carried out over enough seasons to make comparisons.

The third area is an investigation of the relationship between *Viola* and sheep (Salz and Fartmann 2017), in the context of the role that extensive sheep grazing has had in shaping landscapes, particularly highlighted in the conservation of *Maculinea arion* (Warren 1993, Spitzer et al. 2009). Observation in the field further showed that sheep tracks through dwarf shrub heath provided an environment favourable to *Viola* growth. The wide-ranging nature of Falklands sheep over nutrient-poor grassland suggest that endozoochorous dispersal might have supported the spread of *Viola* through the islands, although studies in other environments have shown low establishment rates of vegetation through sheep faeces pellets, either through desiccation or through being outcompeted.

4.4.5 Implications for Yramea cytheris

The wide range of *Y. cytheris* in South America, both in terms of latitude and elevation, suggests a range of climatic tolerance. The variety of habitats and potential habitats at its current range in the Falklands gives it scope to vary niches in response to climate change, although the threats of stochastic events such as inundation due to a change in maritime currents, or a tsunami, are less easy to mitigate. Although the adaptability suggested by the extent of *Y. cytheris's* distribution in South America does not necessarily mean it can cope with rapid climate change, action at an early stage to maintain a diverse dwarf shrub heath habitat, and to ensure that open *Viola* patches continue to flourish across the islands, will maximise its chances of survival.

4.5 References

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Chapter 5: Conservation genetics

5.1 Introduction

This chapter outlines the developing use of genetic analysis in conservation, in particular as applied to isolated populations of butterflies. It then investigates the genetics of *Yramea cytheris* in the Falkland Islands, focusing on those elements of importance in conservation planning, and suggests how these might be incorporated in future work.

5.1.1 The contribution of genetic analysis to conservation

As genetic analysis has become more sophisticated, and cheaper, it has become increasingly used in conservation work. While warnings that it might become an end in itself (Saarinen 2015), and that, done poorly, it might damage rather than assist conservation efforts (Morin et al. 2010) need to be heeded, genetic analysis is now indispensable in helping to underpin an evidence-based approach to a wide, and widening, range of conservation tasks (Frankham 2010, Habel et al. 2015, Fenster et al. 2018, Holderegger et al. 2019).

In considering an evidence-based approach to conservation of a single species and the way in which genetics can inform it, the usual terminology of extinction (Benson et al. 2019, Figueiredo et al. 2019) has been rejected in this chapter in favour of a framework adapted from conflict management (Ackermann 2003, 2010), which moves from prevention to management, then to resolution and, finally, rehabilitation. This is to reflect that, at the local level, and leaving aside what Swart (2018) calls resurrection conservation, extinction is not necessarily for ever (even if the local evolutionary lineages have gone), as the examples of the butterflies *Maculinea arion* in south-west England (Thomas et al. 2009) and *Neonympha mitchellii francisci* in North Carolina, USA (Haddad 2018) demonstrate.

(i) **Prevention**

Addressing potential extinction of a local population is a combination of information gathering, establishing a baseline, developing indicators of possible deterioration, and setting up monitoring mechanisms. In the case of butterflies much of this work will be focused on habitat, but genetic analysis can be important in establishing baselines, such as the taxonomy of the study species, the relationship between its populations, and its genetic health, in particular its capacity to evolve in response to environmental change.

Allocation to taxa

Taxonomic approaches based on morphological characters have been considered in the introduction (1.3), in particular in identifying possible subspecies of *Yramea cytheris.*

Since the introduction of genetic barcoding (Hebert et al. 2003, Jinbo et al. 2011), based on the mitochondrial gene *Cytochrome Oxidase subunit I* (*COI*), DNA analysis has been an essential element in determining taxonomic status. Barcoding was envisaged as a preliminary step in species delimitation, aimed at producing an initial taxonomic framework as a basis for further analysis (Kekkonen and Hebert 2014). Ratnasingham and Hebert (2013), setting out a Barcode Index Numbering System, noted that a >2.2% (14 bp) variation between samples would suggest they were different species. As an indication of delimitation in Lepidoptera, Hajibabaei et al. (2006) found that congeneric species of skipper (Hesperiidae) showed average divergences in *COI* of 4.58% (30bp), with average within-species divergences of 0.17% (1 bp). Wiemers and Fiedler (2007), however, argued that *COI* was insufficient for species delimitation, giving examples of accepted Lycaenidae species with no *COI* variation (23 *Agrodiaetus* and three *Maculinea* species pairs), and others which showed intraspecific variation greater than this (e.g. *Polyommatus amandus*, with 3.8% divergence, and *Polyommatus icarus*, with 5.7–6.8%).

Even with questions about the sufficiency of *COI* for species delimitation (Wiemers and Fiedler 2007, Õunap and Viidalepp 2009), its utility as a starting point has been acknowledged (Galtier et al. 2009). Increasingly, in genetic analysis of Lepidoptera, in addition to *COI* a 604 bp section of the nuclear gene *Elongation Factor-1a* (*EF-1α*), and a 355 bp section of the nuclear gene *wingless* have been used (Peña et al. 2006, Wahlberg and Wheat 2008, Simonsen et al. 2010, Wilson 2010, Aduse-Poku et al. 2015, Chazot et al. 2016, Kozlov et al. 2017). *EF-1α* has been proposed as an alternative to *COI* barcoding in Lepidoptera (Kim et al. 2017). The *wingless* gene has shown a rapid rate of substitution in nymphalids, with a <400 bp length comparable to, or exceeding, 1200 bp of *EF-1α* (Cho et al. 1995, Brower and DeSalle 1998, Campbell et al. 2000). These sequences can be concatenated: while differing rates of evolution of the three genes has led to some concern about the robustness of doing so (Monteiro and Pierce 2001), the process has become generally accepted (Elias et al. 2007, Rubinoff, San Jose, et al. 2012, Ortiz-Acevedo and Willmott 2013).

Next generation sequencing affords a step beyond the use of nuclear genes to supplement *COI* in considering species delimitation. Hinojosa et al. (2019), investigating the complexity of lineages in the skipper *Thymelicus sylvestris* through double-digest restriction-site associated DNA, showed how nuclear markers could help resolve phylogenetic complexity, in particular by highlighting population and geographic linkages.

The possibility of cryptic species, which are identified genetically, needs to be considered (Bickford et al. 2007) although, whilst these have been found in Lepidoptera (Hebert et al. 2004, Dincă et al. 2011), there is no evidence of their having appeared in fritillaries. Delimitation is more uncertain when lower taxonomic levels are involved, as suggested by the weak capacity of *COI* analysis to allocate specimens correctly to currently established subspecies (Austerlitz et al. 2009), although this could be viewed as evidence that the subspecies in question are not valid (Braby et al. 2012). This dichotomy is central to the argument about the utility of the subspecies concept, considered in Chapter 8.

Assigning the correct taxonym is not simply based on a desire for order (for an entertaining account of this impulse see Ritvo 1997). An endangered species is likely to be viewed by policy makers as a priority for conservation: the species persists as "the currency of biology" (Agapow et al. 2004). At a lower level, the extent of separation from the binomial nominate may determine the level of material support a taxon has for conservation work. An isolated, endemic species, for example, is likely to be a higher priority than a subspecies (however defined) or a small outlying population of a widespread taxon. Other issues then become important, such as morphological or behavioural variation; economic, historical or cultural value; remoteness of populations and potential evolutionary significance (Gompert et al. 2006). Here the debate about Evolutionarily Significant Units and Management Units *sensu* Funk et al. (2012), and the extent to which these should be based on genetic factors, is relevant (Moritz 1994, Paetkau 1999). In this context, prioritisation of island species for conservation has been advocated Robertson et al. (2014).

The relationship between populations

Understanding whether small, isolated colonies are the product of single founder events, occasional immigrations or regular immigrations can help in assessing the level of threat they face (Vandewoestijne et al. 2004, Papadopoulou and Knowles 2016, Maresova et al. 2019). Genetics-based phylogeography can then suggest lines of further investigation, such as habitat requirements and larval host plant preferences, which need to be considered in any conservation plan requiring a donor population.

Island populations present particular challenges. Work on island biogeography has focused on the assemblage of species on islands and the processes which affect them (MacArthur and Wilson 2001, Lomolino et al. 2010). This approach has been reflected in work on butterflies, with genetic information increasingly suggesting connections between community composition and patterns of genetic differentiation (Vodă et al. 2016, Dapporto et al. 2017, Dennis and Hardy 2018). Thus some genetic structures might be expected to reflect membership of a largely stable community, while others might suggest a tendency towards dispersal. Genetic analysis has further helped illuminate work on species that have become part of wide-ranging studies, such as those on *Maniola jurtina* in the Isles of Scilly (Dowdeswell and Ford 1955, Creed et al. 1964, Dowdeswell 1981, Baxter et al. 2017) and those on *Melitaea cinxia* in the Åland Islands (Hanski 1998, Saccheri et al. 1998, Nonaka et al. 2019).

Genetic health of populations

Small and fragmented populations, particularly those on small islands, are particularly susceptible to a decline in genetic health (Lynch et al. 1995, Mattila et al. 2012, Frankham et al. 2017). Island populations are likely to suffer increased inbreeding relative to mainland populations reflecting a single founding event or a severe genetic bottleneck. Low dispersal rates, particularly amongst relatively immobile taxa, reduce gene flow, bringing the risk of genetic drift. As a consequence, inbreeding depression can leave the butterfly with a reduced capacity to evolve in response to environmental change, with the attendant risk of extinction (Frankham 1998, Saccheri et al. 1998, Spielman et al. 2004, Roitman et al. 2017).

The response of small island populations can, however, vary. Mattila et al. (2012), investigating the *Melitaea cinxia* population on the small (1km x 2km) Baltic Island of Pikku Tytärsaari, found a high genetic load but no evidence of inbreeding depression. This was attributed to a single founder event resulting in one generation of full-sib mating. Windig et al. (2004), investigating *Pararge aegeria* on Madeira, which resulted from a possible single founder event, found no detectable effects of inbreeding depression or high genetic load.

(ii) **Management**

Genetic health

Genetic diversity is not a guarantee of genetic health: Saarinen et al. (2014) observed an unpredicted increase in allelic richness and heterozygosity in one of the last two surviving populations of the Miami blue butterfly (*Cyclargus thomasi bethunebakeri*) shortly before that population went extinct. It is, however, essential for the adaptability of a population in which inherited, fitness-related traits are crucial (Booy et al. 2000)

In the case of inbreeding and bottlenecks, genetic rescue, through the introduction of individuals from other populations, can be considered. Frankham (2011) addressed concerns about outbreeding depression and the loss of local adaptions through genetic swamping by proposing a decision tree for possible introductions. The decision-tree approach has become part of the Open Standards for the Practice of Conservation and its software platform Miradi (Schwartz et al. 2012, and, for its application to Lepidoptera, see Daniels et al. 2018). Frankham (2015) subsequent meta-analysis suggested that, provided appropriate guidelines were followed, outcrossing with other populations could be successful, citing examples from a range of taxa (though not Lepidoptera).

Wootton and Pfister (2015), working experimentally on flour beetles (*Tribolium castaneum*), suggested three ways of addressing genetic health. The first, evolutionary rescue, relied on standing variation in the population which would lead to adaptation to a changing environment; the second, demographic rescue, would mitigate against possible Allee effects by expanding the size of the population; the third, genetic rescue, would increase the genetic diversity, and therefore fitness, of the population through translocations from donor populations.

(iii) **Resolution**

Reintroduction

Reintroductions have increasingly been used in conservation programmes, whether, in the terms used by Wootton and Pfister (2015), to augment the process of evolutionary rescue; to expand the population to mitigate the Allee effect; or to improve the genetic health of the population. More eye-catchingly, and often controversially, they have been used where the taxon has gone extinct: for example the reintroduction into the UK of the large blue butterfly, *Maculinea arion* (Thomas et al. 2009); the Eurasian beaver, *Castor* *fiber* (Gaywood 2018, Auster et al. 2019); and the potential reintroductions of the Eurasian lynx, *Lynx lynx* (Lipscombe et al. 2018) and the wolf *Canis lupus* (Nilsen et al. 2007).

The IUCN recognised that reintroductions (IUCN Wildlife Health Specialist Group 2013) needed to address a broad range of social and scientific considerations, but emphasised the need to consider genetic health. They recognised two potentially divergent approaches: replacing with something as close as possible to that which went extinct, and replacing with a large number of individuals with a wide genetic base. There is, however, the danger that reintroductions to augment a declining population, or replace an extinct one, fail to take into account adaptations to local conditions (Aardema et al. 2011).

There is the further risk that the introductions carry pathogens. Daniels et al. (2018), refocusing the IUCN guidelines on the particular needs of Lepidoptera, stressed the importance of genetic testing of source populations for pathogens, particularly *Wolbachia*, with its high incidence and potentially (though not inevitably) deleterious effects in Lepidoptera (Hamm et al. 2014, Ahmed et al. 2015).

Captive breeding

Captive breeding is one possible way of avoiding some of the potential problems from a poor choice of donor populations. It can establish a refuge population in case of extinction in the wild; provide individuals for reintroduction; and provide a population for experimental work (Longcore and Bonebrake 2012). Selection of a founding population needs to take into account variation in genotype, although adaptations both in morphology, and as a response to habitat, such as choice of larval host plant, will also need consideration. Any captive breeding programme will further need to mitigate against possible changes in its population resulting from adaptation to a captive environment, thus making it potentially less able to survive in the wild. Lewis and Thomas (2001), for example, observed that a captive population of large white butterflies, *Pieris brassicae*, had, over 100 - 150 generations, become heavier, with smaller wings and lower wing aspect ratios, impacting on their dispersal ability.

Whether it is better in introductions to use individuals from other populations, or to collect butterflies from focal populations and captively rear offspring, will need to be judged on the extent to which local adaptations are important. In this context, the extent to which local adaptations are important.

(iv) **Rehabilitation**

The rehabilitation process after successful management intervention, or wide-scale reintroduction, reverts to a monitoring programme based to some extent on the prevention approaches at 5.1.1(i). This is backed up by work to address the biotic or abiotic factors which led to the decline or extinction. Daniels et al. (2018) suggest regular genetic monitoring of released individuals or reinforced populations, particularly if there is a limited number of initial founders, and evaluation of genetic rescue or reinforcement.

5.1.2 Concerns related to the Falklands

Little is known about the distribution and genetic health of the Falkland populations of *Y. cytheris.* Those populations recorded (1.4.2) are widely separated, although the widespread distribution of *Viola spp.*, the larval host plant, around the islands (Figure 1.11) suggests that others may exist. There is nothing known about their connectivity. Aside from the few specimens from Latin America with sequences recorded in GenBank (Table 5.2) and those papers dealing with the wider genetics of fritillaries (Simonsen 2006a, Simonsen et al. 2006, 2010, 2010), there has been no genetic analysis of the butterfly on which to base further work. The Latin American populations, which would be potential donors, appear to be similarly widely distributed, and equally poorly understood.

5.1.3 Research questions

This genetic investigation, addressing the practical conservation questions discussed above, has been based on four research questions (RQs).

RQ1: to what extent does DNA analysis clarify the relationship between the Falkland Islands and Latin American populations of *Y. cytheris*?
RQ2: to what extent does genetic data suggest either differentiation between, or subgrouping of, separate populations in the Falkland Islands?
RQ3: what evidence does DNA analysis provide for the genetic health of the Falkland Islands population?
RQ4: what are the implications of the results of DNA analysis for conservation management of *Y. cytheris*?

5.2 Materials and methods

5.2.1 Study populations

The principal analyses were carried out on samples from four Falkland Islands study sites, Bleaker Island, the Frying Pan, Roy Cove and Sea Lion Island, with additional material from Punta Arenas, Torres del Paine, Puerto Williams and two unnamed sites, all in Chile (Figure 5.1, Table 5.1 Table 5.2).

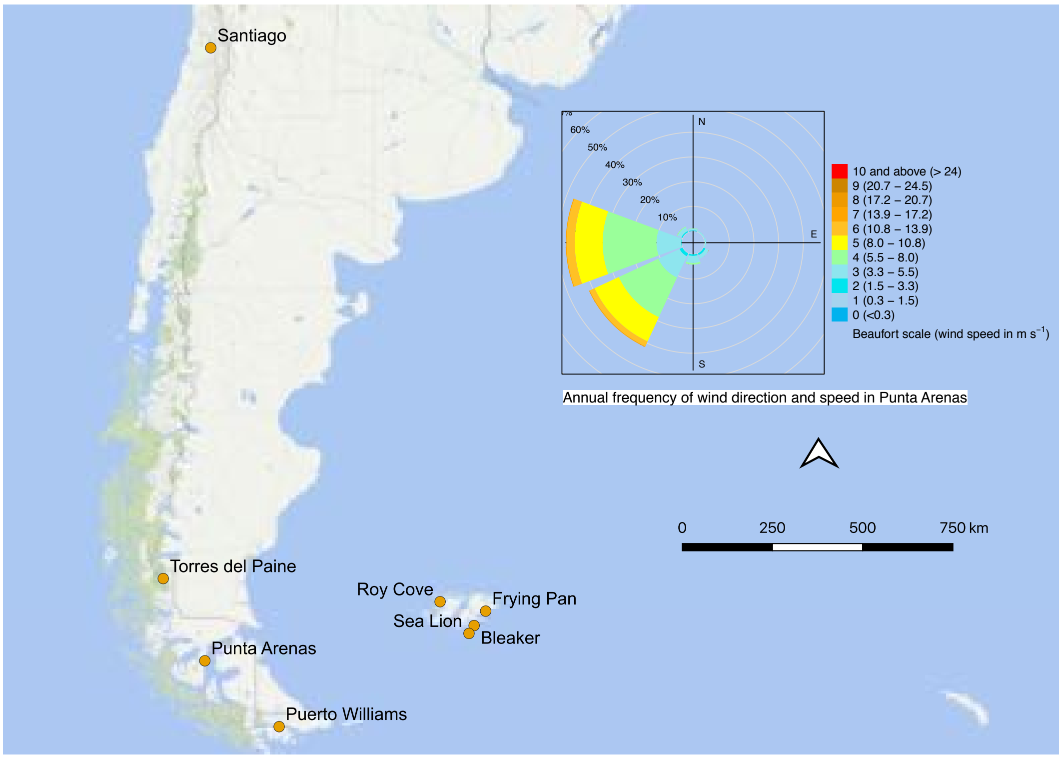

Figure 5.1 Sites of origin of samples used in genetic analysis (Table 5.1). The wind rose shows the predominant wind direction (south and south-west) and speed (> 60% at Beaufort Scale 4 and over) at Punta Arenas, a potential factor in population movement. Data used were 2014-2018, from the Ogimet website (Valor and López 2017).

5.2.2 Collection of samples

(i) **Falkland Islands**

The Falkland Islands Government’s Conservation Committee allowed eight butterflies to be taken, with no more than two from an individual site, during the first field visit (January-February 2016), and twelve, again with no more than two from a site, during the second (December 2016- February 2017) and third (January- February 2018) visits respectively.

Non-lethal sampling was permitted by removal of a leg, which was also used for claw analysis (Koscinski et al. 2011, Crawford et al. 2013) or a small section of wing, of approximately 2 mm x 2mm (Hamm et al. 2009).

(ii) **Latin America**

Four Latin American specimens, from four different sites in Chile (Table 5.1), collected between 2000 and 2003, were kindly donated by Alvaro Zúñiga-Reinoso These were compared with three specimens from GenBank (Table 5.2), only one of which had a clear attribution, to Santiago, Chile, although the two other specimens were attributable to Chile by their voucher codes. A specimen of *Yramea inca* (Staudinger 1894), attributed to Peru by Simonsen et al. (2006), was used as the outgroup. A field visit to Punta Arenas to try to capture further samples, 3–8 February 2018, was unsuccessful.

5.2.3 Data sets analysed

Samples from *Yramea cytheris* were collected from the Falkland Islands for DNA analysis between 2016 and 2018. To these were added samples from Latin America, from the collection of Alvaro Zúñiga-Reinoso (Table 5.1) and GenBank (Table 5.2).

Table 5.1 Number of individuals of *Yramea cytheris* successfully sequenced for the genes *COI*, *EF-1α* and *wingless*. The Falkland specimens were collected on field visits between 2016 and 2018; the Latin American specimens were collected by Alvaro Zúñiga-Reinoso between 2000 and 2003.

		available sequences			
land mass	population	*COI*	*EF-1α*	*wingless*	*CO I + EF-1α + wingless*
Falklands	Bleaker	11	8	7	5
Falklands	Frying Pan	5	5	4	3
Falklands	Roy Cove	8	6	6	5
Falklands	Sea Lion	4	6	5	2
Latin America	Punta Arenas, Chile	1	1	1	1
Latin America	Santiago, Chile	-	1	-	-
Latin America	Puerto Williams, Chile	1	1	1	1
Latin America	Torres del Paine, Chile	1	1	1	1

Table 5.2 Sequences from GenBank for the genes *COI*, *EF-1α* and *wingless* for *Y. cytheris* and, as an outgroup, *Y. inca*. Attributions of *Y. cytheris* samples CH-10B-4 and CH-8A-3 to Chile and *Y. inca* sample PE-22-2 to Peru were inferred from the country codes in Simonsen et al. (2006).

			GenBank accession number		
species	origin	GenBank voucher	*COI*	*EF-1α*	*wingless*
Y. cytheris	Chile	CH-8A-3	KM012985	KM013045	KM013113
Y. cytheris	Chile	CH-10B-4	DQ922858	DQ922890	DQ922826
Y. cytheris	Santiago, Chile	AYK-04-0543-08	KF492178	NA	NA
Y. inca	Peru	PE-22-2	DQ922859	DQ922891	DQ922827

5.2.4 Phylogenetic methods

(i) **Primers**

Primers (Table 5.3) were LCO1490 and HCO2198 (Monteiro and Pierce 2001) for *COI*; ef2F and efrcM4R (Monteiro and Pierce 2001) for *EF-1α*, and LepWG1 and LepWG2 (Brower and DeSalle 1998) for *wingless*.

(ii) **DNA extraction**

DNA was extracted from using the QIAGEN DNeasy Blood & Tissue Kit, following the manufacturer's protocol DNA concentration was determined using a Nanodrop spectrophotometer (Thermo Scientific). DNA was stored and used at a concentration of 10 ng/µl.

Table 5.3 Primers used for the amplification of *COI*, *EF-1α* and *wingless* genes

Gene	primer name	primer sequence
COI	LCO1490-J-1514F	GGT CAA CAA ATC ATA AAG ATA TTG G
	HCO2198-N-2175	TAA ACT TCA GGG TGA CCA AAA AAT CA
EF-1α	ef2F	AAA ATG CCC TGG TTC AAG GGA
	efrcM4R	ACA GCV ACK GTY TGY CTC ATR TC
wingless	LepWG1	GAR TGY AAR TGY CAY GGY ATG TCT GG
	LepWG2	ACT HCG CAR CAC CAR TGG AAT GTR CA

(iii) **PCR and sequencing**

The primers used were LCO1490 and HCO2198 (Folmer et al. 1994) to amplify *COI*; ef2F and efrcM4R (Monteiro and Pierce 2001) to amplify *EF-1α*, and LepWG1 and LepWG2 (Brower and DeSalle 1998) to amplify *wingless* (Table 5.3). Primers were used at a concentration of 20 µmol.

For *COI* and *EF-1α*, MyTaq DNA Polymerase was used in 50µl volume reactions (2µl DNA, 1µl forward primer, 1µl reverse primer, 0.25µl MyTaqDNA, 10µl MyTaq Buffer and 35.75µl water). For *wingless*, which had proved less amenable, and for *COI* and *EF-1α* which had only shown faint signs of amplification, the QIAGEN Multiplex PCR Kit was used. Varying dilutions of DNA were tried, with the best amplification occurring with a 10µl reaction and a high concentration of DNA (4.6µl DNA, 0.2µl forward primer, 0.2µl reverse primer, 5 µml QIAGEN Multiplex PCR Master Mix).

Reactions were run through PCR programmes in either GeneTouch or GenePro Thermal Cyclers.

The basic cycling profile for *COI* and *EF-1α* was 95°C for 5 min; 40 cycles of 94°C for 30 sec, 50°C for 30 sec, 72°C for 1 min 30 sec; and a final extension period of 72°C for 10 min, based on Wahlberg et al. (2016). For *wingless* it was 95°C for 15 min, 35 cycles of 94°C for 30 sec, 55°C for 90 sec, 72°C for 60 sec, with a final extension period of 72°C for 10 min, based on QIAGEN Multiplex PCR Kit protocols. Gradients from 50ºC to 60ºC were used for the annealing stages to optimise the possibilities for successful amplification.

PCR products were stained with 5x Green GoTaq Flexi Buffer. They were then analysed through electrophoresis in 1.5% TAE agarose gel, coloured with SBYR DNA stain and accompanied by a Promega 1kb DNA ladder. Products were visualised under ultra-violet light in a Bio-Rad Gel Doc Imaging System to identify successful amplifications.

Samples successfully amplified were purified and sequenced by Genewiz Europe, Takely, Essex, UK, using ABI Prism 3730xl DNA analysers.

(iv) **Sequence alignment**

Sequences were visualised and edited in Geneious version 9.1.5 (Kearse et al. 2012). Each fragment was aligned using ClustalW (Larkin et al. 2007) within Geneious and then checked by eye.

The sequences were then examined to determine haplotype groupings using DnaSP 5 (Librado and Rozas 2009). Subsequent work was carried out using single representatives of each haplotype. In all cases trees were rooted using GenBank data from *Y. cytheris's* nearest known relative, *Y. inca* (Simonsen 2006): GenBank voucher PE-22-2 (Table 5.2).

Analyses were run on each of the genes *COI*, *EF-1α* and *wingless* individually. All three genes were also concatenated to form single 1596 bp data sets.

(v) **Selection of substitution models**

Possible substitution models for the nucleotide sequence data were considered through jModelTest 2.1.10 (Darriba et al. 2012). All potential models were assessed on Akaike and Bayesian information criteria, with the former adjusted for small data sets (AICc and BIC), and also using performance-based decision theory (DT). In each case, BIC, AICc and DT produced different optimum models.

The utility of selecting a range of models on the basis of model testing packages has been questioned (Ripplinger and Sullivan 2008, Abadi et al. 2019, Spielman 2020), with Abadi et al. suggesting that the General Time Reversible model (Tavaré 1986), together with gamma distribution and invariant sites (GTR+Γ+I) produced an effective outcome in most cases.

Gamma distribution was particularly important given the difficulty of potentially uneven evolutionary rates between mitochondrial and nuclear genes noted by Wahlberg (2006). GTR+Γ+I was accordingly assessed for all Maximum Likelihood and Bayesian analyses by considering its position under the various tests. It was also considered without invariant sites (GTR+Γ), given criticism of possible damage caused by the strong correlation between the proportion of invariable sites and the gamma shape parameter (Sullivan and Swofford 2001, Ren et al. 2005).

As both GTR+Γ+I and GTR+Γ were in the 100% confidence intervals for all models for all data sets, and returned values in the middle of the pack for AICc, BIC and DT, GTR+Γ , given the concerns about GTR+Γ+I, was selected for all analyses.

(vi) **Dendrograms and cladograms**

Sequence data were first analysed through a Bayesian approach. In each case the General Time Reversible method was used, together with gamma distribution (GTR+Γ+I). Bayesian analysis was carried out in Geneious 11.1.4 (Kearse et al. 2012) using the MrBayes 3.2.6 plugin (Ronquist et al. 2012). Four coupled chains were run at a temperature of 0.2 for 5,000,000 generations, with a sample frequency every 1000th generation. The first 1,000,000 samples were discarded as burn-in.

Maximum Likelihood and Maximum Parsimony analyses were also carried out in MEGA X (De Moya et al. 2017) to explore the sensitivity of the data to different evolutionary models. 1000 replicate bootstrap tests were used to determine the most parsimonious tree (Felsenstein 1985).

Dendrograms and cladograms were produced in Geneious 11.1.4 (Kearse et al. 2012) and MEGA X (Kumar et al. 2018), then exported as Newick files to allow visualisation through FigTree 1.4.4 (Rambaut and Drummond 2012) and PDF Expert 2.4.2 (Readdle 2019).

(vii) **Intraspecific genealogies**

The relationship between haplotypes was also considered through median joining networks, recognising that tokogenetic relationships are not hierarchical, and that events affecting populations, such as hybridization, recombination, or gene duplication and loss may not be best considered in a dendrogram (Posada and Buckley 2004, Huson and Bryant 2005, Huson and Scornavacca 2011). Networks were visualised using PopArt (Leigh and Bryant 2015).

(viii) **Genetic distances**

The genetic distances between sampling sites were estimated through the fixation index (F_{ST}), using pairwise genetic differentiation in Arlequin 3.5 (Excoffier and Lischer 2010). Genetic variation of the concatenated genes between individual sites was mapped in QGIS 3.4 (QGIS Development Team 2018) using a base map from Bing Aerial, with pie charts drawn in *R* (R Core Team 2018) using the package *ggplot2* (Wickham 2017).

5.3 Results

5.3.1 COI

The *COI* gene, with three segregation sites, did not show enough variation to allow clear separation of haplotypes (Figure 5.2). Bayesian analysis placed all four haplotypes as a single cluster, and the bootstrap values separating the haplotypes in the Maximum Likelihood approach were not high enough to support differentiation. There were no parsimony-informative sites to allow a Maximum Parsimony approach.

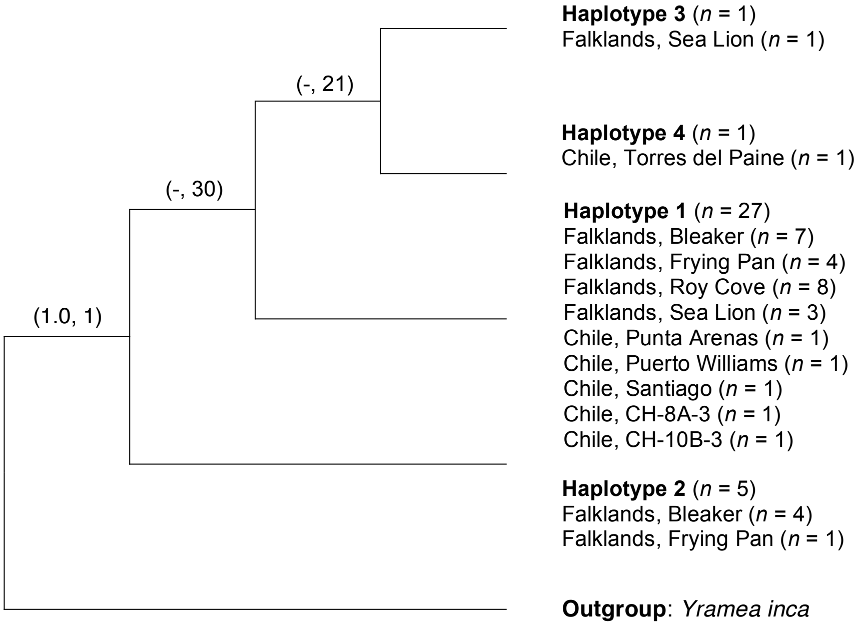

Figure 5.2 *COI* cladogram, based on (i) Bayesian and (ii) Maximum Likelihood approaches using the GTR+Γ model. The numbers in brackets represent, first, the Bayesian posterior probabilities for the node to the right of the brackets and second, the percentage of trees in which the associated taxa clustered together in a 1000 replicate bootstrap test. For Maximum Likelihood, the tree with the highest log likelihood, -979.89, was selected. The low bootstrap values suggest no significant distinction between the four haplotypes, which the Bayesian posterior had not differentiated between. It was not possible to calculate a Maximum Parsimony tree as there were no parsimony-informative sites. There were 644 positions in the final dataset.

Haplotype 1 contained the greatest number of specimens (*n* = 27), from both Latin America and the Falklands, with all Falkland Island sites represented. It also had a wide geographic spread in Latin America, from Santiago in northern Chile to Puerto Williams in the south, a distance of around 2,400 km. Haplotype 2 contained five samples from Bleaker and the Frying Pan; together with Haplotype 3, a single specimen from Sea Lion, they formed a distinct East Falkland, isolated site grouping. The only separate Latin American specimen was Haplotype 4, a single sample from Torres del Paine, which the Maximum Likelihood approach paired with Sea Lion.

A median joining network for *COI* showed single mutations between Haplotyope 1 and Haplotypes 2, 3 and 4 respectively (Figure 5.3).

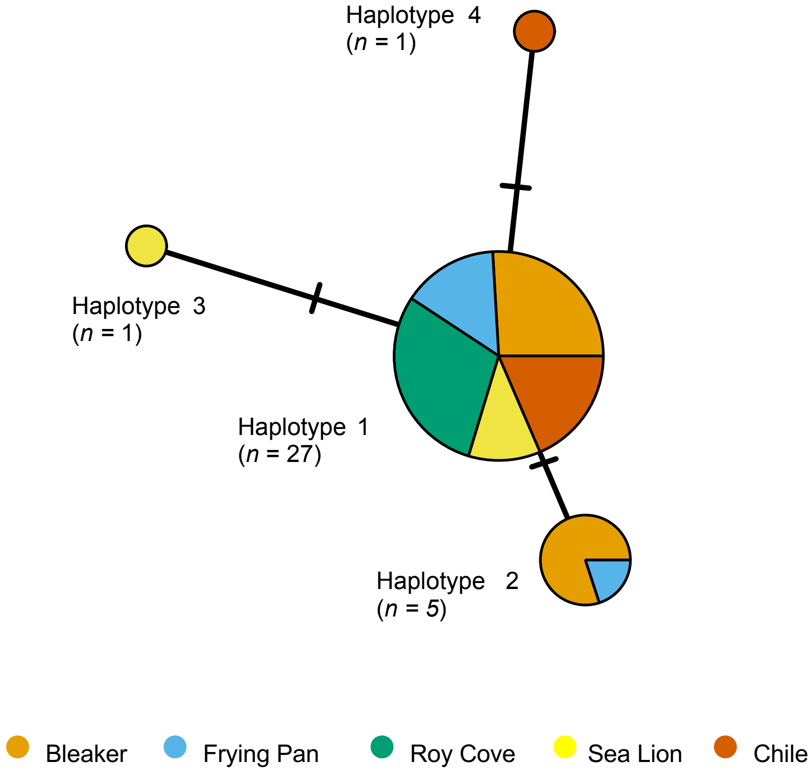

Figure 5.3 Median joining network of *COI* Haplotypes 1 - 4. Haplotype disc colours represent sites. Disc size reflects the number of samples, which are also noted in brackets under the corresponding Haplotype label. Hatch-marks on connecting lines represent the number of nucleotide substitutions.

Pairwise F_{ST} analysis confirmed there were no significant differences between the sampling sites for *COI* (Table 5.4).

Table 5.4 Pairwise F_{ST} obtained for *COI* sequences.

	Bleaker	Frying Pan	Roy Cove	Sea Lion	Chile	
Bleaker	*	0.630	0.135	0.108	0.171	*p*-value for pairwise F_{ST}
Frying Pan	0.000	*	0.405	0.676	0.703	
Roy Cove	0.250	0.101	*	0.495	0.324	
Sea Lion	0.176	0.006	0.186	*	0.703	
Chile	0.185	0.003	0.051	0.019	*	

pairwise F_{ST}

The section below the diagonal shows pairwise F_{ST} values; the section above shows the *p*-value for each pairwise F_{ST} comparison. Negative scores are shown as 0.000. There were no significant ($p < 0.05$) pairwise comparisons.

5.3.2 EF-1α

EF-1α, with seven segregation sites, showed a dominant haplotype, Haplotype 1 (*n* = 26), with a comparable spread to that of Haplotype 1 for *COI* (Figure 5.4). *EF-1α*

Haplotypes 2 and 3, like Haplotypes 2 and 3 in *COI*, came from isolated East Falkland sites.

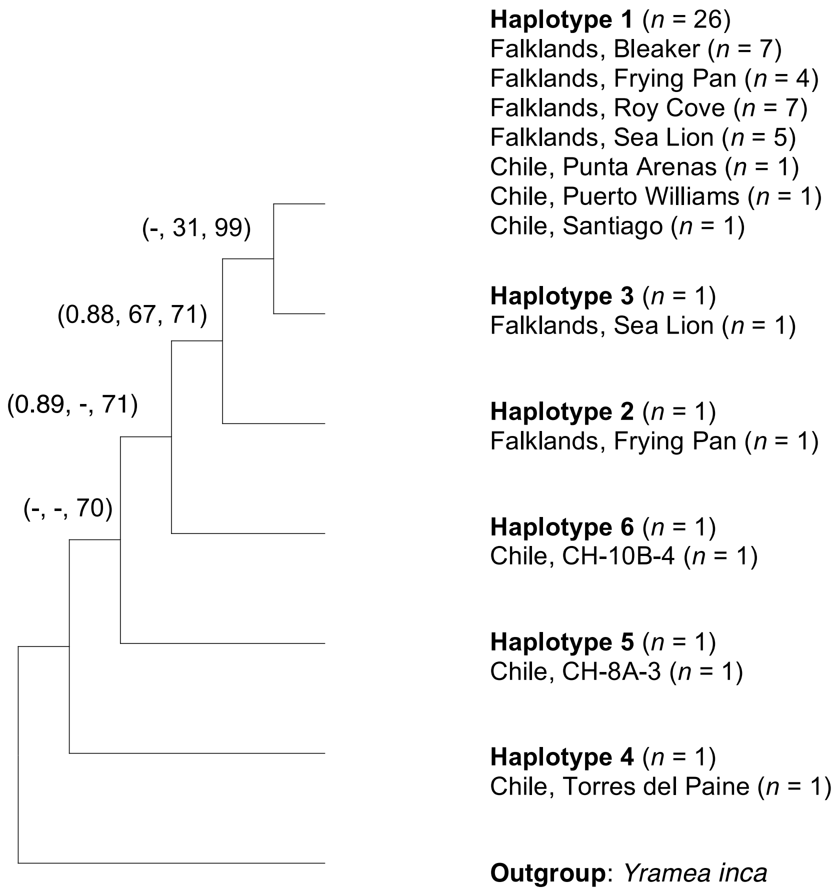

Figure 5.4 *EF-1*α cladogram, based on (i) Bayesian, (ii) Maximum Likelihood and (iii) Maximum Parsimony approaches using the GTR+Γ model. The first number in brackets shows the Bayesian posterior probability. The second and third numbers show the percentage of trees in which the associated haplotypes clustered, following a 1000 replicate bootstrap test, in the Maximum Likelihood and Maximum Parsimony methods respectively. The tree with the highest log likelihood, -979.89, was selected for Maximum Likelihood; the first of the three most parsimonious trees was selected for Maximum Parsimony. There was no Bayesian support for the separation of Haplotypes 1 and 3. The three methods did not agree on the relative placements of Haplotypes 4, 5 and 6, although all split them from Haplotypes 1 - 3. There were 607 positions in the final data set.

The two Chilean samples taken from GenBank, with origin only recorded at the country level, showed clear separation (a Bayesian posterior of 0.89) from Haplotypes 1-3, whereas they were placed in the largest haplotype in *COI*. Each was distinct from the other, as well as from the Torres del Paine sample, although each of the models placed them at different positions in the dendrogram.

A median joining network for *EF-1α* (Figure 5.5) showed single nucleotide substitutions between Haplotyope 1 and Haplotypes 2 and 6 (with dependent lineages for Haplotypes 4 and 5) respectively, and two substitutions between Haplotype 1 and Haplotype 3.

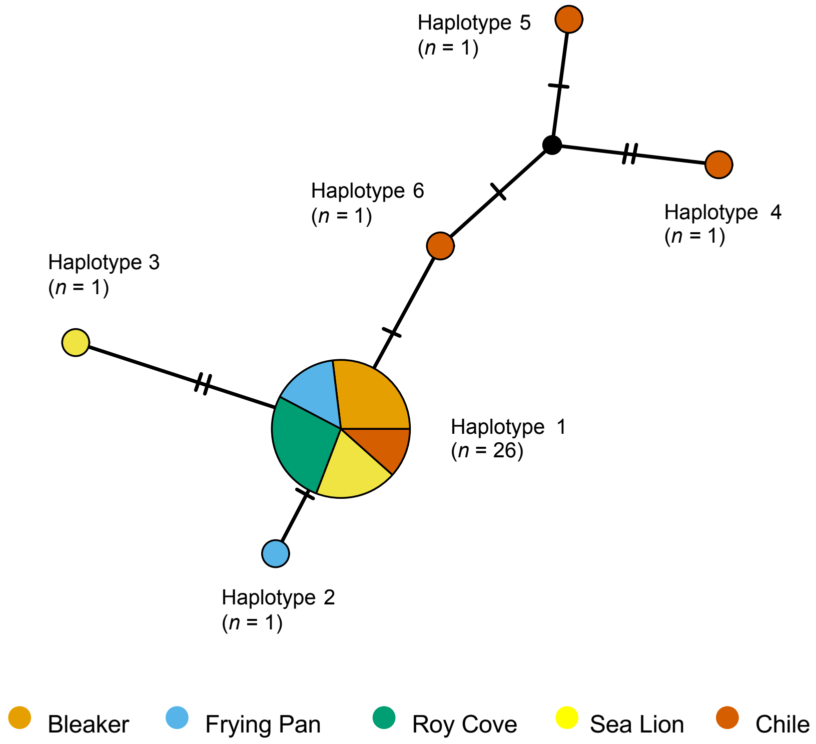

Figure 5.5 Median joining network of *EF-1*α Haplotypes 1 - 6. Haplotype disc colours represent sites. Disc size reflects the number of samples, which are also noted in brackets under the corresponding Haplotype label. Hatch-marks on connecting lines represent the number of nucleotide substitutions. The black disc represents an inferred missing haplotype.

Pairwise F_{ST} analysis confirmed that there were no significant differences between the the sampling sites for *EF-1α* (

Table 5.5)

Table 5.5 Pairwise F_{ST} obtained for *EF-1α* sequences

	Bleaker	Frying Pan	Roy Cove	Sea Lion	Chile	
Bleaker	*	0.446	0.999	0.459	0.090	p-value for pairwise F_{ST}
Frying Pan	0.073	*	0.437	0.718	0.110	
Roy Cove	0.000	0.073	*	0.464	0.073	
Sea Lion	0.028	0.000	0.028	*	0.186	
Chile	0.233	0.147	0.233	0.160	*	
			pairwise F_{ST}			

The section below the diagonal shows pairwise F_{ST} values; the section above shows the p-value for each pairwise F_{ST} comparison. Negative scores are shown as 0.000. There were no significant ($p < 0.05$) pairwise comparisons.

5.3.3 wingless

wingless, with four segregation sites, also showed a dominant haplotype, Haplotype 1 (n = 25), which, as with *COI* and *EF-1α*, comprised Chilean and Falklands samples (Figure 5.6). Haplotypes 1 and 4 were paired, separately from Haplotypes 2 and 3, which each of the models placed differently in the tree.

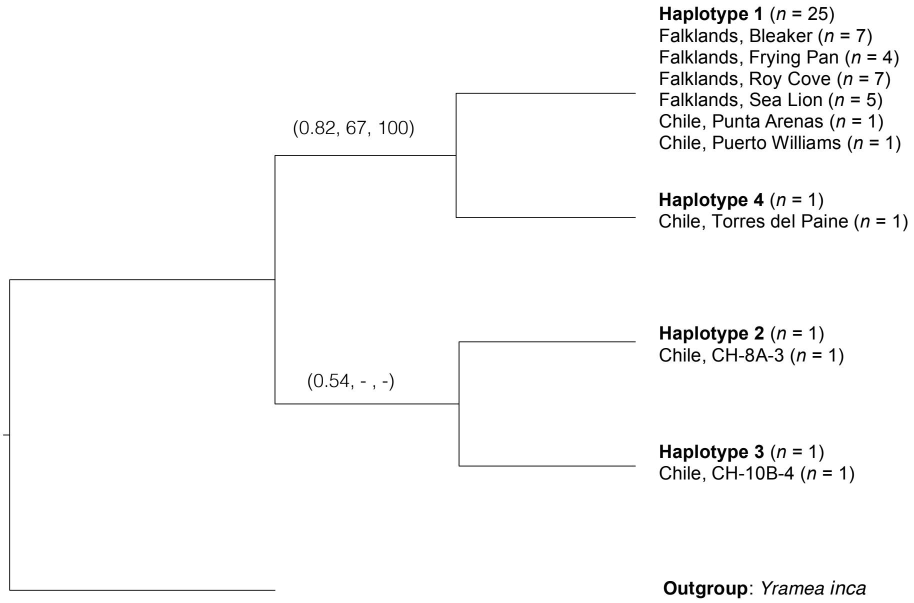

Figure 5.6 *wingless* cladogram, based on (i) Bayesian, (ii) Maximum Likelihood and (iii) Maximum Parsimony approaches using the GTR+Γ model. The first number in brackets shows the Bayesian posterior probability. The second and third numbers show the percentage of trees in which the associated haplotypes clustered, following a 1000 replicate bootstrap test, in the Maximum Likelihood and Maximum Parsimony methods respectively. The tree with the highest log likelihood, -546.5, was selected for Maximum Likelihood; the first of the three most parsimonious trees was selected for Maximum Parsimony. The separation of Haplotypes 1 and 4 from Haplotypes 2 and 3 was supported by all three methods. They did not, however, agree on the relative placements of Haplotypes 2 and 3. The division shown was supported by the Bayesian approach; Maximum Likelihood had a node separating Haplotypes 1 and 4 from Haplotype 2, though with a bootstrap support of only 18%, while Maximum Parsimony had a node separating Haplotypes 1 and 4 from Haplotype 3, with a bootstrap support of 99%. There were 357 positions in the final dataset.

A median joining network for *wingless* (Figure 5.7) showed a single nucleotide substitution between Haplotyope 1 and Haplotypes 4, and two substitutions between Haplotyope 1 and Haplotype 2. Haplotype 3 was linked through missing inferred haplotypes to Haplotypes 1, 2 and 4.

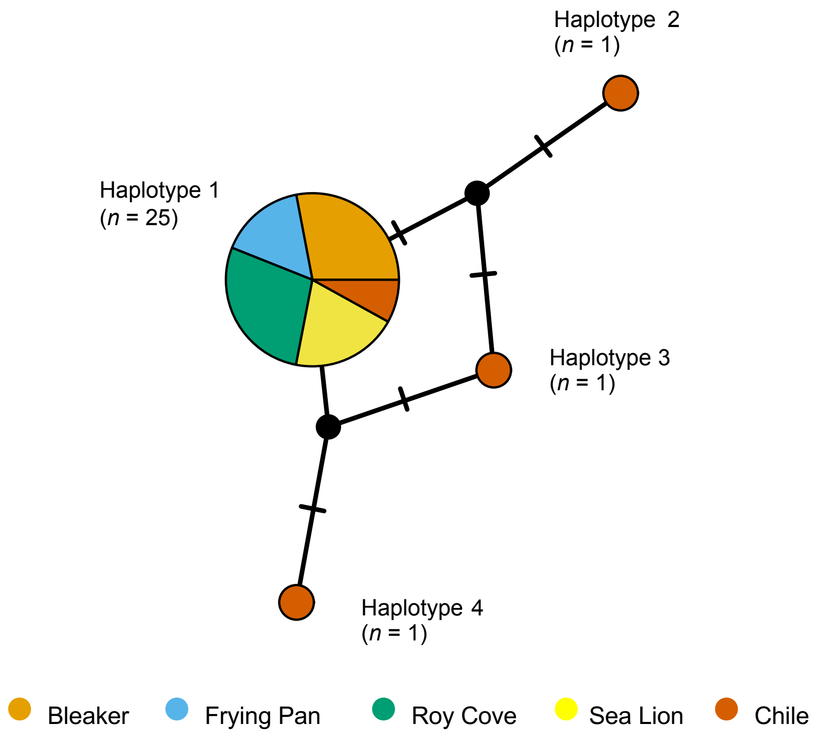

Figure 5.7 Median joining network of *wingless* Haplotypes 1 - 4. Haplotype disc colours represent sites. Disc size reflects the number of samples, which are also noted in brackets under the corresponding Haplotype label. Hatch-marks on connecting lines represent the number of nucleotide substitutions. The black discs represent an inferred missing haplotype.

Pairwise F_{ST} analysis showed significant differences for *wingless* between the Chilean populations and those of Roy Cove and Bleaker respectively (Table 5.6), although this is likely to be distorted by inadequate sampling size (numbers are shown in the cladogram at Figure 5.6).

Table 5.6 Pairwise F_{ST} obtained for *wingless* sequences

	Bleaker	Frying Pan	Roy Cove	Sea Lion	Chile	
Bleaker	*	0.999	0.999	0.999	**0.048**	
Frying Pan	0.000	*	0.999	0.999	0.165	*p*-value for pairwise F_{ST}
Roy Cove	0.000	0.000	*	0.999	**0.043**	
Sea Lion	0.000	0.000	0.000	*	0.165	
Chile	**0.247**	0.111	**0.247**	0.166	*	
			pairwise F_{ST}			

The section below the diagonal shows pairwise F_{ST} values; the section above shows the *p*-value for each pairwise F_{ST} comparison. Negative scores are shown as 0.000. Numbers in bold show significant ($p < 0.05$) pairwise comparisons.

5.3.4 Concatenation

Concatenation of all three genes confirmed the clustering of those haplotypes which included samples from the Falklands, with a clear separation from the Torres del Paine and two GenBank specimens (Figure 5.9). Haplotype 1, containing the largest number of specimens (*n* = 13) included samples from Punta Arenas and Puerto Williams with the Falklands specimens. The separation of Haplotypes 1 - 4 in Figure 5.9 derived from the Maximum Parsimony tree, although the Maximum Likelihood tree also separated them in a different order, with Haplotypes 3 and 4 paired (24% bootstrap value), then 2, 3 and 4 clustered (21% bootstrap value), before the clustering of 1 - 4.

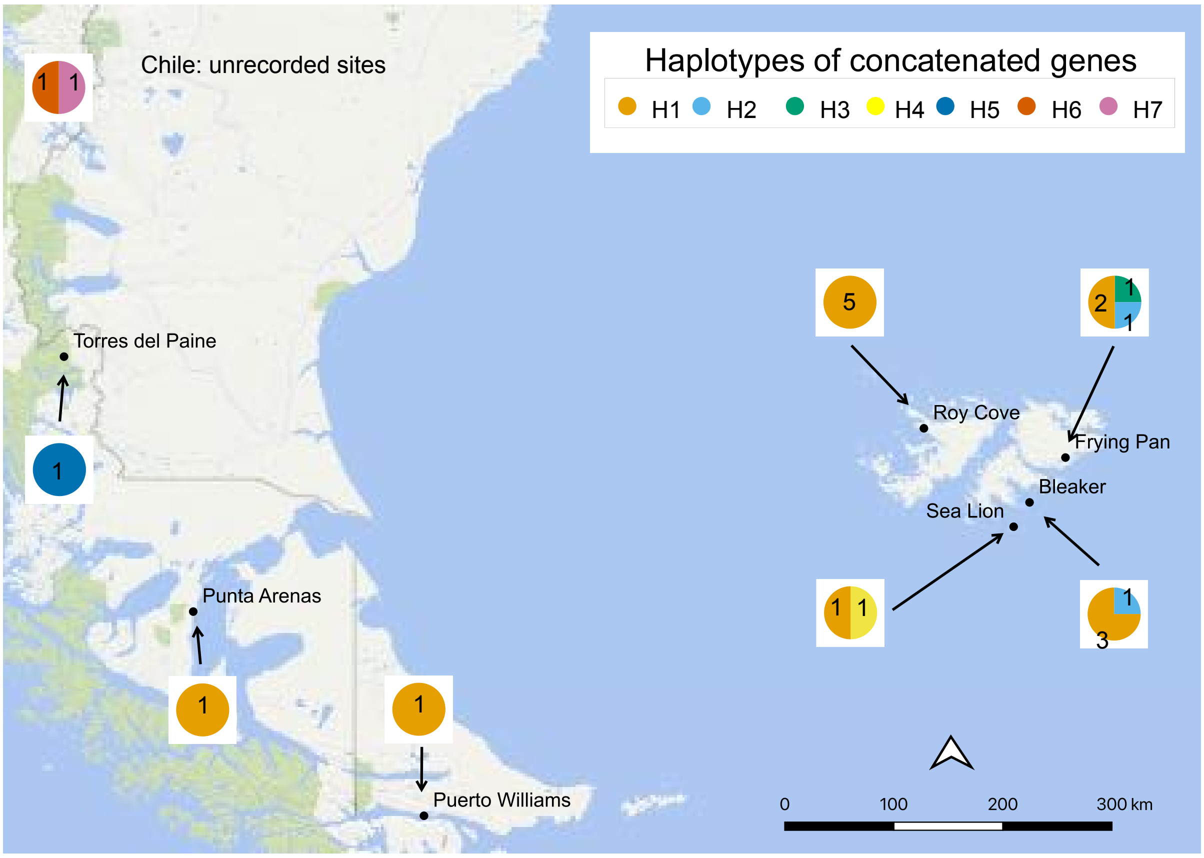

Figure 5.8 distribution of *Y.cytheris* haplotypes for concatenated *COI*, *EF-1α* and *wingless* genes. H1 to H7 correspond to Haplotypes 1 - 7 in Figure 5.9 and Figure 5.10. The discs show the haplotypes of samples from each site. Numbers of samples for each haplotype are shown in the appropriate colour in each disc. The map shows the prevalence of Haplotype 1 in the Punta Arenas, Puerto Williams and Roy Cove triangle, and the increased haplotype variation away from it.

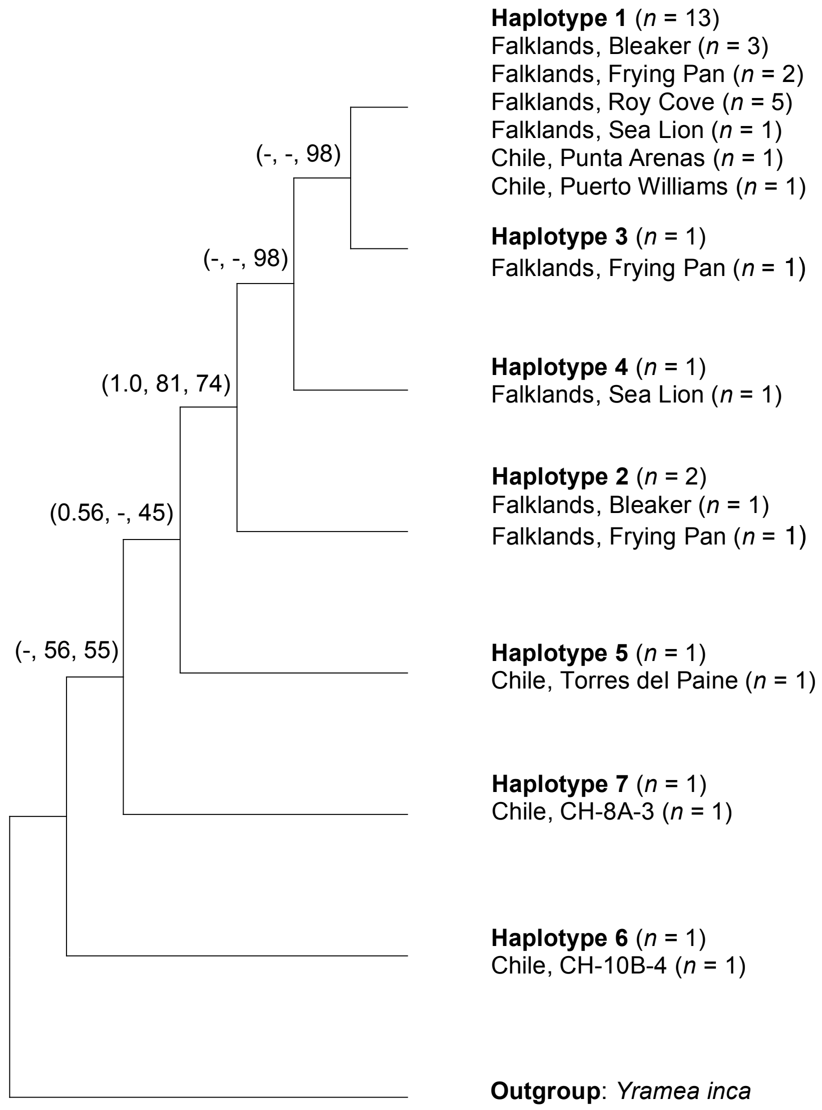

Figure 5.9 Concatenated *COI*, *EF-1α* and *wingless* cladogram based on (i) Bayesian, (ii) Maximum Likelihood and (iii) Maximum Parsimony approaches using the GTR+Γ model. The first number in brackets shows the Bayesian posterior probability. The second and third numbers show the percentage of trees in which the associated haplotypes clustered, following a 1000 replicate bootstrap test, in the Maximum Likelihood and Maximum Parsimony methods respectively. The tree with the highest log likelihood, -2520.88, was selected for Maximum Likelihood; the first of the 12 most parsimonious trees was selected for Maximum Parsimony. There was no Bayesian support for the separation of Haplotypes 1, 3 and 4, although there was a posterior probability of 1.0 for the separation of Haplotypes 1 - 4 from Haplotypes 5 - 7. The three methods did not agree on the relative placements of Haplotypes 5 - 7, although all split them from Haplotypes 1 - 4. There were 1596 positions in the final dataset.

A median joining network for the concatenated genes (Figure 5.10) showed Haplotypes 5 - 7, each represented by a single Chilean sample, as a distinct group, with an inferred common haplotype two mutations from Haplotype 1. The East Falkland grouping of

Bleaker, Sea Lion and Frying Pan each showed at least one other haplotype alongside Haplotype 1, with the Frying Pan most diverse at three haplotypes overall.

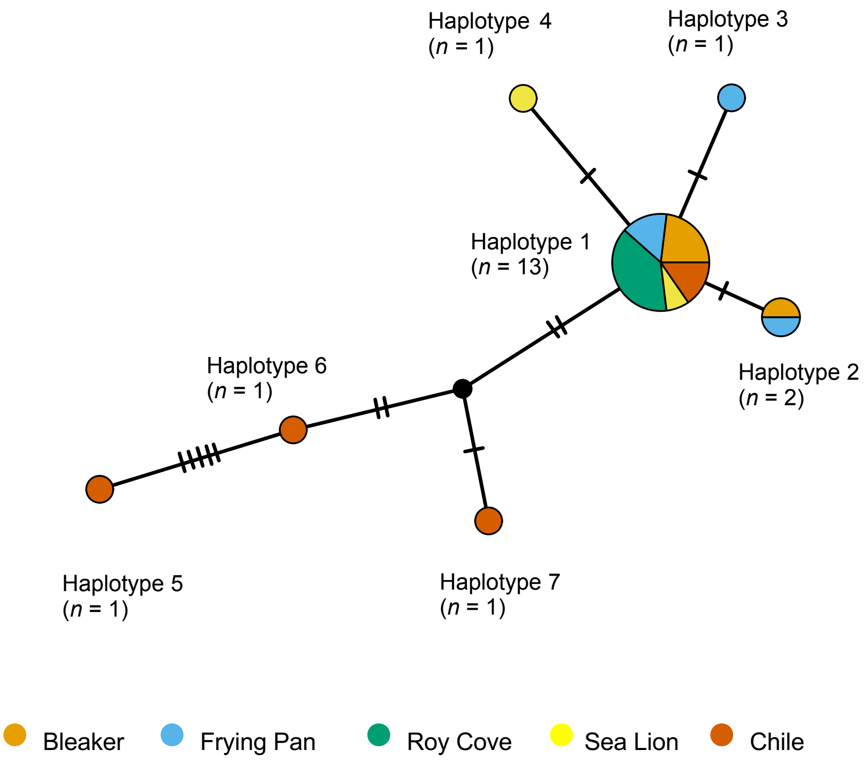

Figure 5.10 Median joining network of Haplotypes 1 - 7 derived from concatenation of the *COI*, *EF-1α* and *wingless* genes. Haplotype disc colours represent sites. Disc size reflects the number of samples, which are also noted in brackets under the corresponding Haplotype label. Hatch-marks on connecting lines represent the number of nucleotide substitutions. The black disc represents an inferred missing haplotype.

Pairwise F_{ST} analysis (Table 5.7) confirmed that there were no significant differences between the the sampling sites for the concatenation of the three genes.

Table 5.7 Pairwise F_{ST} obtained for concatenated *COI, EF-1α* and *wingless* sequences

	Bleaker	Frying Pan	Roy Cove	Sea Lion	Chile	
Bleaker	*	0.999	0.437	0.557	0.134	*p*-value for pairwise F_{ST}
Frying Pan	0.000	*	0.138	0.791	0.086	
Roy Cove	0.062	0.062	*	0.239	0.168	
Sea Lion	0.111	0.000	0.474	*	0.466	
Chile	0.127	0.120	0.187	0.000	*	
			pairwise F_{ST}			

The section below the diagonal shows pairwise F_{ST} values; the section above shows the *p*-value for each pairwise F_{ST} comparison. Negative scores are shown as 0.000. There were no significant ($p < 0.05$) pairwise comparisons.

5.4 Discussion

5.4.1 Genetic relationship between Falklands and Latin American populations

(i) **Findings**

The Falklands and Latin American populations were too close genetically to enable a clear distinction to be made between the two, with the most populated haplotype in each gene comprising specimens from both populations.

(ii) **Discussion**

The absence of clear genetic differentiation between the two populations suggested that the split of *Y. cytheris* into two subspecies was more an expression of geography than of biology (Mayr 1982, Phillimore and Owens 2006). There was, however, some evidence for divergence, in that, other than in the most common haplotype (85% of samples) none of the other haplotypes contained specimens from more than one population. The median joining networks all showed uniquely Latin American haplotypes as directly connected either to the most common haplotype for each gene, or to each other (in the case of *wingless* and the three concatenated genes, through inferred missing haplotypes). In no case was a uniquely Latin American haplotype connected directly to a uniquely Falkland Islands one.

The most common haplotype for each gene contained the two single specimens from Punta Arenas and Puerto Williams, the two nearest sites to the Falkland Islands. They are also upwind in the prevailing strong, westerly and southwesterly air flow (Figure 5.1), suggesting a climatic element to dispersal (Gatehouse 1997, Saastamoinen et al. 2018). Of the other Chilean specimens, that from Torres del Paine specimen was in a separate haplotype for each gene. The two unattributed Chilean specimens were also in their own respective haplotypes, other than for *COI*, where they were part of the largest haplotype*.* Perhaps less expectedly, given its geographic situation (1500 km to the north west), Santiago's two specimens were also in the most common haplotypes, one in *COI*, the other in *EF-1α* and *wingless.*

This is an area that requires further investigation. The Latin American analysis was based on single samples from a limited number of sites, and GenBank samples, with one exception, were only attributed at the country level. A wider range of Latin American samples should be collected and investigated, until which time it is necessary to be cautious about over-extrapolation.

5.4.2 Genetics of Falkland Island populations

(i) **Findings**

The Falkland Island specimens showed a variety of haplotypes: three in both *COI* and *EF-1α* and four in the concatenated genes. Only *wingless* showed a single haplotype in the Falklands.

(ii) **Discussion**

Phillimore and Owens (2006) observed that the level of genetic distinctness among subspecies was higher on islands than on continents. Mayr (1963) had noted that populations on islands encountered a physical impediment to gene flow to and from other populations, and therefore could diverge in isolation. Phillimore and Owen concluded that, as island populations tended to be smaller than those on continents, they might show more rapid fixation of genes.

There was some evidence of genetic variation between sites within the Falklands populations, which showed three haplotypes not found in Latin American samples. At the level of the three genes investigated, however, it was not possible to infer the nature of any relationships between Falkland Island sites. *wingless*, with its rapid rate of substitution in nymphalids (Cho et al. 1995, Brower and DeSalle 1998, Campbell et al. 2000), was particularly uninformative, with all Falklands samples belonging to a single haplotype.

The Roy Cove specimens were, in every case, members of the most common haplotype (Haplotype 1 in each gene, and in the concatenation). Other sites showed more than one haplotype in a number of cases but other than the most populated for each gene, only one haplotype contained samples from more than one site: Haplotype 2 in *COI*, comprising four samples from Bleaker and one from the Frying Pan. While four out of the 11 Bleaker specimens were from this haplotype, there was inadequate evidence to suggest an emerging Bleaker genotype. Sea Lion, also a small, isolated population, showed a separate haplotype in each of the three genes, but from a small sample size.

The Frying Pan population, with one specimen sharing a separate haplotype with Bleaker in *COI* and *wingless,* might have been augmented by Bleaker specimens blown in by the prevailing wind. If that was the case, a similar flow from Sea Lion to Bleaker might be expected, although no supporting evidence for this was found.

Overall, however, the picture was that of a prevalent haplotype, accounting for 85% of all samples, found in all the Falklands study sites. With the single exception of a Sea Lion haplotype in *EF-1α*, which differed by two substitutions, all other Falkland Island haplotypes only differed from the most common haplotype by a single substitution.

There was not enough evidence from the limited data to assess the genetic health of the Falklands population. The populations of *Y. cytheris* in the Falklands appeared small and scattered, and the butterfly itself was not mobile (Chapter 3). There is therefore a permanent risk of stochastic events causing local extinctions, whatever the genetic health of the butterfly.

5.4.3 Conservation implications

The existence of a widespread haplotype in the concatenated genes suggests that introductions or captive breeding, at least as far as genetics are concerned, can be considered. The most populated haplotype is found in all the Falkland sites, as well as in the Magallanes region of Chile, so there is a range of options in identifying potential donor populations. If the Falklands population can be considered an insular part of the Latin American population, conservation measures in cooperation with Chile and Argentina, such as augmentation and reintroduction, become feasible.

Anything other than tentative conclusions are hampered by the small sample sizes. Further work should begin with more non-lethal sampling, particularly of Latin American specimens, to allow a clearer picture of haplotype distribution, including the relationship of the Santiago population with that of the Magallanes region. This should be augmented by sampling museum specimens (Harper et al. 2006, Saarinen and Daniels 2012) to try to get a historical perspective, which would enable an understanding of the migratory processes.

While further work on *COI*, *EF-1α* and *wingless* genes might help elucidate the relationship between populations, to get a clearer picture of population relationships and history at a site level microsatellite analysis coupled with next generation sequencing will be necessary. There are challenges to genetic work on Lepidoptera. The difficulty in isolating microsatellites, and their low inter- and intra-specific transferability have been pointed out by e.g. Meglecz et al. (2004); Zhang (2004) and Vandewoestijne et al. (2012). Thus, although primers from related species (Lamas and Grados 2004, Simonsen 2006) exist, such as *Boloria acrocnema* (Monroe et al. 2016), *Boloria aquilonaris* (Vandewoestijne et al. 2012) and *Boloria eunomia* (Legrand et al. 2014), it is likely that many will resist amplification, and only a small proportion of those that do will show polymorphic sites.

Next generation sequencing offers a more promising approach through, for example, restriction site associated DNA genotyping, and analysis across the whole genome of single-nucleotide polymorphism markers (Saarinen and Austin 2010, Ekblom and Galindo 2011, Turlure et al. 2014, Richards and Murali 2015). When supported by comparable developments in statistical analysis, it should make questions such as the origins of and relationships between individual populations, and their viability in terms of inbreeding and loss of genetic diversity, more tractable (Selkoe and Toonen 2006; Frankham 2010).

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Chapter 6: Wings: function, shape and size

6.1 Introduction

Vallentin, in Boyson (1924), cited in the epigraph to this thesis, wondered how such an apparently delicate insect as *Yramea cytheris* could survive in such a windswept environment as the Falkland Islands This chapter, and the following chapter on claws, consider the extent to which shape is a factor.

This chapter considers the extent to which wing size and shape are driven by biomechanical responses to flight patterns. Differences between sexes and populations are examined through responses to behaviours (breeding, nectaring, dispersal, signalling and predation), and to the environment (elevation, latitude, temperature and wind speed).

Species, and populations, may reflect differing selection pressures (Shreeve et al. 2009). Le Roy et al (2019), in a study of the adaptive evolution of butterfly shape, pointed out that, even when correlations between wing shape and ecological factors could be established, the underlying selective processes were often unclear. Breuker et al. (2007), concluded that, to understand fully how factors such as dispersal were associated with wing shape, it would be necessary to identify *all* the different selection pressures in operation. An examination of the various factors shows how similar outcomes might be arrived at through a variety of routes. So shorter wings, with their weight nearer the body, might, for example, be a response to the need for manoeuvrability when faced with high levels of predation; the threat of being blown away by high winds in an island setting; or reduced larval development times at higher latitudes.

6.1.1 Relationship between wing shape and behavioural patterns

(i) **Breeding**

Sexual size dimorphism (SSD)

Teder and Tammaru (2005) found that, in over 80% of the insects they studied, females were bigger than males. This trait was confirmed in temperate zone butterfly species by Wiklund and Forsberg (1991) and Nève and Singer (2008). Two origins, not mutually exclusive, have been identified for sexual size dimorphism (SSD). Fecundity selection theory (Pincheira-Donoso and Hunt 2015) predicts a larger body for egg-carrying females. Protandry, in the sense of the eclosion of males before females, suggests shorter male larval developmental and pupal times resulting in smaller bodies (Fischer and Fiedler 2001, Nève and Singer 2008). As wing size in butterflies has been shown to be closely correlated with body size (Dudley 1990, Kingsolver 1999, Sullivan and Miller 2007) larger bodies would be predicted to have larger wings within a species.

Mating behaviour

Males generally have one of two approaches in seeking a mate: perching or patrolling (Shreeve 1987, Berwaerts et al. 2002, Dudley 2002). A perching male typically waits on vegetation, taking off to inspect either a potential mate, or to fight off another male moving into its territory (Wickman 1992). This requires acceleration, manoeuvrability and speed, associated with a low first moment of area ($\hat{r}_1$) (Dennis and Shreeve 1988) and low wing aspect ratios (*AR*) (Jugovic et al. 2018). A patrolling male requires a more sustained flight at a lower speed. This might predict a high *AR*, but little evidence for this has been found (Dudley 1990, Wickman 1992, Shreeve et al. 2009).

Oviposition

Female butterflies in search of oviposition sites use a number of flight modes, characterised by slow, deliberate flight and hovering (Dudley 1990, 2002). This is a similar pattern to patrolling in males, and is associated with similar morphological traits, including a high value of the radius of $\hat{r}_1$. Wings with high $\hat{r}_1$ values have their area distributed more distally than those with low $\hat{r}_1$ values. Wang et al. (2014) showed that an increased area of wing towards the wing tip led to more effective hovering.

(ii) **Nectaring and dispersal**

The relationship between wing shape and range is unclear. Sekar (2012), in a meta-analysis of traits affecting dispersal, concluded that wingspan was directly correlated with dispersal ability, although Dennis and Hardy (2018) observed that to understand dispersal, other metrics, such as aspect ratio and wing loading needed to be taken into account. Prolonged fast flight, under experimental conditions, was linked to high *AR* and high $\hat{r}_1$ in the Malaysian butterfly *Troides radamantus* (Betts and Wootton 1988). Li et al. (2016) found that migratory monarchs *Danaus plexippus* had longer wings than non-migratory, although with no significant difference in *AR*. Breuker et al. (2007) found that variation in forewing shape in *Melitaea cinxia* was associated with dispersal in females, which had a more rounded forewing, but not males; and that the sexes did not differ in dispersal rate despite sexual size and shape dimorphism. Against that, hoewever, Hill et al. (1999) found that colonising (as opposed to resident) specimens of *Pararge aegeria* tended towards wings with low *AR*, which might otherwise be linked with acceleration and manoeuvrabilty. The situation was further complicated by an underlying paradox identified by Singer and Hanski (2004): the fritillary *Euphydryas editha* had the physical capacity to fly long distances, but most individuals of most species failed to do so. A study by Viljur et al. (2019) of largely immobile butterflies on open land in Estonia recast the point: virtually all species in their landscapes represented, in their phrase, 'dispersal phenotypes'.

Turning from the characteristics of mobile butterflies to those of sedentary ones, it might be expected, following Darwin (1859) and MacArthur and Wilson (2001), that adaptation to an insular environment would lead to a loss of dispersal power, that is to say reduction in wing size (brachyptery), and possible flightlessness (aptery) (Heppner 1991). The evidence is mixed. Kotze (2008) found a significantly higher proportion of brachypterous carabid beetles on Baltic islands compared with the mainland; Roff (1990), however, found no significantly increased incidence of flightlessness in island populations, although observed an increase with elevation and latitude. In this context it should be noted that while flightlessness and brachyptery is known in moths, including in the tussac moth *Borkhausenia* in the Falklands (Bradley 1965, Jones and Lewington 2004), there appears to be only one genus of butterflies to exhibit it, in the female *Redonda*, at 3000m in the Venezuelan Andes (Viloria et al. 2003, 2015), in cold and windy conditions. There is some evidence of brachyptery at a local level in the populations of *Plebejus argus caernensis* and *Hipparchia semele thyone* on Great Orme's Head in North Wales, which, although now a headland, had long been an island (Dennis and Hardy 2018, Middlebrook et al. 2019).

(iii) **Signalling and mimicry**

Distinctive wing patterns can be driven by Batesian mimicry (Jones et al. 2013); Müllerian mimicry or aposematic colouration (Srygley 2007); or sexual signalling (Oliver et al. 2009). For maximum visibility, a large colour patch at the end of a wing could select for a broad wing shape, with a greater $\hat{r}_1$ (Outomuro et al. 2013) than would be predicted for a more muted, or camouflaged, wing. Syrgley (2007) further suggested that wing motion might also be a feature subject to mimicry, which would potentially have an impact on wing shape.

(iv) **Avoiding predation**

The ability to escape would be the last resort in the failure of a wing pattern either to act as camouflage or as a deterrent. Evidence from other taxa suggests that, for those unable to outpace a potential predator, high turning acceleration, linked with high $\hat{r}_1$, which can enable zigzagging, can be effective. Howland (1974) provided a model, explored further, in relation to moths evading bats, by Corcoran and Conner (2016). Experimental removal of butterfly hind wings resulted in a sharp reduction in linear and turning acceleration, suggesting that a high ratio of hind wing area to total wing area gave an advantage in evading predation (Jantzen and Eisner 2008). It also showed that the butterfly could fly without hind wings, but not without forewings.

6.1.2 Environmental responses

(i) **Elevation**

Sullivan and Miller (2007), investigating the relationship between body size and site elevation of macromoths in the Americas, observed that comparable studies of butterflies showed inconsistency, with size decreasing, increasing or switching erratically as elevation increased. They concluded that intraspecific variability in body size showed numerous ramifications, ambiguities and inconsistencies in study methods. Hawkins and DeVries (1996), investigating Costa Rican butterflies, found that Pieridae wings were smaller as elevation increased. Papilionidae, Nymphalidae and Riodinidae, however, showed some evidence, albeit irregular and not always statistically significant, of larger wings at greater elevations. Alves et al. (2016) found the Latin American fly *Polietina orbitalis* had broader wings below 900m, and narrower above. They suggested that the narrower wing could be an advantage at elevation because reduced air density could interfere with aerodynamic forces.

(ii) **Latitude**

Bergmann's rule (Bergmann 1847), that larger species within a genus are found in colder locations, has been viewed as having intra- as well as inter-specific applicability (Blackburn et al. 1999). Its applicability of the rule to ectotherms, however, is unclear (Partridge and Coyne 1997), and an inverse Bergmann's rule has also been observed (Ramírez-Delgado et al. 2016), with the suggestion that shorter season length, leaving reduced time for larval development, might lead to smaller adults (Mousseau 1997). The findings of Sanzana et al. (2013), that females of the Nymphalid *Auca coctei* in Chile were smaller at higher latitudes, gives support to an inverse Bergmann's rule applying to butterflies.

(iii) **Temperature**

Hassall (2015), investigating *AR* in damsel flies, found that higher wing *AR* was associated with colder regions as they generated more lift. The pattern is less clear with butterflies (Betts and Wootton, 1988), where lower *AR* has also been found at lower temperatures (Vandewoestijne and Van Dyck, 2011).

(iv) **Wind speed**

The impact of wind on butterfly wings has principally been studied in relation to flight performance (Srygley and Thomas 2002, Park et al. 2010, Ortega Ancel et al. 2017) and is also related the propensity to disperse (Singer and Hanski 2004, DeVries et al. 2010, Rossato et al. 2018). High winds on islands are a potential threat to populations (MacArthur and Wilson 2001), and, as noted in 6.1.1(ii), are likely to be an element in diminished wing size in island populations (Dennis and Shreeve 1989, Dennis and Hardy 2018).

6.1.3 Application to Y. cytheris

Y. cytheris's wing shape and size were explored for sexual dimorphism and differences between the Latin American and Falkland Island populations, with male wings predicted to be smaller than female (6.1.1 (i)), and Falkland butterflies, as island populations, expected to be smaller than Latin American (6.1.1 (ii)). Differences between Falkland Island populations were investigated to determine whether butterflies from windier sites had smaller wings (6.1.1 (ii)), or other attributes, such as a lower first moment of area ($\hat{r}_1$) or aspect ratio (*AR*) (6.1.4 (i)), which might benefit tenacity rather than dispersal ability. Differences in wings of Latin American populations were explored to assess any effects of latitude and elevation, which had produced varying responses in other taxa (6.1.2 (i) and (ii)).

6.1.4 Analytical approach

The basis of analysis was the planform, the two-dimensional projection of wing area (Dudley 1990). While further understanding might have been gained through body measurements, and particularly of thoracic weight (Chai and Srygley 1990, Dudley 1990, Shreeve et al. 2009), forewing length has been found to be an adequate proxy (Nylin and Svard 1991, Sullivan and Miller 2007, Sekar 2012), with the caveat that there is a slight allometric trend, with comparatively lighter bodies at the largest body sizes and relatively heavier ones at the shortest body sizes (García-Barros 2015) More widely, Dudley (1990), measuring 15 species of Panamanian butterfly, found that as body mass increased, so, isometrically, did wing span, wing length, body length, wing area, thoracic mass and wing mass, all significant at $p < 0.001$.

Two approaches were taken to analysis of the planform: traditional and geometric morphometrics. Traditional morphometrics, the analysis of measurements taken on a continuous scale, relies on the establishment of fixed points, or landmarks, as a basis for comparative measurement (Marcus 1990). Geometric morphometrics (Bookstein 1982), also landmark-based, is the analysis of shape, defined through the placement of landmarks, from which the element of size has been removed.

(i) **Traditional morphometrics**

While traditional morphometrics relies on the establishment of fixed points, the process is complicated by a lack of either clarity or agreement on what those points should be, and how other metrics should be derived from them. This can make comparisons of studies by different investigators difficult.

The measurements which underpin analyses in this chapter are wing length, width, base, area and angle. These are defined in relation to wing venation and described in the Methods section (6.2.5). Two metrics, aspect ratio (AR) and the first moment of area ($\hat{r}_1$) need more detailed explanation.

Aspect ratio AR

AR has historically been used in aeronautics as the primary indicator of wing efficiency (Raymer 1989), and defined as R^2 / S, where R is the wing span and S the wing area (Kermode et al. 2006, Breuker et al. 2007, Hassall 2015).

A variety of other formulas have been used. Le Roy et al. (2019) define AR as the ratio of wing span to mean wing width or chord, which would only produce the same result as R^2 / S if the wing was square. Others combine measurements from both forewings, following a more conventional definition of wing span: $4R^2/2S$ (Dudley 2002, Hamm et al. 2009, Cespedes et al. 2015); $4R^2/S$ (Betts and Wootton 1988, Lancaster and Downes 2017). These formulas do not include hind wings. Dudley (1990) suggested they should be incorporated in the formula $4R^2/S$, where S was the total area of all four wings. The difficulty with this is the use of combined area of forewing and overlapped hind wing. At a practical level, as butterflies, unlike moths, have no frenulum connecting the wings, it is too dependent on the way in which wings are viewed, or museum specimens mounted. In theoretical terms, it only makes sense if both wings have essentially a dipteral function in flight, which, although the wings move in phase through amplexiform coupling (Dudley 2002) is not the case.

In this chapter forewings and hind wings are analysed singly and separately, with the *AR* of each calculated as R^2 / S.

First moment of area $\hat{r}_1$,

AR has shortcomings in the description of insect wings, as it does not address the distribution of wing area along the wing's span (Betts and Wootton, 1988; Wang et al., 2014). This is a potentially important factor in behavioural flight strategies (6.1.1). Thus two imaginary triangular wings might have identical planforms, and therefore *AR*. As, however, they are attached by different points, they function differently, with the main area of the wing closer to the body in one, and closer to the tip in another (Figure 6.1)

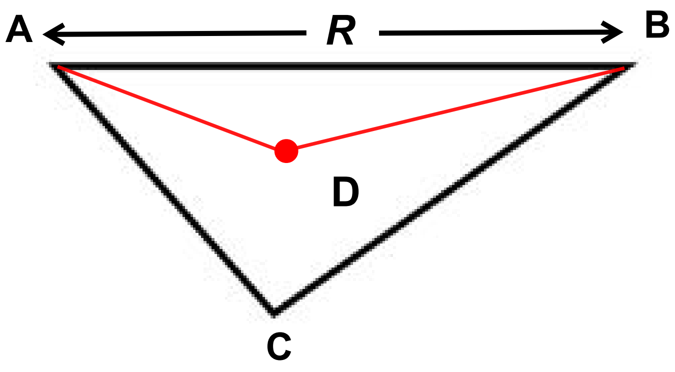

Figure 6.1 Aspect ratio (*AR*) compared with first moment of area ($\hat{r}_1$) of a wing. Triangle ABC represents two possible wing shapes, one attached to the thorax at A, the other at B. Point D represents the centroid and AD the wing length, *R*, of both shapes. The area of the triangle ABC is *S*. *AR*, R^2 / S, is the same for both wings. $\hat{r}_1$, of each wing is different. $\hat{r}_1$ is calculated as the distance from the attachment point to the centroid, divided by *R*. Thus, for a wing attached at A, $\hat{r}_1$ is AD/*R*, and, for a wing attached at B, BD/*R*. A wing with its centre of area closer to the body therefore has an $\hat{r}_1$ smaller than a wing with its centre of area further away.

$\hat{r}_1$ is calculated as the distance between the proximal end of the wing and the spanwise position of the centroid (Ellington 1984, Betts and Wootton 1988, Dudley 1990, Wang et al. 2014). It is a measure of the concentration of wing area away from the thorax: thus two wings of identical shape and size but attached to the thorax by different ends of the main wing length would have the same *AR*, but a different $\hat{r}_1$. Thus, depending on the point of attachment, in Figure 6.1 it is either AD/*R* or BD/*R*. Le Roy et al. (2019) use the second moment of area, $\hat{r}_2$, following Ellington (1984), for the same purpose. In this chapter $\hat{r}_1$ is used.

Relationship between forewings and hind wings

While considering forewings and hind wings as having a dipteral function is conceptually challenging, Dudley's (2002) characterisation of the wings moving in phase through amplexiform coupling is a reminder that forewings and hind wings neither operate, nor should therefore be analysed, in isolation. Each wing has a different function, therefore it is possible that the impact of behaviour and the environment may lead to differences in the size relationship between the two.

(ii) **Geometric morphometrics**

Geometric morphometrics, through landmarks calculated as Cartesian coordinates, enables the analysis of shape at a finer scale than traditional morphometrics, providing a quantitative method for making comparisons (Zelditch et al. 2012). It has a particular value in visualising differences between samples through principal component analysis and the application of Procrustes distances (the square root of squared distances between the corresponding landmarks of two shapes), and between groupings through canonical variate analysis and the application of Mahalanobis distances (the squared distance between the means of pre-allocated groups divided by pooled variance/covariance matrices) (Klingenberg and McIntyre 1998, Klingenberg and Monteiro 2005).

6.1.5 Research questions

RQ1: to what extent do *Y. cytheris* wings vary with sex, land mass or site?
RQ2: to what extent can either behavioural or environmental factors explain any observed differences?

6.2 Materials and methods

6.2.1 Ethics statement

Research work in the Falklands was carried out under Licence R19/2015 from the Falkland Islands Government. *Y. cytheris* is a protected species in the Falkland Islands (Falkland Islands Government 1999) and lethal collection of specimens was limited by quota. In all cases research took place with the permission of land owners.

6.2.2 Data set

In addition to specimens collected in the Falkland Islands, data were collected from specimens in the collections of the British Museum of Natural History Museum, London (BMNH), and of the Oxford University Museum of Natural History (OUMNH). Alvaro Zúñiga-Reinoso also provided five Chilean specimens from his collection. The data set comprised 46 male and 53 female specimens, both forewing and hind wing, from the Falklands, and 30 female and 47 male forewings, together with 13 female and 26 male hind wings, from Latin America (Table 6.1). A more detailed table, giving sources of material, is at Appendix Table C.1).

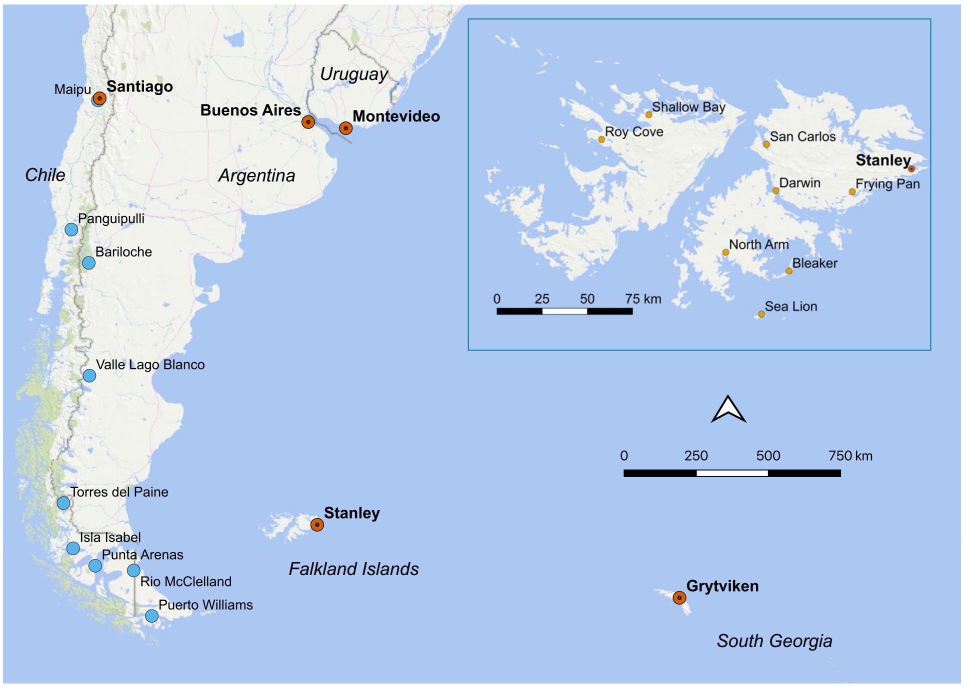

Figure 6.2 Sites of origin of wing samples. Falkland Island sites are shown on the inset map. Country and British Overseas Territory names are in italic font, principal settlements and capital cities are in bold.

Table 6.1 Sites of origin of wing data by sex and wing pair. Falkland Island data came from specimens collected during the 2016-2018 field visits, as well as from specimens in BMNH and OUMNH. Latin American data come from specimens in BMNH, OUMNH and the collection of Alvaro Zúñiga-Reinoso.

land mass	site	latitude	longitude	elevation (m)	forewing		hind wing	
					F	M	F	M
FK	Bleaker	-52.2101	-58.8518	5	3	4	4	4
FK	Darwin	-51.8069	-58.9592	0	5	12	5	12
FK	Frying Pan	-51.8118	-58.3350	7	3	4	3	4
FK	North Arm	-52.1165	-59.3689	12	5	5	5	4
FK	Roy Cove	-51.5484	-60.3832	20	8	9	7	10
FK	San Carlos	-51.5730	-59.0350	19	3	1	3	1
FK	Sea Lion	-52.4250	-59.0767	14	2	2	2	2
FK	Shallow Bay	-51.4230	-59.9980	19	17	16	17	16
LA	Bariloche, Chile	-41.1335	-71.3103	893	0	2	0	0
LA	Chubut, Argentina	-45.9340	-71.2731	604	9	9	5	4
LA	Isla Isabel, Chile	-52.5500	-72.2330	47	2	0	2	0
LA	Panguipulli, Chile	-39.6416	-72.3370	130	4	0	5	0
LA	Puerto Williams, Chile	-54.9000	-67.6000	30	0	0	1	1
LA	Punta Arenas, Chile	-53.1667	-70.9333	34	5	5	5	4
LA	Rio McClelland, Chile	-53.3330	-68.6670	50	8	24	1	14
LA	Santiago, Chile	-33.5500	-70.7700	538	2	0	2	0
LA	Torres del Paine, Chile	-50.9000	-72.8000	175	0	1	0	1

FK = Falkland Islands, LA = Latin America

6.2.3 Image acquisition

Wings from butterflies caught in the field were photographed between microscope slides, cemented together to mitigate against possible measurement error due to wing curvature or angle. Museum specimens were photographed individually, from both sides, on their original pins (a requirement of the loans). They were positioned horizontally by eye using the lens's depth of field at wide aperture for critical adjustment. All photographs included a scale, positioned at the same focal distance as the wing. Images were recorded using a Nikon D800 single lens reflex camera and Nikon 105mm AF Micro Nikkor lens and stored as lossless TIFF files.

An additional set of forewing dorsal images of Latin American *Y. cytheris* was supplied by Geoff Martin of the BMNH. These had been photographed using the SatScan tray scanner system (Blagoderov et al. 2010), a method that Johnson et al (2013) had found to be as accurate as photographing pinned specimens. They were calibrated against corresponding images taken under preceding protocols. A limitation of the whole tray scan was that hind wings were partially covered by forewings in setting and therefore could provide no relevant data. Further details of image acquisition and handling are in Appendix C.2

6.2.4 Image preparation for traditional morphometrics

(i) **Wing morphology and landmark setting**

Forewings

Three landmarks were established on the forewings to enable measurement of length, width, base and forewing angle (Figure 6.3). These were (using the letters in the figure):
A: the proximal end of the anal 1 vein;
B: the distal end of the radius 4 vein;
C: the distal end of the anal 1 vein.

Landmarks B and C conformed with Bookstein's (1990, 1991) main criterion for the optimal, type 1, landmark, the discrete juxtaposition of tissues. Both points were situated where the vein met the outer edge of the wing. Landmark A was more problematic. To enable a length measurement of the wing to be taken, a landmark was necessary where the wing joined the thorax. Although such a landmark would have met Bookstein's criteria, the difficulty was that there was no junction between the proximal ends of the anal, medial/cubital and radial veins which would have given a clearly visible reference point. The proximal end of the anal 2 vein was selected as the best approximation.

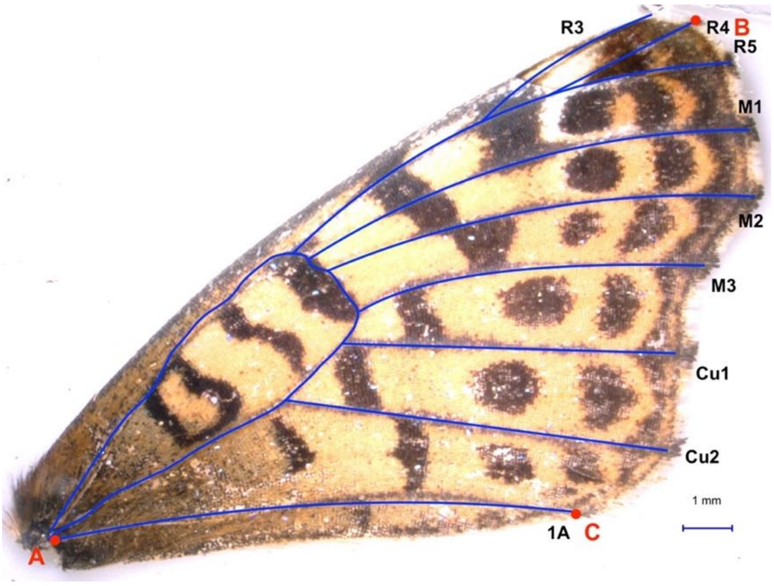

Figure 6.3 The forewing of *Yramea cytheris* showing venation and landmarks used for traditional morphometric measurements. The alphanumerics refer to the vein's position in the wing's wider structure: R = radius; M = media; Cu = cubitus; A = anal. The larger letters A, B and C, in red, show the landmarks selected as a basis for measurements in the traditional morphometric analysis. Photograph by the author.

Hind wings

Three major landmarks were established on the hind wings to enable measurement of length, width, base and hind wing angle (Figure 6.4). These were (using the letters in the figure):

A: the discal cell junction of the cubital and radial structures;
B: the distal end of the subcostal and radius 1 vein;
C: the distal end of the anal 1 + 2 vein.

All three landmarks represented the discrete juxtaposition of tissues which corresponded with Bookstein's (1990, 1991) optimal type 1 landmark. The discal cell junction of the cubital and radial structures provided the clear proximal landmark lacking in the forewing.

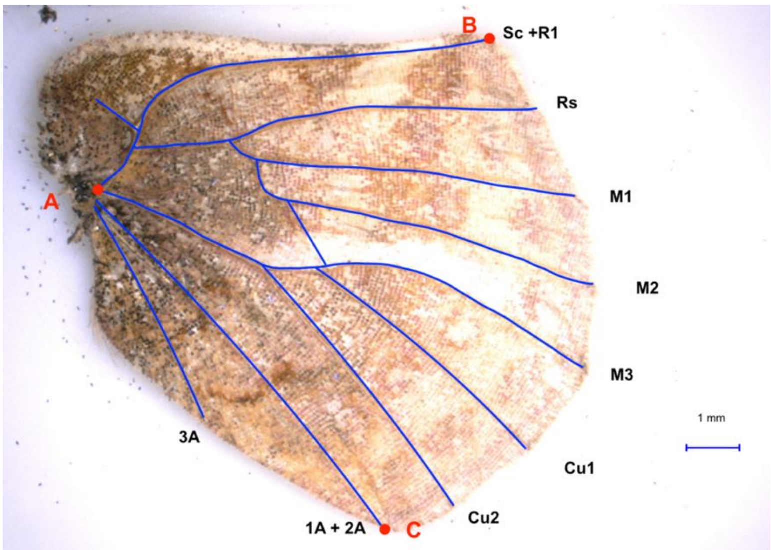

Figure 6.4 The hind wing of a male *Yramea cytheris* showing venation and landmarks for traditional morphometrics. The alphanumerics refer to the vein's position in the wing's wider structure: Sc = subcosta; R = radius; M = media; Cu = cubitus; A = anal. The letters in red show the landmarks used in the traditional morphometric analysis. Photograph by the author.

6.2.5 Wing measurements

(i) **Measurements: size**

All straight line measurements were made with the straight line tool in FIJI (Schindelin et al. 2012), with the scale recalibrated for each image.

Wing length (R)

Forewing length *R* was measured from the proximal end of the anal 1 vein to the distal end of radial 4 (A and B in Figure 6.3). Hind wing length *R* was measured from the proximal end of the combined anal 1 and 2 vein, at its intersection with the disc, to the distal end of the combined subcosta and radial 1 (A and B in Figure 6.4).

Wing area (S)

Wing area was computed in FIJI. The freehand selection tool was used to produce an outline of the wing, with the Measurement tool set to calculate area automatically. The outcome was used in the calculation of the wing's *AR.*

Wing width

Forewing width was measured as the distance between the distal end of the radial 4 vein and the distal end of the anal 1, effectively the farthest points from the thorax on the wing's leading and lower trailing edges (B and C in Figure 6.3). Hind wing width was measured as the distance between the distal end of the combined subcosta and radial 1 vein and the distal end of the combined anal 1 and 2 vein, effectively the farthest points from the thorax on the wing's leading and lower trailing edges (B and C in Figure 6.4).

Wing base

Wing base was measured as the length of the anal 1 vein for the forewing, and anal 1+2 for the hind wing (A and C in Figure 6.3 and Figure 6.4)

Length as a proxy for size

Dudley's (1990) finding of a relationship between wing span, wing length and wing area, discussed at 6.1.4, was tested for both forewings and hind wings of *Y.cytheris* through correlation tests appropriate to the data. Wing base was also considered for both wings.

(ii) **Wing angle**

The angle between the base and the length of both forewing and hind wing was measured as a potential index of shape in its own right.

(iii) **Aspect ratio (*AR*)**

Aspect ratio was measured as R^2/S

(iv) **First moment of area ($\hat{r}_1$)**

$\hat{r}_1$ was calculated as the distance between the proximal end of the wing and the centroid, divided by *R.* The position of the centroid was calculated automatically using the measurement tool in FIJI as part of the wing area calculation.

(v) **The relationship between forewings and hind wings**

The proportion of total wing area made up by the hind wing was assessed for sex and land mass, whether Falkland Islands or Latin America. This was followed up by an examination of correlation between forewing and hind wing lengths.

6.2.6 Image preparation for geometric morphometrics

(i) **Image processing**

TIFF files were initially processed in the TPS suite of software (Rohlf 2015). They were first read into a TPS file in tpsUtil 1.70x64. The resulting files were transferred to tpsDig ver.2.26, where the scale was set in accordance with the measurement scale incorporated in the images.

(ii) **Wing structure and landmark selection**

Sets of landmarks were digitised on both forewings and hind wings (Figure 6.5 and Figure 6.6, Table 6.2). Sites selected were either the intersections of veins, or the points where a vein met the edge of a wing. Both conform with Bookstein's (1991, cited in Zelditch et al., 2012) type 1 landmarks, those derived from the discrete juxtaposition of tissues.

Forewing landmarks were generally registered on the dorsal surface. Hind wing venation was less clearly visible on the dorsal surface of hind wings, in which cases the ventral surface was used, with the dorsal only used when landmarks were obscured, for example by legs. If necessary, the image was adjusted using the left/right flip in tpsDig to ensure all images were oriented in the same way.

The difficulty of identifying a type 1 landmark at the proximal end of the forewing was considered at 6.2.4(i). A number of geometric morphometric studies have, despite the difficulties, used as landmarks the points at which the radial or cubital veins join the thorax (Benítez, 2013; Sanzana et al., 2013; Juhász et al., 2016; Zhong et al., 2016). While a landmark at the proximal end of the forewing was important for traditional morphometrics, it is arguably less so for geometric morphometrics as wing shape, in particular the relationship between the disc and the outer edges of the forewing, can be analysed using the type 1 landmarks.

The problem does not arise with hindwings, where there is a clear junction on the disc between the cubital and radial vein structures (Figure 6.6: landmark 7).

Figure 6.5 Ventral side of the left forewing of *Yramea cytheris* showing landmarks for geometric morphometric analysis. The proximal ends of the anal, medial/cubital and radial veins, which do not provide a robust type 1 landmark, are circled. Photograph by the author.

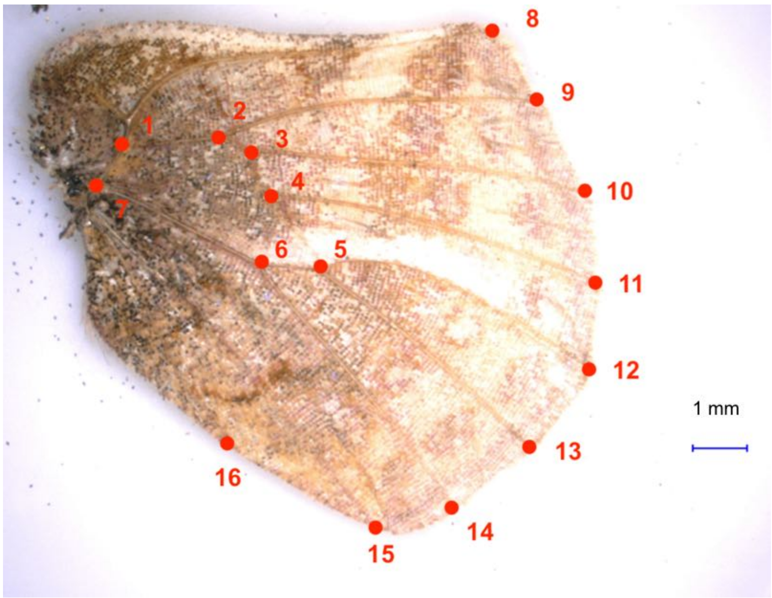

Figure 6.6 Ventral side of the left hindwing of *Yramea cytheris* showing landmarks digitised for geometric morphometric analysis. Photograph by the author.

Table 6.2 Landmarks of forewings and hindwings, following the Comstock - Needham system.

forewing		hind wing	
No.	Location	No.	Location
1	disc - radius (3-5) junction	1	disc - subcosta / radius 1 junction
2	disc - medius 2 junction	2	disc - radial sector junction
3	disc - medius 3 junction	3	disc - medius 1 junction
4	disc - cubitus 1 junction	4	disc - medius 2 junction
5	disc - cubitus 2 junction	5	disc - cubitus 1 junction
6	radius 4 - radius 5 junction	6	disc - cubitus 2 junction
7	distal end of radius 3	7	discal junction of cubital and radial structures
8	distal end of radius 4	8	distal end of subcosta / radius 1
9	distal end of radius 5	9	distal end of radial sector
10	distal end of medius 1	10	distal end of medius 1
11	distal end of medius 2	11	distal end of medius 2
12	distal end of medius 3	12	distal end of medius 3
13	distal end of cubitus 1	13	distal end of cubitus 1
14	distal end of cubitus 2	14	distal end of cubitus 2
15	distal end of anal 1	15	distal end of anal 2
		16	distal end of anal 3

Numbers refer to landmarks shown at Figure 6.5 (forewing) and Figure 6.6 (hind wing)

TPS files were read into MorphoJ 1.06d (Klingenberg 2011). The landmark coordinates were then subjected to Procrustes superimposition (Klingenberg and McIntyre 1998). With all images oriented in the same direction, this process involves scaling images to unit centroid size, which removes variation due to scale and position. Centroids of all images are then superimposed upon each other, then those images are rotated around their centroid to produce the optimal fit.

(iii) **Warped outline drawings**

Shape changes are visualised in warped outline drawings. As Klingenberg (2013) noted, human perception requires a shape as the context for making sense of shape change. Shape changes, moreover, are only interpretable in the context of the structure which holds them, and in conjuction with the shape of that structure.

An outline file was made from a wing image with its landmarks marked. Lines were constructed using a series of intermediate landmarks to delineate wing outlines and the major veins. The file was then imported into MorphoJ as a basis for warped outline drawings, which show initial and target shapes of a shape variation using the information provided by the landmarks.

The theoretical basis for the warped outline drawing is the thin-plate spline (Bookstein 1989). The frequent usage of a deformation grid, based on the spline, has not been followed here. The grid has underpinned much morphometric work since it was devised (as a transformation grid) by Thompson (1917). It is, however, less relatable to an overall wing structure than a warped outline.

It should be noted that the warped outline drawing is an aid to visualisation only. While the original file follows a semi-landmark structure, none of the subsequent images does. Therefore, while the landmarks carry biological information, the warped outline drawings (as is also true of deformation grids) do not.

(iv) **PCAs and CVAs**

The main tool for distinguishing between individual specimens was the PCA. Separation was measured through Procrustes distances, with α set at 0.05. Predetermined groupings were analysed through CVAs using both Procrustes and Mahalanobis distances, with α for the latter set at 0.001 (Tabachnick and Fidell 2013). *p*-values for both distances were derived from 10,000 round permutation tests.

PCs 1 and 2, and CVs 1 and 2, and their distributions, are shown as Figures in the results section. Other PCs and CVs contributing over 8 % to the overall analysis, and their distributions, are shown in Appendix D.

6.2.7 Approach to RQs

The approach to RQs 1 (6.1.5) utilised traditional and geometric morphometrics from the whole data set.

The approach to RQ2 was dependent on RQ1, as it had to take into account any significant differences between female and male samples, as well as between those from the Falkland Islands and Latin America.

Comparison of latitude and elevation was based on Latin American samples. These came from a range of latitudes from -54.9º to -33.5º, and elevations from 30m to 893m, whereas the Falklands range for latitude was only -52.4º to -51.4º, and, for elevation, sea level to 20m.

Elevation and latitude for the locations cited for Latin American samples were read from Bing Terrain maps at 1:1,000,000 accessed through XYZ tiles in QGIS 3.4 (QGIS Development Team, 2018). Wind speed was more complex, as butterfly habitats are potentially more sensitive to local geography, taking into account the effects of slope, aspect and shelter in relation to the prevailing wind (4.3.1). Data from Latin American meteorological stations were therefore not used, as being too broad-brush. Analysis of Falkland Island samples was based on data from the four study sites, using wind speeds at 30cm above the ground, an observed approximation to the butterfly's habitual patrolling height (4.2.3 (i)).

6.2.8 Data analysis

TIFF images for angle and chord measurement were processed in *ImageJ* using the *FIJI* platform (Schindelin et al. 2012, Schneider et al. 2012), with the aid of the macro *bissect* [*sic*] (Burri 2016).

TIFF images for geometric morphometrics were processed in the *tps* software suite (Rohlf 2010, 2015). Initial grouping and importing were done in *tpsUtil*. *tpsDig2* was then used to produce landmark files. Subsequent processing of landmark files through Procrustes fits (Mitteroecker and Gunz 2009), the production of covariance matrices, PCAs and CVAs was undertaken in *MorphoJ* (Klingenberg 2011, 2013).

The majority of calculations were done in *R* (R Core Team 2018). The *tidyverse* package, in particular *ggplot2* and *dplyer* (Wickham 2017), provided the principal resource for visualisations, supplemented through *github* (Makiyama 2018) and *ggfortify* (Tang and Horikoshi 2016). ANOVAs, t-tests, correlations and regressions were carried out using elements of *car* (Fox and Weisberg 2018); *corrplot* (Wei and Simko 2017); *effsize* (Torchiano 2017); *EnvStats* (Millard 2013); *Hmisc* (Harrell 2019); *lmerTest* (Kuznetsova et al. 2017); *lsr* (Navarro 2015); *multcomp* and *PerformanceAnalytics* (Hothorn et al. 2016); and *Psych* (Revelle 2018).

All mapping was done in *QGIS 3.4* (QGIS Development Team 2018).

6.3 Results

6.3.1 Forewings: traditional morphometrics

(i) **Comparison of forewing length, width, base and area**

Kolmogorov-Smirnov tests supported the assumption of normal distribution for the three direct measurements (length, width, base) as well as area. Pearson's product-moment correlation test was used to assess the relationship between forewing length and each of the other measurements to determine the extent to which it could be used as a proxy for size. The tests showed a very strong positive association between: length and width ($r = 0.95$, $p < 0.001$); length and base ($r = 0.95$, $p < 0.001$); and length and area ($r = 0.98$, $p < 0.001$). Accordingly, length was used as a proxy for forewing size.

(ii) **Forewing length**

A two-way ANOVA (Appendix Table C.2) showed that both land mass and sex were highly significant factors in forewing length, with a large effect size. The interaction between sex and land mass was not significant. Visualisation of forewing length by land mass and sex (Figure 6.7), together with post-hoc *t*-tests (Table 6.3), showed that Falkland Island forewings were significantly shorter than Latin American, and that female forewings were significantly longer than male, both with a very large effect size.

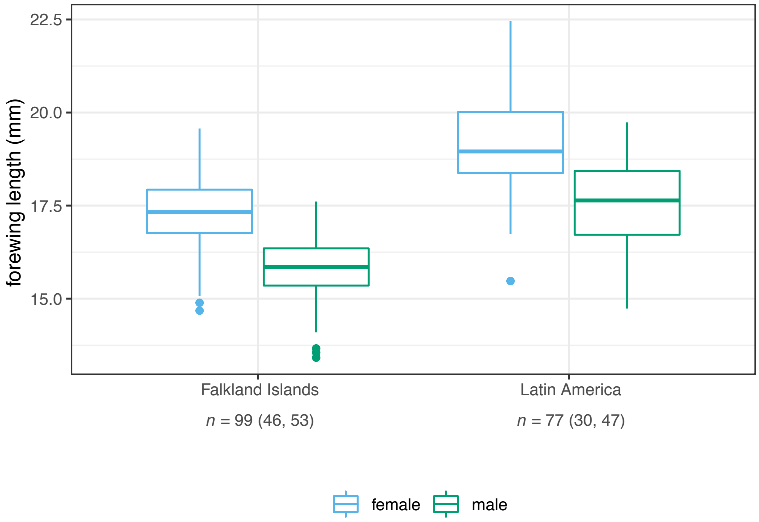

Figure 6.7 Forewing length compared by land mass and sex. Numbers in brackets refer to female and male forewings respectively.

Table 6.3 *t*-test comparisons of forewing lengths by land mass by sex.

factor	*n*	*M*	*SD*	*df*	*t*	*p*-value	95% CI	*d*
FK: F	46	17.30	1.11	97	7.78	**<0.001**	1.16, 1.96	**1.57**
FK: M	53	15.74	0.88					
LA: F	30	19.15	1.58	75	5.40	**<0.001**	1.09, 2.37	**1.26**
LA: M	47	17.41	1.23					
FK: F	46	17.30	1.11	74	-5.98	**<0.001**	-2.46, -1.23	**1.40**
LA: F	30	19.15	1.58					
FK: M	53	15.74	0.88	98	-7.84	**<0.001**	-2.09, -1.25	**1.57**
LA: M	47	17.41	1.23					

factors: FK = Falkland Islands, LA = Latin America; F = female, M = male. Significance at $p < 0.05$ and large effect size at $d > 0.8$ are shown in bold.

(iii) **Forewing angle**

A two-way ANOVA (Appendix Table C.3) showed that land mass was a significant factor in forewing angle, although with a small to medium effect size. Sex and the interaction between sex and land mass were not significant. Visualisation of forewing angle by land mass and sex (Figure 6.8), together with post-hoc *t*-tests (Table 6.4), showed that, in both females and males, Falkland Island forewing angles were significantly smaller than Latin American.

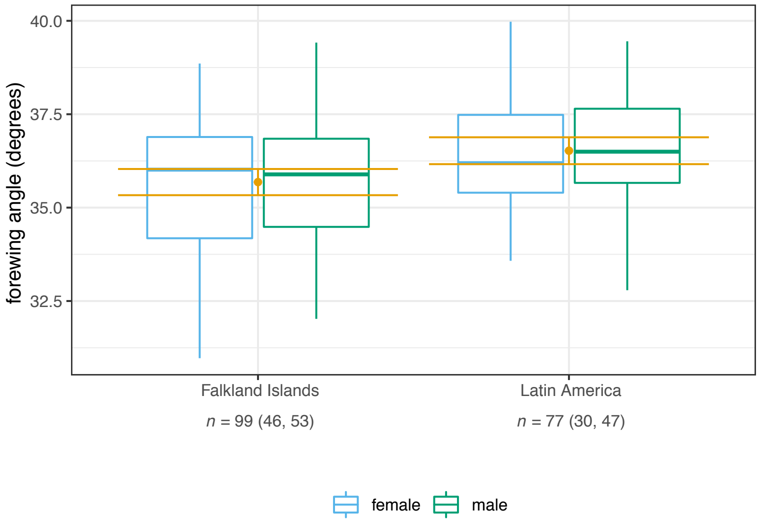

Figure 6.8 Forewing angles compared by land mass and sex. 95% confidence intervals for the means of the two populations, with sex pooled, were added (orange bars). Numbers in brackets refer to female and male forewings respectively.

Table 6.4 *t*-test comparisons of forewing angles by land mass and sex.

factor	*n*	*M*	*SD*	*df*	*t*	*p*-value	95% CI	*d*
FK: F	46	35.64	1.81	74	-2.08	**0.041**	-1.72, -0.04	0.49
LA: F	30	36.52	1.77					
FK: M	53	35.72	1.72	98	-2.49	**0.014**	-1.44, -0.16	0.50
LA: M	47	36.53	1.47					

factors: FK = Falkland Islands, LA = Latin America; F = female, M = male. Significance at < 0.05 is shown in bold.

(iv) **Forewing aspect ratio (*AR*)**

A two-way ANOVA (Appendix Table C.4) showed that land mass was a significant factor in forewing *AR*, with a medium effect size. Sex and the interaction between sex and land mass were not significant. Visualisation of forewing *AR* by land mass and sex (Figure 6.9), together with post-hoc *t*-tests, showed that forewings of Falkland Island specimens of both sexes had larger *AR* (for a given area, they were longer and narrower) than their Latin American counterparts (Table 6.5).

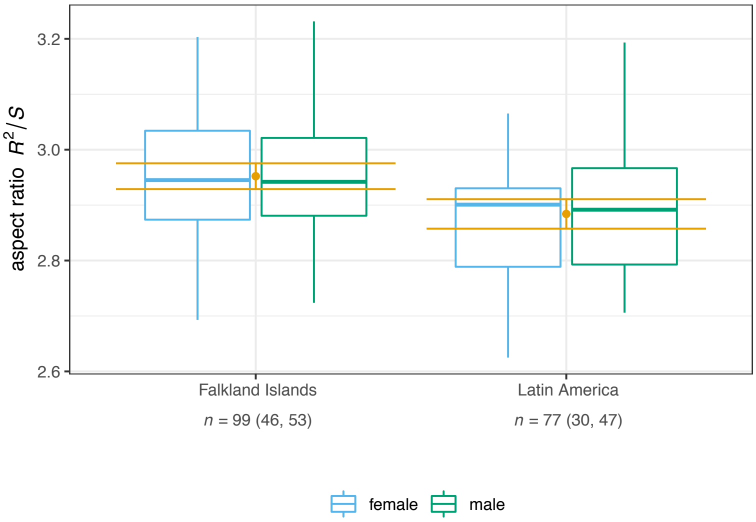

Figure 6.9. Forewing *AR* compared by land mass and sex. 95% confidence intervals for the means of the two populations, with sex pooled, were added (orange bars). Numbers in brackets refer to female and male forewings respectively.

Table 6.5 *t*-test comparisons of forewing *AR* by land mass and sex.

factor	*n*	*M*	*SD*	*df*	*t*	*p*-value	95% CI	*d*
FK: F	46	2.95	0.11	74	2.70	**0.009**	0.02, 0.13	0.63
LA: F	30	2.87	0.11					
FK: M	53	2.96	0.11	98	2.79	**0.006**	0.02, 0.11	0.56
LA: M	47	2.89	0.12					

factors: FK = Falkland Islands, LA = Latin America; F = female, M = male. Significance at $p < 0.05$ is shown in bold.

Correlation between angle and AR

The correlation between angle and *AR* was tested, given that, with the close correlation of length, width, base and area, they should be testing the same aspect of wing area. Pearson's product moment showed that *AR* was strongly negatively correlated with forewing angle $p < 0.001$, 95% CI [-0.78, -0.64], $r = -0.72$. Angle was therefore not pursued further as a metric.

(v) **Forewing first moment of area ($\hat{r}_1$)**

A two-way ANOVA (Appendix Table C.5) showed that both land mass (Falkland Islands or Latin America) and sex were highly significant factors in forewing $\hat{r}_1$, but with only a small effect size. The interaction between sex and land mass was not significant.

Visualisation of forewing $\hat{r}_1$ by land mass and sex (Figure 6.10), together with post-hoc *t*-tests (Table 6.6), showed that it varied with a high degree of significance and a large effect size between Falkland Island and Latin American males and also between Falkland Island females and males; and significantly, with a medium effect size, between Latin American females and males. The only contrast not significant at $p < 0.05$ was between Falkland Island and Latin American females.

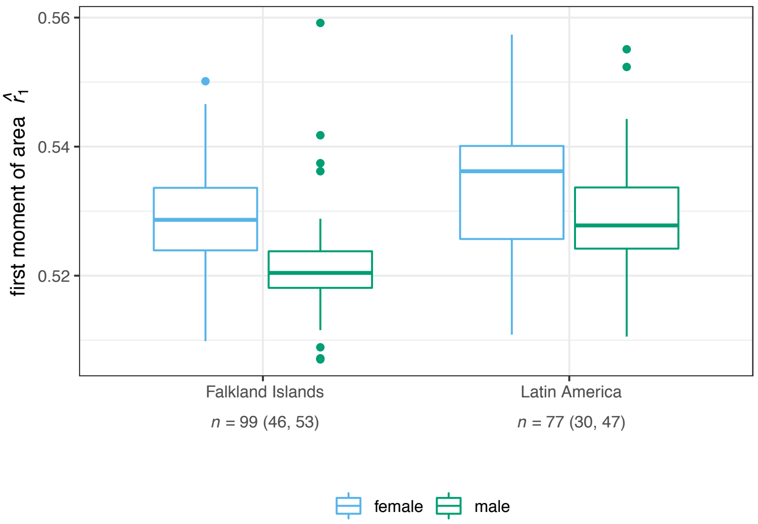

Figure 6.10. Forewing $\hat{r}_1$ compared by land mass and sex. Numbers in brackets refer to female and male forewings respectively.

Table 6.6 *t*-test comparisons of $\hat{r}_1$ by land mass by sex.

factor	*n*	*M*	*SD*	*df*	*t*	*p*-value	95% CI	*d*
FK: F	46	0.529	0.008	97	4.67	**<0.001**	0.005, 0.011	**0.94**
FK: M	53	0.521	0.009					
LA: F	30	0.533	0.011	75	2.03	**0.046**	<0.001, 0.009	0.47
LA: M	47	0.529	0.009					
FK: F	46	0.529	0.008	74	-1.82	0.072	-0.008, <0.001	0.42
LA: F	30	0.533	0.011					
FK: M	53	0.521	0.009	98	-4.20	**<0.001**	-0.01, -0.004	**0.84**
LA: M	47	0.529	0.009					

factors: FK = Falkland Islands, LA = Latin America; F = female, M = male. *p*-values significant at < 0.05, and large effect sizes at *d* > 0.8, are shown in bold font.

6.3.2 Forewings: geometric morphometrics

(i) **Principal Component Analysis (PCA) of forewings**

A PCA of the forewings showed that the first two PCs contributed 41% to the analysis (Figure 6.11). The target shape of PC1 (25.7%) had a narrower wing, as between the distal ends of the veins radius 4 and anal 1A, but with longer medials 1, 2 and 3 and cubitals 1 and 2. There was a distinctive scalloping in the target shape linking the distal ends of medial 2, medial 3 and cubital 1. PC2 (15.3%) had longer cubitals in the starting shape, with no distinctive angle in the target shape.

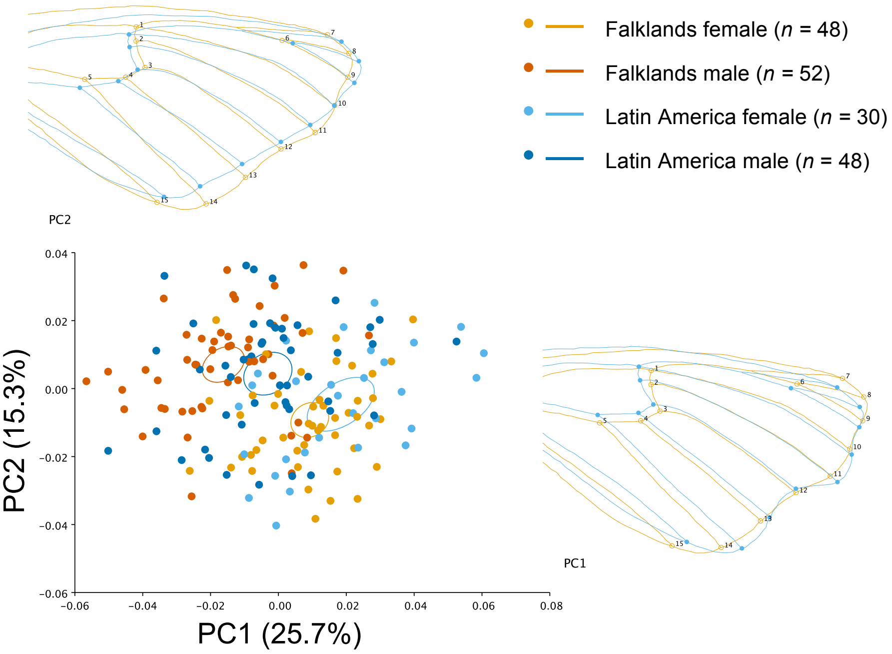

Figure 6.11 PCA of forewings by land mass and sex. 95% confidence ellipses of means are shown in the colour of their respective combination of the two. The warped outlines show, in blue, the target shape of the PC on each axis, compared with the mean shape, in orange. The first two PCs contributed 41% to the analysis.

There was clustering along each of the first two PCs. Females tended towards the target shape on PC1, and away from it on PC2, all in the direction of a narrower and more scalloped wing. Latin American wings, particularly female,tended towards the target shape on PC1.

(ii) **Canonical Variate Analysis (CVA) of forewings**

A CVA of the forewings showed that two CVs contributed 93.8% to the analysis (Figure 6.12). The target shape of CV1 (60.4%) showed a narrower wing, with distinctive scalloping, while that of CV2 (33.4%) was broader, with an overall compression of landmarks around the discal cell (landmarks 1 - 5). When plotted against CVs there was a clear separation of the four groups about the origin. Female forewings tended towards the target shape of CV1, with male forewings tending away from it; Falkland forewings tended away from the target shape of CV2, with Latin American forewings tending towards it.

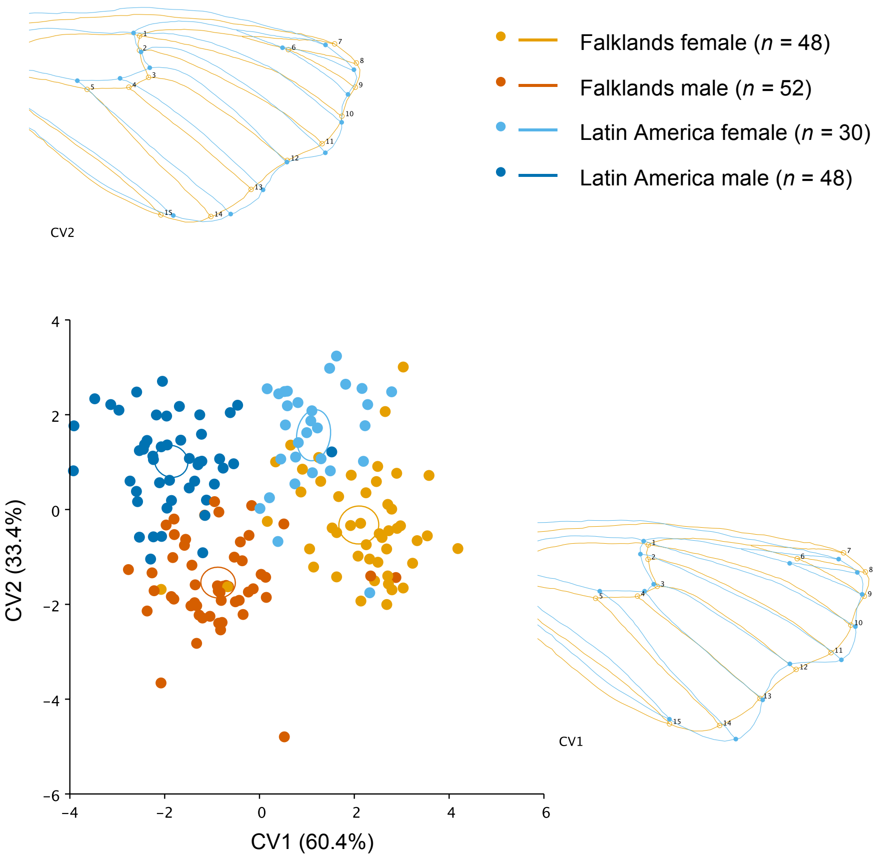

Figure 6.12 CVA of forewings by land mass and sex. 95% confidence ellipses of means are shown in the colour of their respective combination of the two. The warped outlines show, in blue, the target shape of the CV on each axis, compared with the mean shape, in orange. The first two CVs contributed 93.8% to the analysis.

Both Mahalanobis and Procrustes distances (Appendix Table C.10) showed a high significance, at $p \leq 0.0001$, between each contrast.

6.3.3 Forewing contrasts at site level

(i) **Falkland Island forewings**

Length

Analysis by one-way ANOVA of the response of forewing length to site showed a significant effect for Falkland Island males ($F\,(7,\ 45) = 2.98$, $p = 0.012$), with a large effect size ($\eta_p^2 = 0.316$). The effect was not significant for females (*F* (7, 38) = 1.09, *p* = 0.391).

Visualisation (Figure 6.13), together with post-hoc comparisons using the Tukey HSD test, showed that Bleaker male forewings (*M* = 14.4, *SD* = 1.11) were significantly shorter than those from Darwin (*M* = 16.1, *SD* = 0.52), *p* = 0.016 and Shallow Bay (*M* = 15.9, *SD* = 0.74), *p* = 0.035.

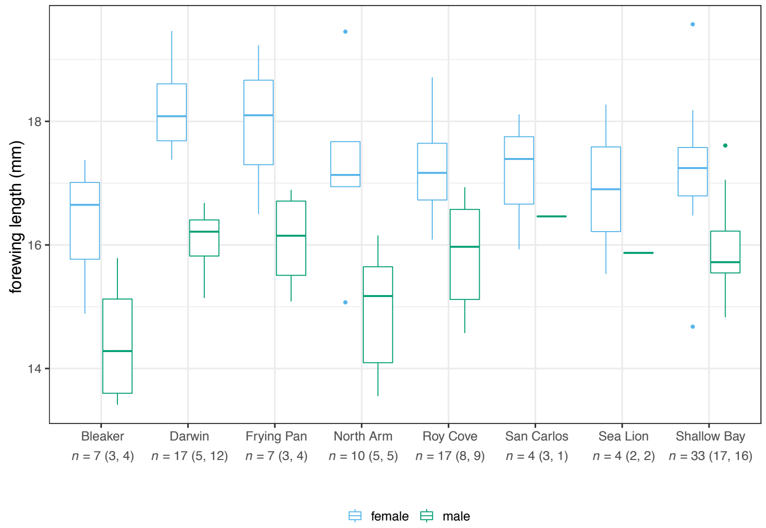

Figure 6.13 Comparison between forewing lengths at Falkland Islands sites. Numbers in brackets refer to female and male forewings respectively.

Aspect ratio (AR)

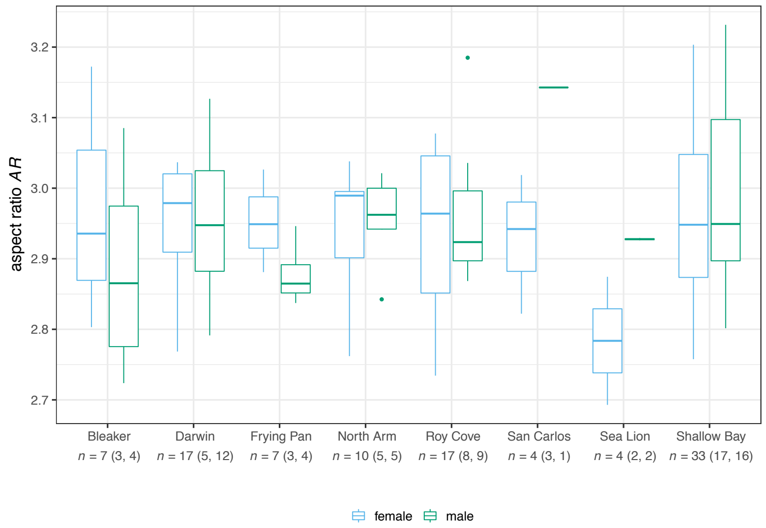

Figure 6.14 Comparison between forewing *AR* at Falkland Islands sites. Numbers in brackets refer to female and male forewings respectively.

Analysis by one-way ANOVA of the response of forewing *AR* to site showed no significant effect for either Falkland Island females (*F* (7, 38) = 0.63, *p* = 0.729) or males (*F* (7, 45) = 1.1, *p* = 0.378). Potentially significant contrasts, such as those involving Sea Lion (Figure 6.14), had insufficient data to support them. No post-hoc testing was therefore undertaken.

First moment of area ($\hat{r}_1$)

Analysis by one-way ANOVA of the response of $\hat{r}_1$ to site showed a significant effect for Falkland Island females (*F* (7, 38) = 2.62, *p* = 0.026), with a large effect size (η_p^2 = 0.326). The effect was not significant for males (*F* (7, 45) = 0.5, *p* = 0.827).

Box plot visualisation (Figure 6.15), together with post-hoc comparisons using the Tukey HSD test, showed that North Arm female forewings (*M* = 0.539, *SD* = 0.01) had a significantly larger $\hat{r}_1$ than Frying Pan (*M* = 0.521, *SD* = 0.005), *p* = 0.049 and Roy Cove (*M* = 0.525, *SD* = 0.014), *p* = 0.048

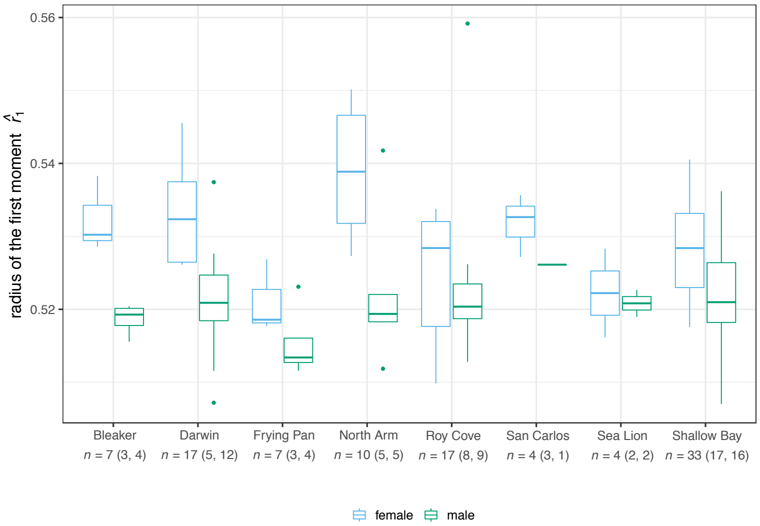

Figure 6.15 Comparison between forewing $\hat{r}_1$ at Falkland Islands sites. Numbers in brackets refer to female and male forewings respectively.

Geometric morphometrics: female

A CVA of Falkland Island female forewings showed that the first two CVs contributed 64% to the analysis (Figure 6.16). The target shapes of CV1 (47.5%) and CV2 (16.5%) showed a flattening of the distinctive female scalloping, with CV1 close to a straight line between landmarks 11 – 14.

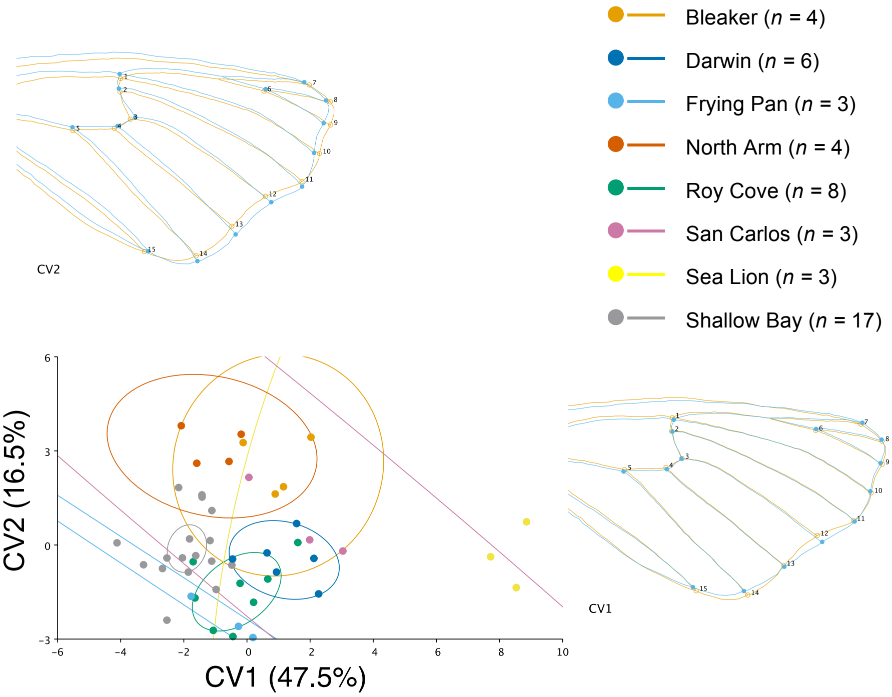

Figure 6.16 CVA of Falkland Island female forewings by site. 95% confidence ellipses of means are shown in the colour of their respective sites. The warped outlines show, in blue, the target shape of the CV on each axis, compared with the mean shape, in orange. The first two CVs contributed 64% to the analysis.

Some separation was visible between the sites, particularly in CV2. Sea Lion, with a small sample size (*n* = 3) was a significant outlier towards the target shape in CV1. An analysis of Mahalanobis and Procrustes distances (Appendix Table C.11) showed significant separation between 14 of the 28 possible pairings, with Shallow Bay in seven pairings, Sea Lion in five and Bleaker, Darwin and Roy Cove in three each.

Geometric morphometrics: male

A CVA of Falkland Island male forewings showed that the first two CVs contributed 64.1% to the analysis (Figure 6.17). The target shapes of CV1 and CV2 showed a broader wing.

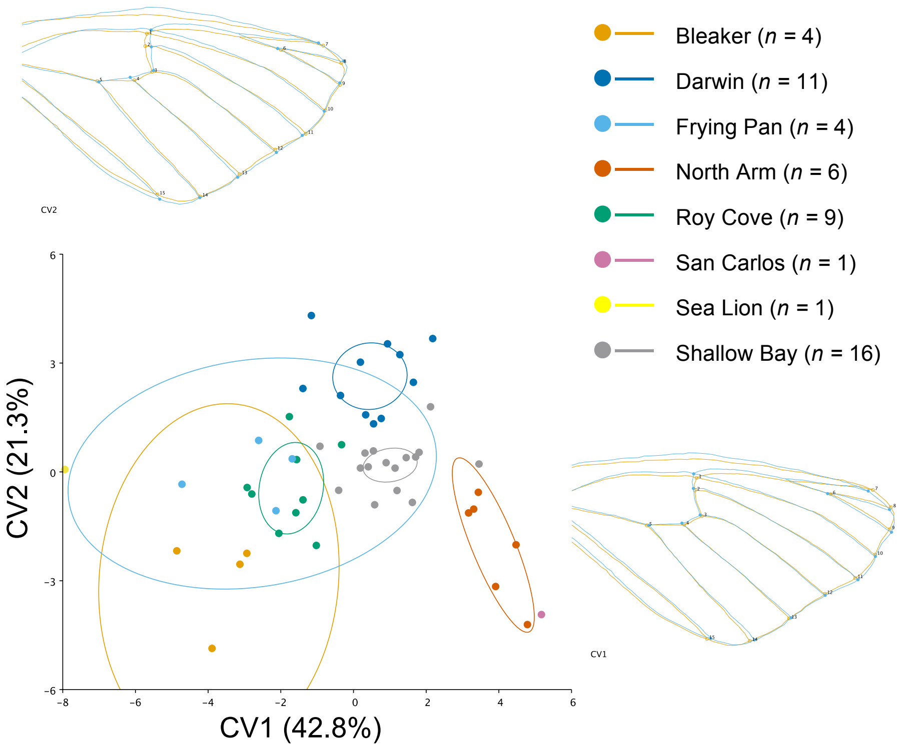

Figure 6.17 CVA of Falkland Island male forewings by site. 95% confidence ellipses of means are shown in the colour of their respective sites. The warped outlines show, in blue, the target shape of the CV on each axis, compared with the mean shape, in orange. The first two CVs contributed 64.1% to the analysis.

Some separation was visible between the sites, particularly in CVs 1 and 2 An analysis of Mahalanobis and Procrustes distances (Appendix Table C.12) showed significant separation of clusters in nine of the 28 possible pairings, with Shallow Bay and Darwin in four pairings, and North Arm and Roy Cove in three each.

(ii) **Latin American forewings**

Length

Analysis by one-way ANOVA of the response of forewing length to site showed a significant effect for Latin American females (F (5, 24) = 2.74, p = 0.042), with a large effect size (η_p^2 = 0.364) and males (F (6, 40) = 6.37, p = 0.00009), also with a large effect size (η_p^2 = 0.489).

Box plot visualisation (Figure 6.18), together with post-hoc comparisons using the Tukey HSD test, showed that Straits of Magellan female forewings (M = 21.5, SD = 1.34) were significantly longer than those from Punta Arenas (M = 17.5, SD = 1.51), p = 0.022. This might be viewed with caution, as these two butterflies are very much an outlier in forewing size.

Panguipulli male forewings ($M = 17.3$, $SD = 0.94$) were significantly longer than Bariloche ($M = 14.8$, $SD = 0.05$), $p = 0.039$, while Rio McClelland ($M = 18.1$, $SD = 0.83$) forewings were significantly longer than: Bariloche ($M = 14.8$, $SD = 0.05$), $p = 0.0004$; Chubut ($M = 16.8$, $SD = 1.39$), $p = 0.02$; and Punta Arenas ($M = 16.6$, $SD = 0.42$), $p = 0.045$.

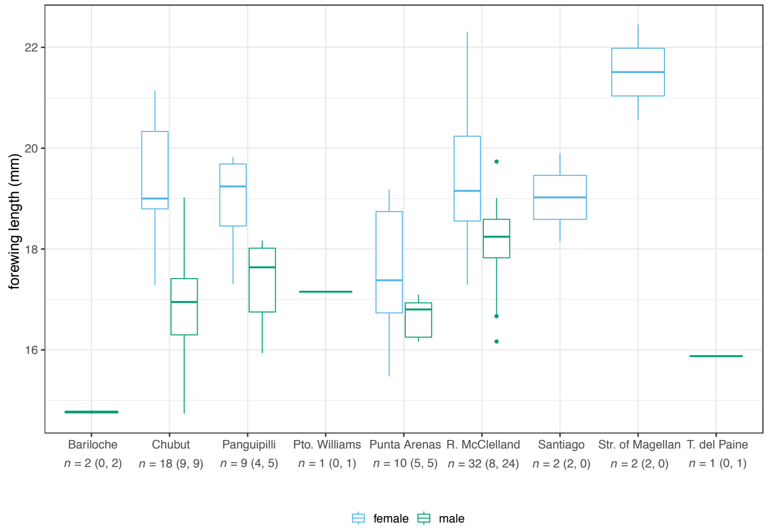

Figure 6.18 Comparison between forewing lengths at Latin American sites. Numbers in brackets refer to female and male forewings respectively.

Aspect ratio (AR)

Analysis by one-way ANOVA of the response of forewing AR to site showed a significant effect for Latin American females ($F\,(5, 24) = 2.79$, $p = 0.04$), with a large effect size ($\eta_p^2 = 0.368$) and males ($F\,(6, 40) = 5.16$, $p = 0.0005$), also with a large effect size ($\eta_p^2 = 0.436$).

Box plot visualisation (Figure 6.19), together with post-hoc comparisons using the Tukey HSD test, did not, despite the ANOVA result, show any individual contrast in female forewing AR significant at $p < 0.\ 05$.

Chubut male forewings ($M = 3.00$, $SD = 0.12$) had a significantly greater AR than Panguipulli ($M = 2.83$, $SD = 0.01$), $p = 0.049$, while Rio McClelland ($M = 2.84$, *SD* = 0.09) forewings had a significantly lower *AR* than: Chubut (*M* = 3.0, *SD* = 0.12), *p* = 0.003; and Punta Arenas (*M* = 3.00 *SD* = 0.10), *p* = 0.023.

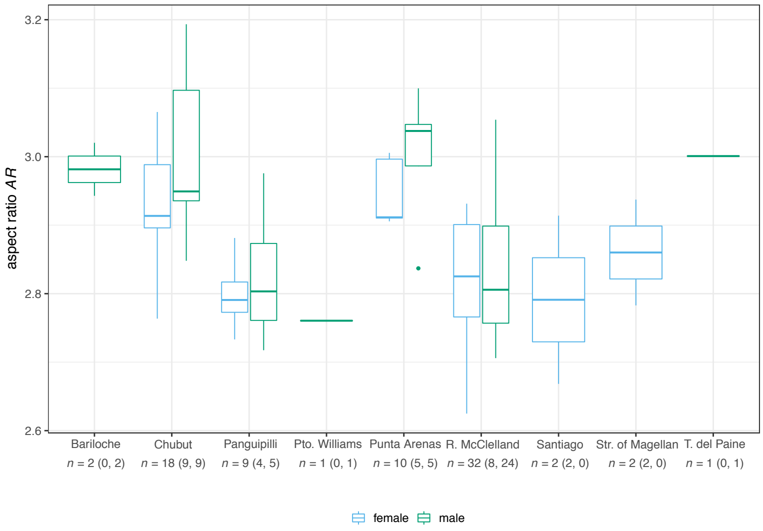

Figure 6.19 Comparison between forewing *AR* at Latin American sites. Numbers in brackets refer to female and male forewings respectively.

First moment of area ($\hat{r}_1$)

There was little contrast in forewing $\hat{r}_1$ between Latin American sites (Figure 6.20). A one-way ANOVA between the four sites gave a *p*-value of 0.365 for females, and 0.096 for males. Analysis by one-way ANOVA of the response of forewing $\hat{r}_1$ to site showed no significant effect for either Latin American females (*F* (5, 24) = 0.64, *p* = 0.671) or males (*F* (6, 40) = 1.19, *p* = 0.329). No post-hoc testing was therefore undertaken.

The significant contrast in $\hat{r}_1$ (*p* = 0.046) between Latin American female and male forewings, with female larger than male, seen at the land mass level (Figure 6.10, Table 6.6), was more nuanced at site level. *t*-tests for four sites showed only one significant variation, with a large effect size, at Rio McClelland, *p* = 0.023, *d* = 0.98.

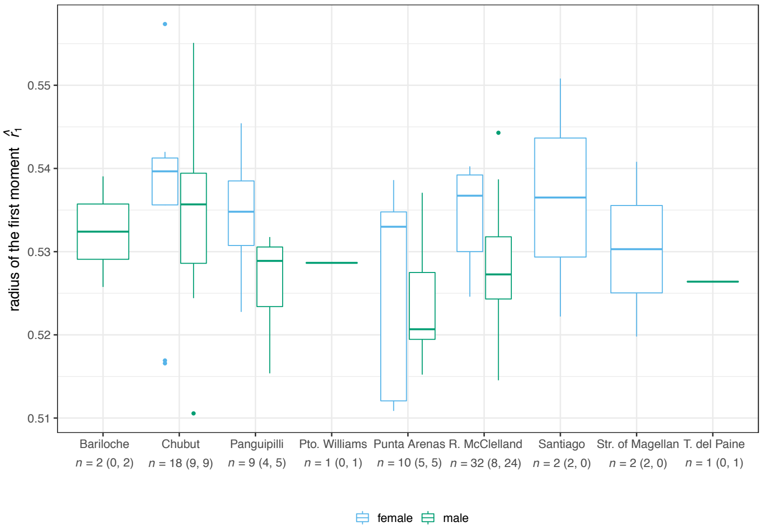

Figure 6.20 Comparison between forewing $\hat{r}_1$ at Latin American sites. Numbers in brackets refer to female and male forewings respectively.

Geometric morphometrics: female

A CVA of Latin American female forewings showed that the first two CVs contributed 89.8% to the analysis (Figure 6.21) The target shape of CV1 (71.2%) showed a slightly more scalloped wing with a more prominent wing tip with the movement of landmarks 8-10. That of CV2 (18.6%) showed a broader wing, with a larger discal cell.

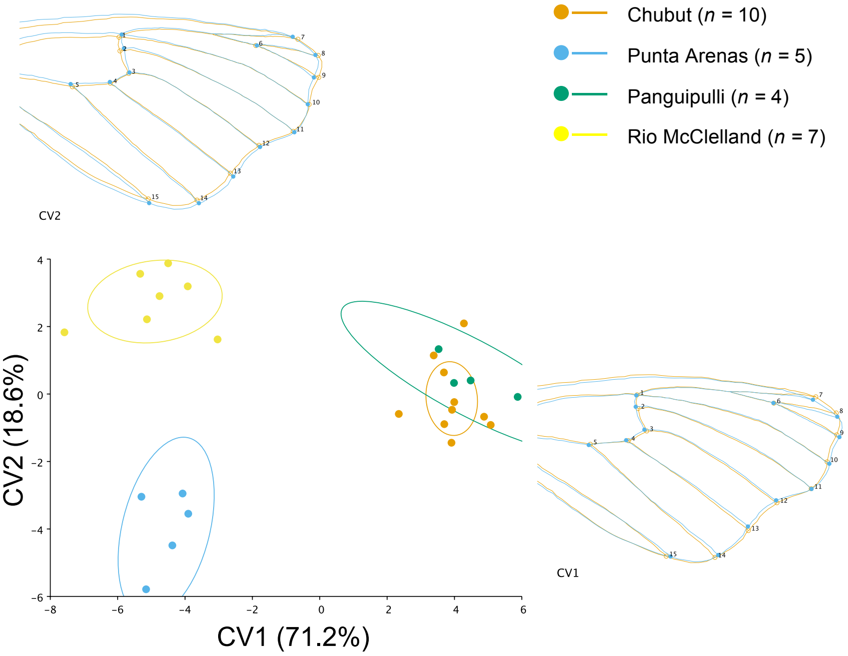

Figure 6.21 CVA of Latin American female forewings by site. 95% confidence ellipses of means are shown in the colour of their respective sites. The warped outlines show, in blue, the target shape of the CV on each axis, compared with the mean shape, in orange. The first two CVs contributed 89.8% to the analysis.

Clear separation was visible between the sites for each of the CVs other than between Chubut and Panguipulli for CVs 1 and 2, and between Punta Arenas and Rio McClelland for CV 1. Chubut showed significant separation in Mahalanobis distance from Punta Arenas, Panguipulli and Rio McClelland, as did Punta Arenas from Rio McClelland (Appendix Table C.13). Only Panguipulli and Rio McClelland showed significant separation by Procrustes distance.

Geometric morphometrics: male

A CVA of Latin American male forewings showed that the first two CVs contributed 71.2% to the analysis (Figure 6.22). The target shape for CV1 (45.4%), showed a lengthened wing tip and a slight scalloping of the medial - cubital wing edge. CV2 (25.8%) showed a narrowing of the wing, with a contraction of the discal cell.

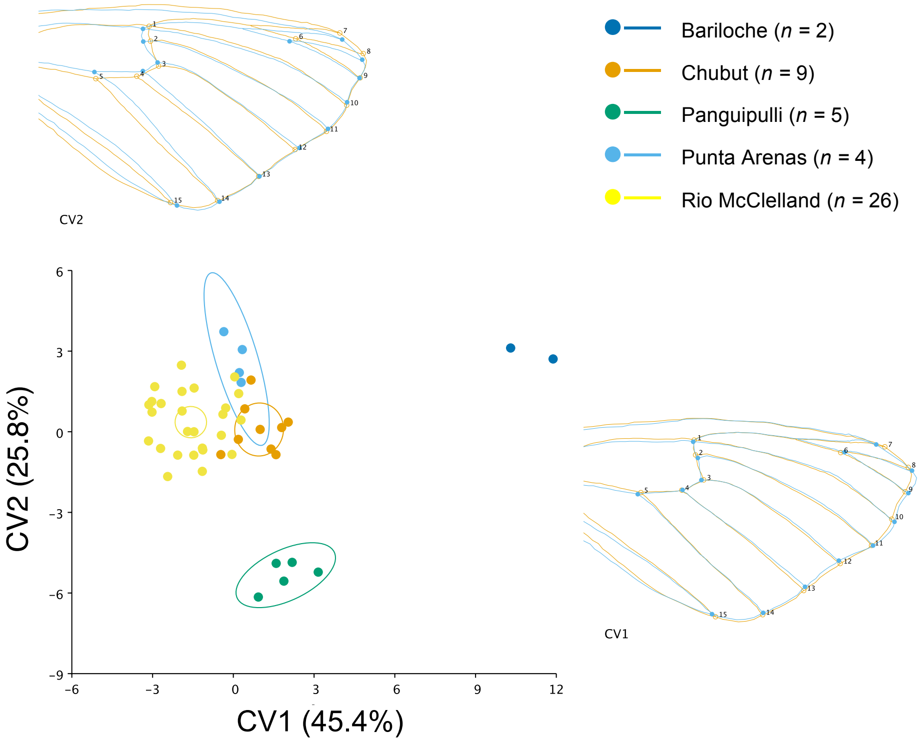

Figure 6.22 CVA of Latin American male forewings by site. 95% confidence ellipses of means are shown in the colour of their respective sites. The warped outlines show, in blue, the target shape of the CV on each axis, compared with the mean shape, in orange. The first two CVs contributed 71.2% to the analysis.

CV1 showed separation between Chubut, Bariloche and the other three sites respectively. CV2 separated Punta Arenas and Panguipulli. Chubut showed significant separation in Mahalanobis distance from Bariloche, Panguipulli and Rio McClelland; Rio McClelland also showed clear separation from Bariloche, Punta Arenas and Panguipulli (Appendix Table C.14); Rio McClelland showed significant separation by Procrustes distance from Bariloche and Panguipulli.

6.3.4 Hind wings: traditional morphometrics

(i) **Relationship between forewing and hind wing**

Ratio of area of hind wing to total area of wing pair

Land mass, sex and the interaction of the two did not have a significant effect on the ratio of hind wing area to the total area of the wing pair (Table 6.7). There was a close correspondence of ratios between sexes and land masses (Figure 6.23).

Table 6.7 The effect of land mass and sex on the ratio of area of hind wing to total area of wing pair. No factor was significant at $p < 0.05$.

Factor	*df*	*SS*	*MS*	*F*-ratio	*p*-value	η_p^2
land mass	1	3.1	3.06	0.603	0.439	0.004
sex	1	<0.01	<0.01	0.001	0.982	<0.001
land mass: sex	1	<0.01	<0.01	0.001	0.978	<0.001
residuals	132	670.8	5.08			

Analysis using type II sum of squares. *p*-values significant at < 0.05, and large effect sizes at $\eta_p^2 > 0.14$, are shown in bold font.

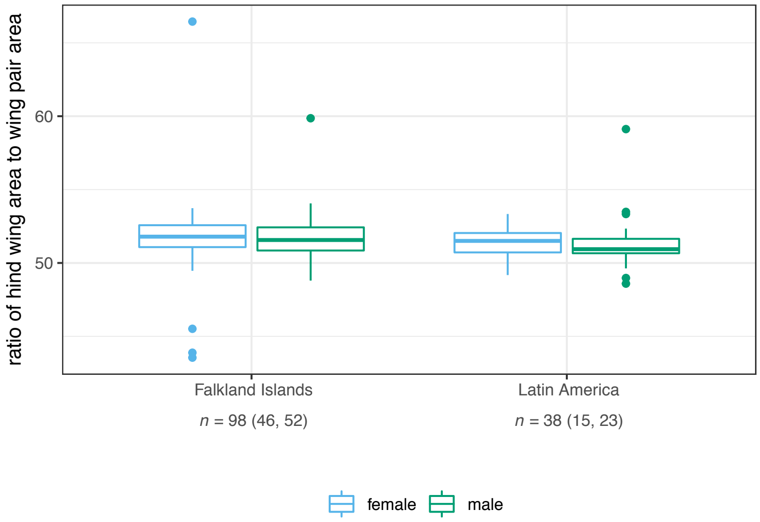

Figure 6.23 The effect on the ratio of hind wing area to overall wing pair area of land mass and sex. There was little observable variation between land masses or sexes.

Correlation between forewing and hind wing length

Kolmogorov-Smirnov tests supported the assumption of normal distribution for forewing and hind wing length. A scatterplot showed a strong association between the forewing length and hind wing length (Figure 6.24), confirmed by Pearson's product-moment correlation test ($r = 0.86$, 95% CI [0.81, 00.90], $df = 134$, $p < 0.001$).

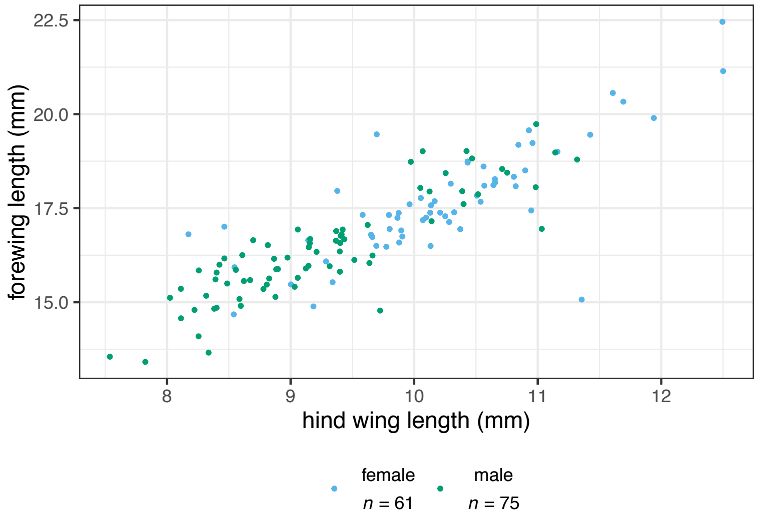

Figure 6.24 Comparison between forewing and hind wing lengths for a combined data set of Falkland and Latin American specimens.

(ii) **Comparison of hind wing length, width, base and area**

Kolmogorov-Smirnov tests supported the assumption of normal distribution for the three direct measurements (length, width, base) as well as area. Pearson's product-moment correlation test was used to assess the relationship between hind wing length and each of the other measurements to determine the extent to which it could be used as a proxy for size. The tests showed a very strong positive association between: length and width ($r = 0.92$, $p < 0.001$); length and base ($r = 0.92$, $p < 0.001$); and length and area ($r = 0.95$, $p < 0.001$). Accordingly, length was used as a proxy for hind wing size.

(iii) **Hind wing length**

A two-way ANOVA (Appendix Table C.6) showed that both land mass and sex were highly significant factors in hind wing length, with a large effect size. The interaction between sex and land mass was not significant. Visualisation of hind wing length by land mass and sex (Figure 6.25) together with post-hoc *t*-tests (Table 6.8), showed that

Falkland Island hind wings were significantly shorter than Latin American, and that female hind wings were significantly longer than male, both with a very large effect size

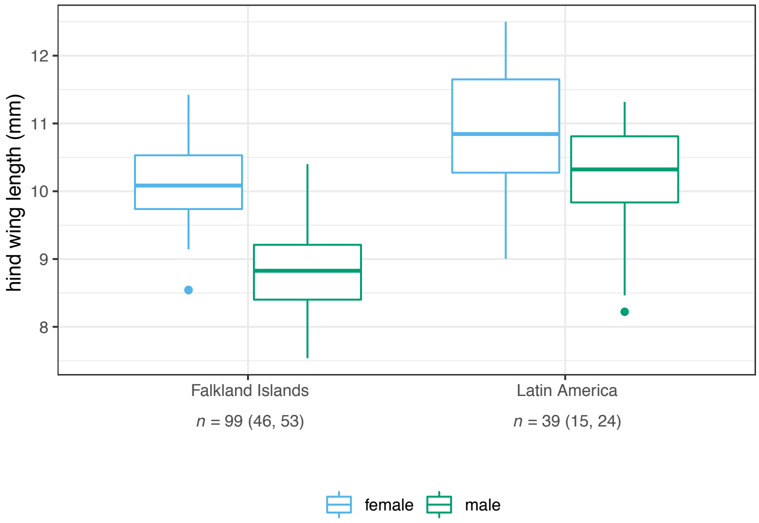

Figure 6.25. Hind wing lengths compared by land mass and sex. Numbers in brackets refer to female and male hind wings respectively.

Table 6.8 *t*-test comparisons of hind wing lengths. Contrasts are land mass by sex, and sex by land mass.

	n	*M*	*SD*	*df*	*t*	*p*-value	95% CI	*d*
FK: F	46	10.07	0.63	97	10.44	**<0.001**	1.00, 1.47	**2.10**
FK: M	53	8.84	0.55					
LA: F	15	10.92	1.00	37	2.55	**0.015**	0.16, 1.40	**0.84**
LA: M	24	10.14	0.88					
FK: F	46	10.07	0.63	59	-3.88	**<0.001**	-1.28, -0.41	**1.15**
LA: F	15	10.92	1.00					
FK: M	53	8.84	0.55	75	-7.90	**<0.001**	-1.63, -0.97	**1.94**
LA: M	24	10.14	0.88					

FK = Falkland Islands, LA = Latin America; F = female, M = male. Significance at $p < 0.05$, and large effect size at $d > 0.8$, are shown in bold.

(iv) **Hind wing angle**

A two-way ANOVA (Appendix Table C.7) and a visualisation (Figure 6.26) showed that neither land mass nor sex had a significant effect on the hind wing angle.

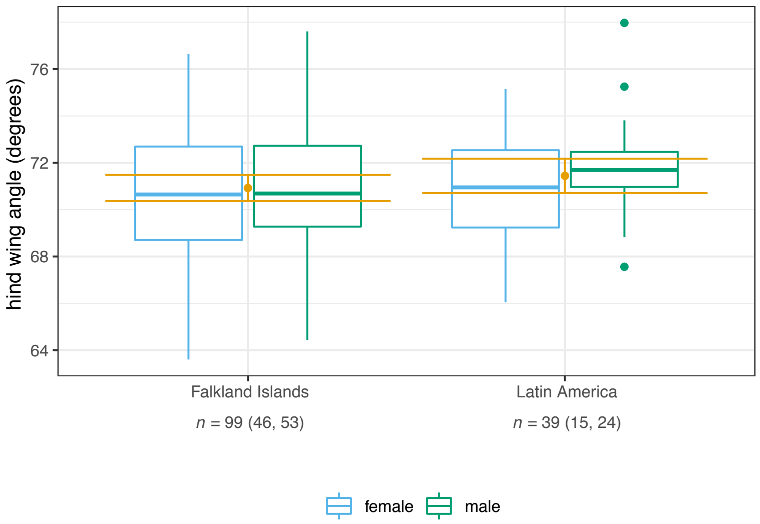

Figure 6.26 Hind wing angles compared by land mass and sex. 95% confidence intervals for the means of the two populations, with sex pooled, were added (orange bars). Numbers in brackets refer to female and male hind wings respectively.

(v) **Hind wing *AR***

A two-way ANOVA (Appendix Table C.8) showed that sex was a significant factor in hind wing *AR*, with a medium effect size. Land mass and the interaction between sex and land mass were not significant. Visualisation of hind wing *AR* by land mass and sex (Figure 6.27), together with post-hoc *t*-tests (Table 6.9), showed that female hind wings from both the Falkland Islands and Latin America had larger *AR* (for a given area, they were longer and narrower) than male.

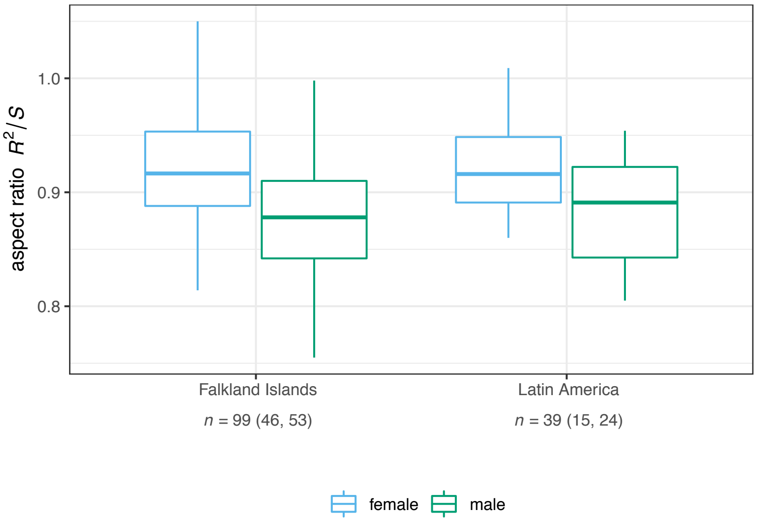

Figure 6.27 Hind wing *AR* compared by land mass and sex. Numbers in brackets refer to female and male hind wings respectively.

Table 6.9 *t*-test comparisons of hind wing *AR* by land mass and sex.

factor	*n*	*M*	*SD*	*df*	*t*	*p*-value	95% CI	*d*
FK: F	46	0.92	0.05	97	3.57	**<0.001**	0.02, 0.06	0.72
FK: M	53	0.88	0.06					
LA: F	15	0.92	0.05	37	2.49	**0.017**	0.92, 0.88	**0.82**
LA: M	24	0.88	0.05					

factors: FK = Falkland Islands, LA = Latin America; F = female, M = male. Significance at $p < 0.05$ and large effect size at $d > 0.8$ are shown in bold.

(vi) **Hind wing $\hat{r}_1$**

A two-way ANOVA (Appendix Table C.9) showed that land mass and sex were not significant factors in hind wing $\hat{r}_1$. Visualisation of $\hat{r}_1$ by land mass and sex (Figure 6.28) suggested a possible contrast between female and male Latin American specimens. This was explored with a *t*-test and found not significant: female (*M* = 0.586, *SD* = 0.018) and male (*M* = 0.574, *SD* = 0.025) *df* = 37, t = 1.65, *p* = 0.108, 95% CI [-0.003, 0.03], *d* = 0.54.

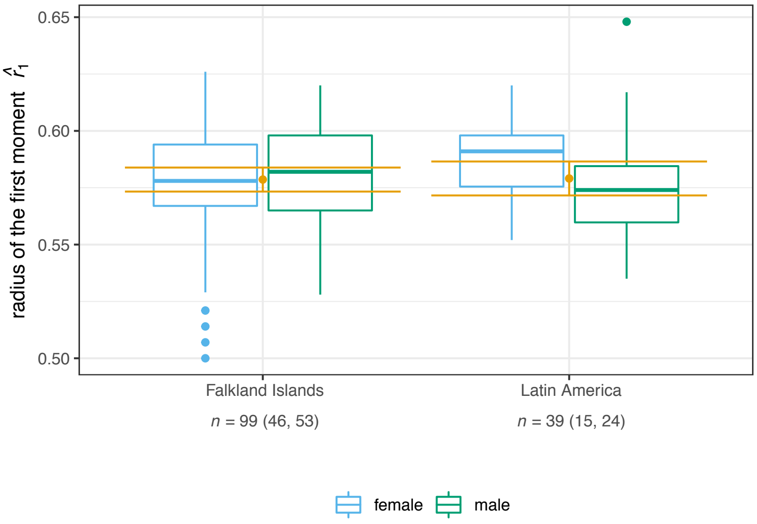

Figure 6.28 Hind wing $\hat{r}_1$ compared by land mass and sex. 95% confidence intervals for the means of the two populations, with sex pooled, were added (orange bars). Numbers in brackets refer to female and male hind wings respectively.

6.3.5 Hind wings: geometric morphometrics

(i) **Principal Component Analysis (PCA) of hind wings**

A CVA of hind wings showed that the first two PCs contributed 45.5% to the analysis (Figure 6.29). PC1 (26.1%) showed a wider wing in the initial shape than in the target shape, as between the distal ends of subcosta/radius1 and anal 1+2 but with shorter medias 1and 2. PC2 (19.4%) had longer cubitals in the initial shape.

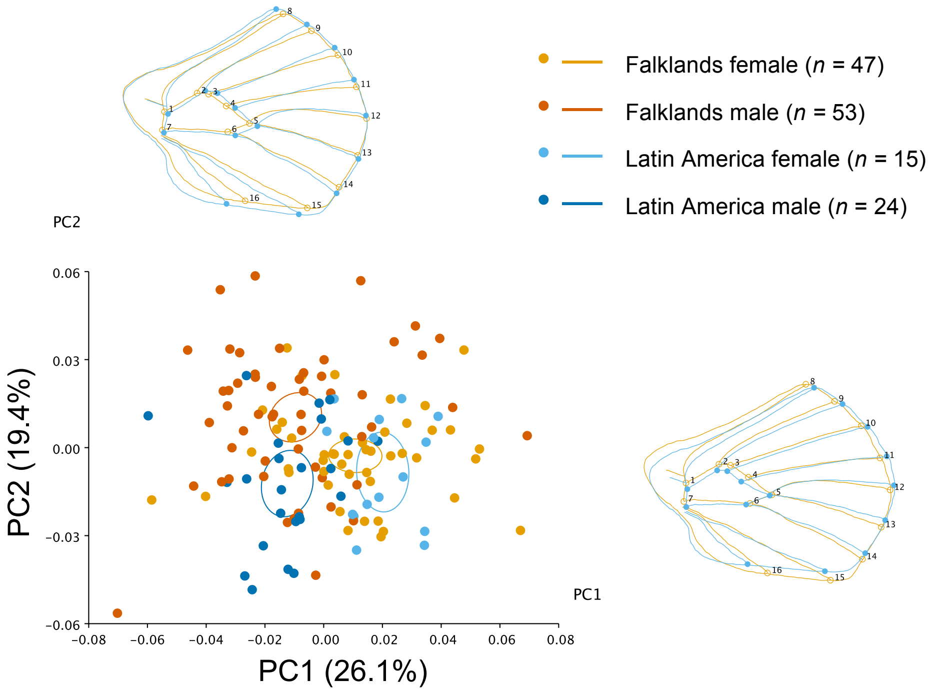

Figure 6.29 PCA of hind wings by land mass and sex. 95% confidence ellipses of means are shown in the colour of their respective combination of the two. The warped outlines show, in blue, the target shape of the PC on each axis, compared with the mean shape, in orange. The first two PCs contributed 45.5% to the analysis.

There was clustering against each of the first two PCs. Females tended towards the target shape in PC1, and away from it in PC2. Falkland Islands specimens, particularly male, tended towards the target shape in PC1, Latin American specimens away from it.

(ii) **Canonical Variate Analysis (CVA) of hind wings**

A CVA of hind wings showed that the first two CVs contributed 91.9% to the analysis (Figure 6.30). The target shape of CV1 (56.7%) showed a wider wing with particularly long medials 1 and 2 (landmarks 10 and 11). The target shape of CV2 (35.2%) was broader, with a larger discal cell.

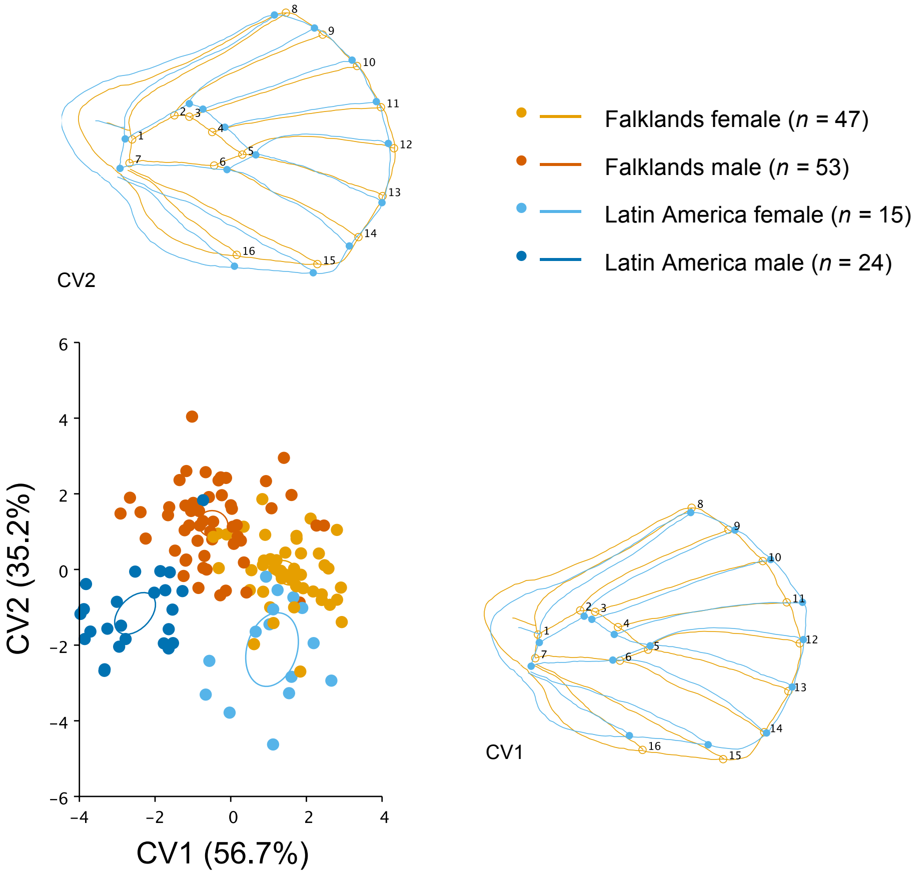

Figure 6.30 CVA of hind wings by land mass and sex. 95% confidence ellipses of means are shown in the colour of their respective combination of the two. The warped outlines show, in blue, the target shape of the CV on each axis, compared with the mean shape, in orange. The first two CVs contributed 91.9% to the analysis.

When plotted against CVs 1 and 2 there was a clear separation of the four groups about the origin. Female hind wings tended towards the target shape of CV1, with male tending away from it. Falklands hind wings tended towards the target shape of CV2, with Latin American forewings tending away from it.

An analysis of Mahalanobis and Procrustes distances (Appendix Table C.15) confirmed the significance of all contrasts at $p \leq 0.0001$ other than for the Procrustes distance between Latin American and Falkland females ($p = 0.034$, with $\alpha = 0.05$).

6.3.6 Hind wing contrasts at site level

(i) **Falkland Island hind wings**

Length

Analysis by one-way ANOVA of the response of hind wing length to site showed a significant effect for Falkland Island males (F (7, 45) = 2.52, p = 0.028), with a large effect size (η_p^2 = 0.281). The effect was not significant for females (F (7, 38) = 1.93, p = 0.091).

Box plot visualisation (Figure 6.13), together with post-hoc comparisons using the Tukey HSD test, showed that North Arm male hind wings (M = 8.24, SD = 0.54) were significantly shorter than those from Darwin (M = 9.16, SD = 0.41), p = 0.048, but that other contrasts were not significant at $p < 0.05$.

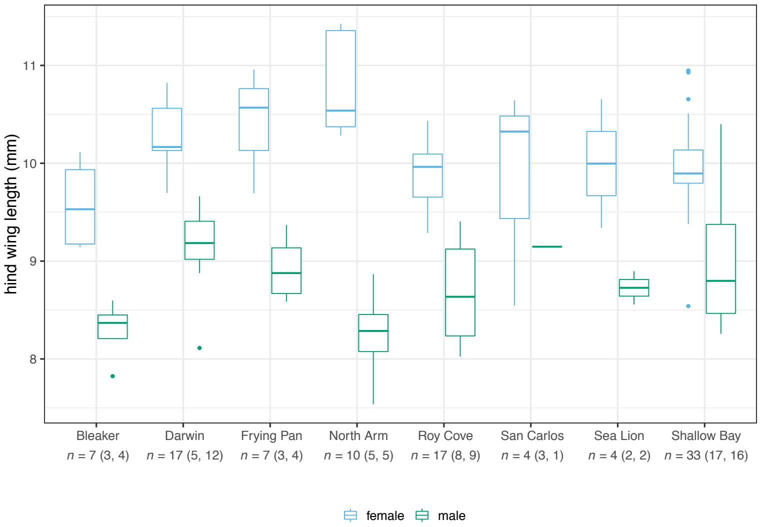

Figure 6.31 Comparison between hind wing lengths at Falkland Islands sites. Numbers in brackets refer to female and male forewings respectively.

Aspect ratio (AR)

Analysis by one-way ANOVA of the response of hind wing *AR* to site showed a significant effect for Falkland Island males (F (7, 45) = 2.48, p = 0.03), with a large effect size (η_p^2 = 0.278). The effect was not significant for females (F (7, 38) = 0.4, p = 0.894).

Box plot visualisation (Figure 6.13), together with post-hoc comparisons using the Tukey HSD test, showed that no contrasts between sites were significant at $p < 0.05$.

The larger *AR* in female, compared with male, hind wings, seen at the land mass level (Figure 6.27, Table 6.9), was also seen at site level (Figure 6.32). Female/male *t*-tests showed significant variation with large effect sizes for Frying Pan, $p = 0.025$, $d = 2.4$ and Shallow Bay, $p = 0.013$, $d = 0.92$.

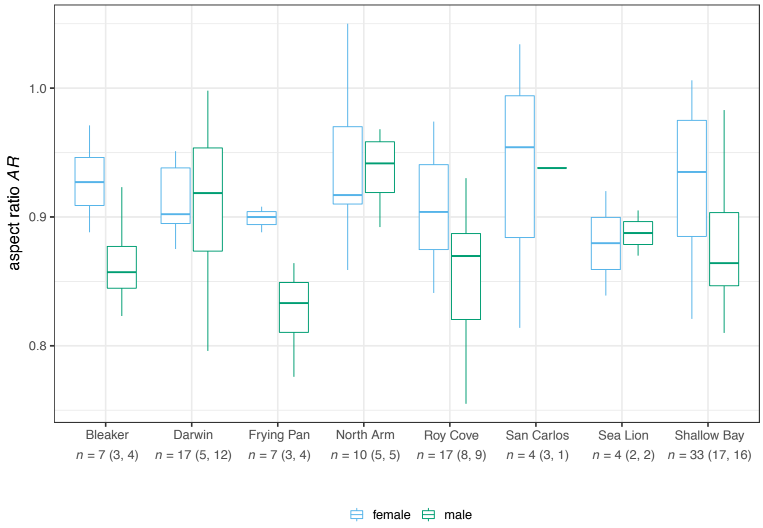

Figure 6.32 *AR* for hind wings from Falkland Island sites. Numbers in brackets refer to female and male forewings respectively.

First moment of area ($\hat{r}_1$)

Analysis by one-way ANOVA of the response of hind wing $\hat{r}_1$ to site showed a significant effect for Falkland Island females (F (7, 38) = 5.97, $p = 0.0001$), with a large effect size ($\eta_p^2 = 0.523$) and for males (F (7, 45) = 6.58, $p = 0.00002$), also with a large effect size ($\eta_p^2 = 0.278$).

Box plot visualisation (Figure 6.33), together with post-hoc comparisons using the Tukey HSD test, showed that Darwin females ($M = 0.6$, $SD = 0.02$) had a significantly higher $\hat{r}_1$ than Bleaker ($M = 0.54$, $SD = 0.04$; $p = 0.003$), Frying Pan ($M = 0.53$, $SD = 0.02$; $p = 0.003$) and Sea Lion ($M = 0.54$, $SD = 0.03$; $p = 0.019$); and Shallow Bay ($M = 0.59$, $SD = 0.02$) than Bleaker ($M = 0.54$, $SD = 0.04$; $p = 0.006$), and Frying Pan ($M = 0.53$, $SD = 0.02$; $p = 0.007$).

Shallow Bay males ($M = 0.6$, $SD = 0.02$) had a significantly higher $\hat{r}_1$ than Bleaker ($M = 0.56$, $SD = 0.02$; $p = 0.005$); Frying Pan ($M = 0.55$, $SD = 0.01$; $p = 0.00008$); Roy Cove ($M = 0.58$, $SD = 0.02$; $p = 0.027$); and Sea Lion ($M = 0.55$, $SD = 0.01$; $p = 0.018$); and North Arm ($M = 0.59$, $SD = 0.02$) than Frying Pan ($M = 0.55$, $SD = 0.01$; $p = 0.037$).

The absence of significant contrast in $\hat{r}_1$ between female and male hind wings, seen at the land mass level (Figure 6.28), was reflected at site level (Figure 6.33), with the exception of Shallow Bay, where male $\hat{r}_1$ was significantly greater than female at $p = 0.019$, with a large effect size $d = 0.86$. In all cases, other than Darwin, male $\hat{r}_1$ was also greater than female at low levels of significance. In Darwin female $\hat{r}_1$ was greater than female, albeit at $p = 0.108$, but with a large effect size, $d = 0.91$.

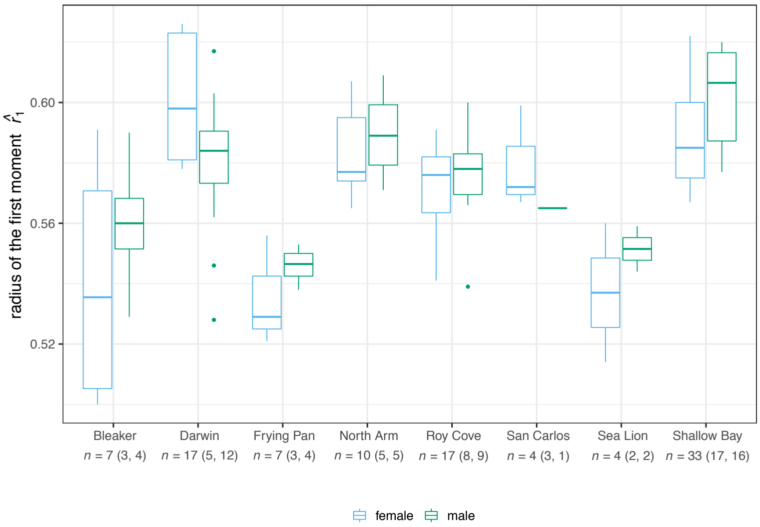

Figure 6.33 $\hat{r}_1$ for hind wings from Falkland Island sites. Numbers in brackets refer to female and male forewings respectively.

Geometric morphometrics: female

A CVA of Falkland Island female hind wings showed that the first two CVs contributed 61% to the analysis (Figure 6.34). The target shape of CV1 (37.3%) showed a smaller discal cell, with an inflection at the proximal end of medial 1 (landmark 3), and a proximal movement of the tornus landmarks (15 and 16). CV2 (23.7%) showed a proximal movement of the radial sector and medials 1 and 2 (landmarks 9 – 11).

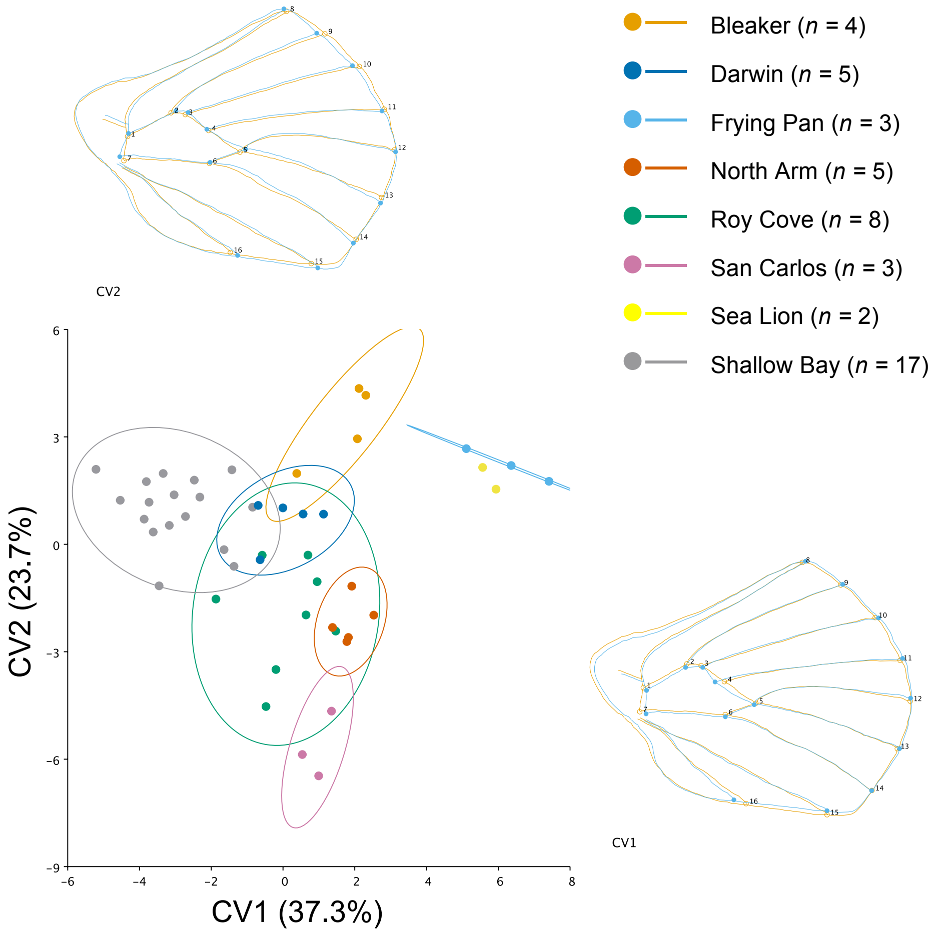

Figure 6.34 CVA of Falkland Island female hind wings by site. 95% confidence ellipses of means are shown in the colour of their respective sites. The warped outlines show, in blue, the target shape of the CV on each axis, compared with the mean shape, in orange. The first two CVs contributed 61% to the analysis.

The CVs showed some clear clustering, with Frying Pan and Sea Lion, with small sample sizes, as outliers at the extremes of the target shapes for CVs 1 and 2. An analysis of Mahalanobis and Procrustes distances (Appendix Table C.16) showed significant separation of clusters in 11 of the 28 possible pairings.

Geometric morphometrics: male

A CVA of Falkland Island male hind wings showed that the first two CVs contributed 61% to the analysis (Figure 6.35). The target shape of CV1 (40.4%) showed a larger discal cell, with the wing shape weighted away from the termen (landmarks 9 - 13) towards the tornus (landmarks 14 - 15). CV2 (22.8%) showed a proximal movement of the tornus landmarks 14 - 16.

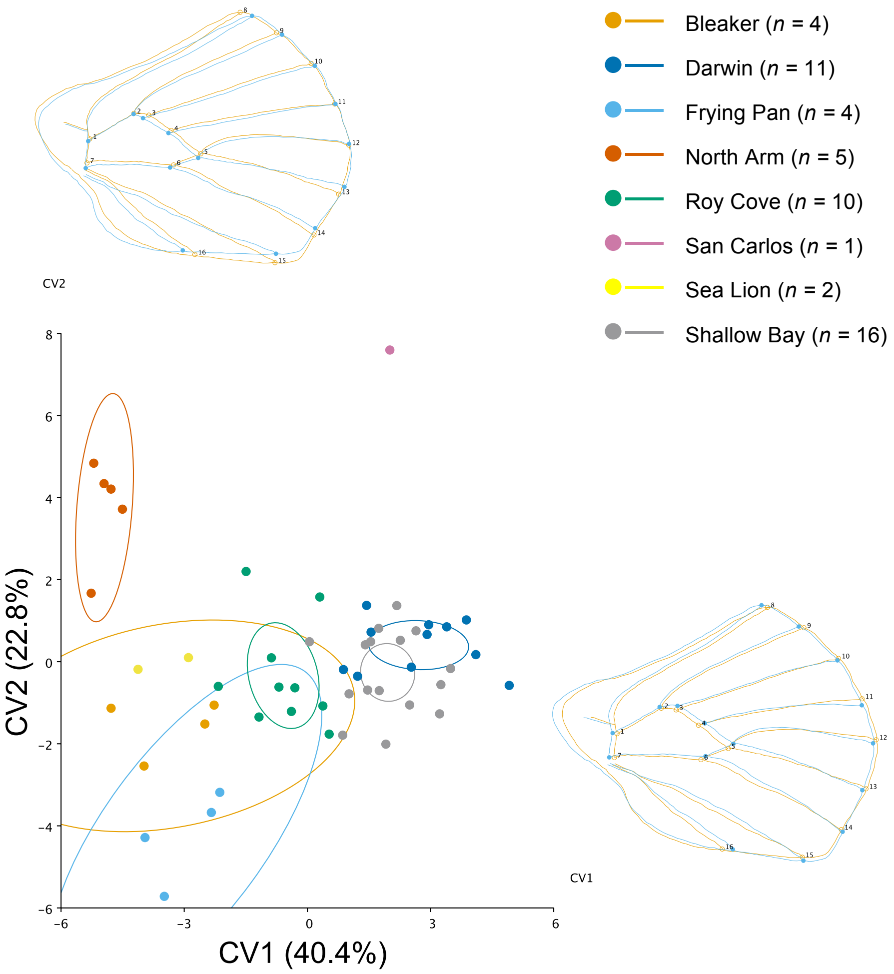

Figure 6.35 CVA of Falkland Island male hind wings by site. 95% confidence ellipses of means are shown in the colour of their respective sites. The warped outlines show, in blue, the target shape of the CV on each axis, compared with the mean shape, in orange. The first two CVs contributed 61% to the analysis.

CVs 1 - 3 showed some clear separations of sites, with North Arm, Darwin, Roy Cove and the single sample from San Carlos at the extremes of target shapes. An analysis of Mahalanobis and Procrustes distances (Appendix Table C.17) showed significant separation of clusters in 11 of the 28 possible pairings.

6.3.7 Summaries of results

(i) **Traditional morphometric analysis of wings by sex and land mass**

Wing size - as forewing and hind wing length - provided the main contrasts between sexes and locations, with AR and $\hat{r}_1$ in forewings, and AR in hind wings, also showing significant variations (Table 6.10).

Table 6.10 Summary of *t*-test comparisons of (a) forewing and (b) hind wing length, angle, *AR* and $\hat{r}_1$. by land mass and sex. Comparisons are made, in pairs of rows with the same greyscale shading, between female and male in the Falklands and Latin America respectively, then between Falklands and Latin American females and males respectively.

(a) forewing

		n	length	angle	*AR*	$\hat{r}_1$
Falkland Islands:	female	46	17.3 ***	35.6^{ns}	2.95^{ns}	0.53***
	male	53	15.7	35.7	2.96	0.52
Latin America:	female	30	19.1***	36.5^{ns}	2.87^{ns}	0.53*
	male	47	17.4	36.5	2.89	0.53
female:	Falkland Islands	46	17.3***	35.6*	2.95**	0.53^{ns}
	Latin America	30	19.1	36.5	2.87	0.53
male:	Falkland Islands	53	15.7***	35.7*	2.96**	0.52***
	Latin America	47	17.4	36.5	2.89	0.53

(b) hind wing

		n	length	angle	*AR*	$\hat{r}_1$
Falkland Islands:	female	46	10.1 ***	ns	0.92***	ns
	male	53	8.8	ns	0.88	ns
Latin America:	female	15	10.9***	ns	0.92*	ns
	male	24	10.1	ns	0.88	ns
female:	Falkland Islands	46	10.1***	ns	ns	ns
	Latin America	15	10.9	ns	ns	ns
male:	Falkland Islands	53	8.8***	ns	ns	ns
	Latin America	24	10.1	ns	ns	ns

significant contrasts are marked: * significant at $p < 0.05$; ** significant at $p < 0.01$; *** significant at $p < 0.001$. Contrasts shown to be non-significant at $p < 0.05$ are marked ns, if not explored after a non-significant ANOVA, and with a superscript ^{ns} for non-significant contrasts in *t*-tests.

(ii) **Site level analysis of wings by traditional and geometric morphometrics**

While sample size was, in most cases, small, the site level analyses in 6.3.3 and 6.3.6 showed some significant contrasts, summarised in Table 6.11 and Table 6.12.

Table 6.11 Summary of Falkland Island forewing and hind wing contrasts between sites. Comparisons of forewings and hind wings are by both traditional and geometric morphometrics. Traditional morphometric results show sex, wing and the measurement used; geometric morphometrics show sex and wing.

site	BL	DA	FP	NO	RC	SC	SL	SW	
BL	-	**M: *R*** **F: $\hat{r}_1$**	ns	ns	ns	ns	ns	**M: *R*** **F: $\hat{r}_1$** **M: $\hat{r}_1$**	
DA	**M**	-	**F: $\hat{r}_1$**	**M: *R***	ns	ns	**F: $\hat{r}_1$**	ns	
FP	ns	**M** **M**	-	**F: $\hat{r}_1$** **M: $\hat{r}_1$**	ns	ns	ns	**F: $\hat{r}_1$** **M: $\hat{r}_1$**	
NO	ns	**M** **F, M**	ns	-	**F: $\hat{r}_1$**	ns	ns	ns	traditional morphometrics
RC	**M** **F, M**	**M** **M**	**M**	**F, M** **F, M**	-	ns	ns	**M: $\hat{r}_1$**	
SC	ns	**F**	ns	ns	ns	-	ns	ns	
SL	**F**	**F** **F**	ns	**F**	**M**	**F**	-	**M: $\hat{r}_1$**	
SW	**F, M** **F, M**	**F, M** **F, M**	**F, M** **F, M**	**F, M** **F, M**	**F** **F, M**	**F** **F, M**	**F** **F, M**	-	

geometric morphometrics

BL = Bleaker; DA = Darwin; FP = Frying Pan; NO = North Arm; RC = Roy Cove; SC = San Carlos; SL = Sea Lion; SW = Shallow Bay. Forewing characters are in orange, hind wing in blue. F = female, M = male. Traditional morphometrics summarise contrasts significant at $p < 0.05$, Geometric morphometrics those with Mahalanobis distances < 0.001. Non-significant contrasts are marked ns.

Table 6.12 Summary of Latin American forewing contrasts between sites. Comparisons are by both traditional and geometric morphometrics. Traditional morphometric results show sex and the measurement used, geometric morphometrics show sex.

site	Bariloche	Chubut	Panguipulli	Punta Arenas	Rio McClelland	
Bariloche	-	ns	M: *R*	ns	M: *R*	
Chubut	ns	-	ns	M: *AR*	M: *R* M: *AR*	
Panguipulli	ns	F, M	-	ns	ns	traditional morphometrics
Punta Arenas	ns	F, M	ns	-	M: *R* M: *AR*	
Rio McClelland	M	F, M	M	F, M	-	

F = female, M = male. Traditional morphometrics summarise contrasts significant at $p < 0.05$, Geometric morphometrics those with Mahalanobis distances < 0.001. Non-significant contrasts are marked ns.

6.3.8 Effect of elevation and latitude on forewings

(i) **Length**

Linear regressions showed that Latin American male forewings were shorter at higher elevations, $p = 0.013$, with a medium effect size, $\eta_p^2 = 0.134$ (Figure 6.36a, Appendix Table C.19), with no clear difference in length attributable to latitude ($p = 0.435$) (Figure 6.36b). Elevation, latitude or the interaction of both had no significant effect on Latin American female forewing length (Appendix Table C.18).

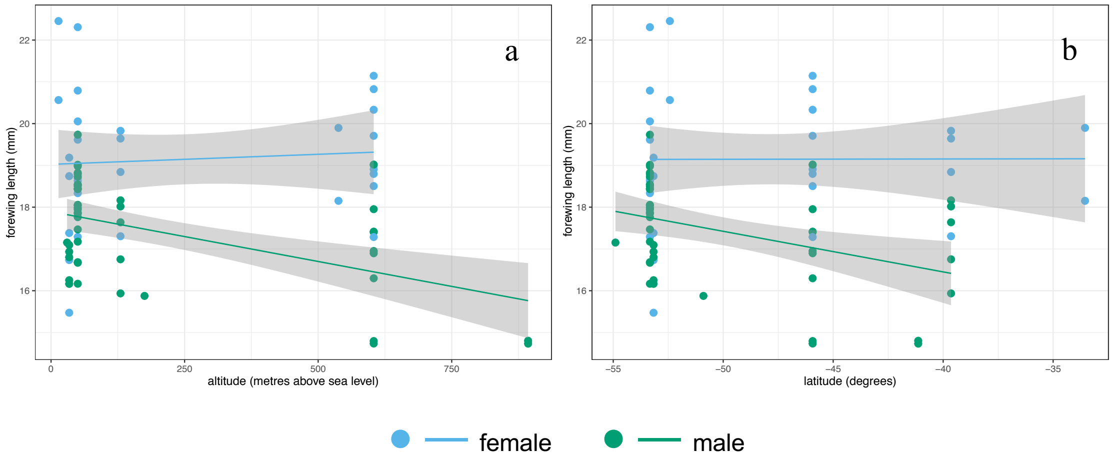

Figure 6.36 effect on forewing length of (a) elevation and (b) latitude in Latin American *Y. cytheris.* The grey areas represent the standard error of the fitted regression line after loess smoothing.

(ii) ***Aspect ratio* (*AR*)**

Linear regressions showed that forewing aspect ratios for both females ($p = 0.027$, $\eta_p^2 = 0.174$) and males ($p = 0.003$, $\eta_p^2 = 0.190$) were significantly higher, with a large effect size, at higher elevations (Figure 6.37a, Appendix Table C.20). The effect of latitude was not significant at $p < 0.05$ for either sex (Figure 6.37b, and C.21).

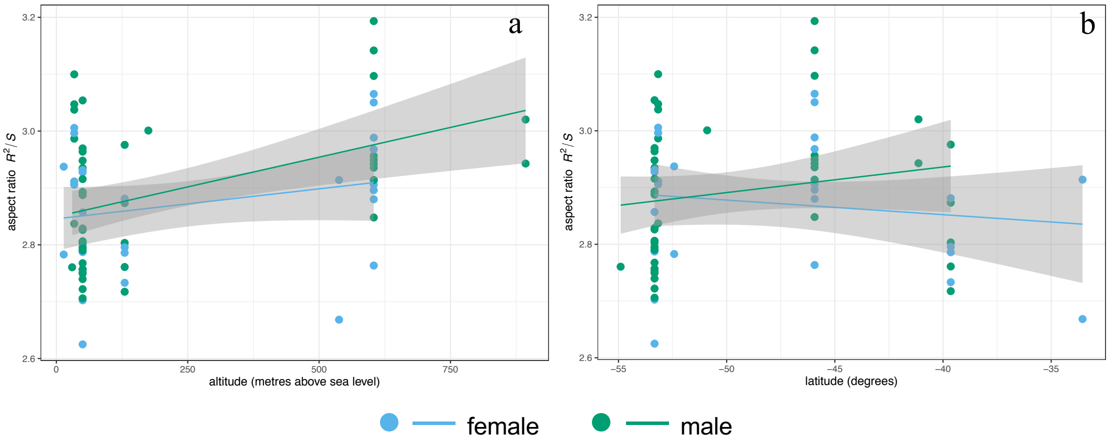

Figure 6.37 effect on forewing *AR* of (a) elevation and (b) latitude in Latin American *Y. cytheris.* The grey areas represent the standard error of the fitted regression line after loess smoothing.

(iii) ***First moment of area* ($\hat{r}_1$)**

Linear regressions showed that $\hat{r}_1$, in male forewings was significantly higher, albeit with a medium effect size, at higher elevations ($p = 0.032$, $\eta_p^2 = 0.032$). Female forewing $\hat{r}_1$ differences were not significant at $p < 0.05$ (Figure 6.38a, Appendix Table C.22) The effect of latitude was not significant at $p < 0.05$ for either sex (Figure 6.38b, Appendix Table C.23).

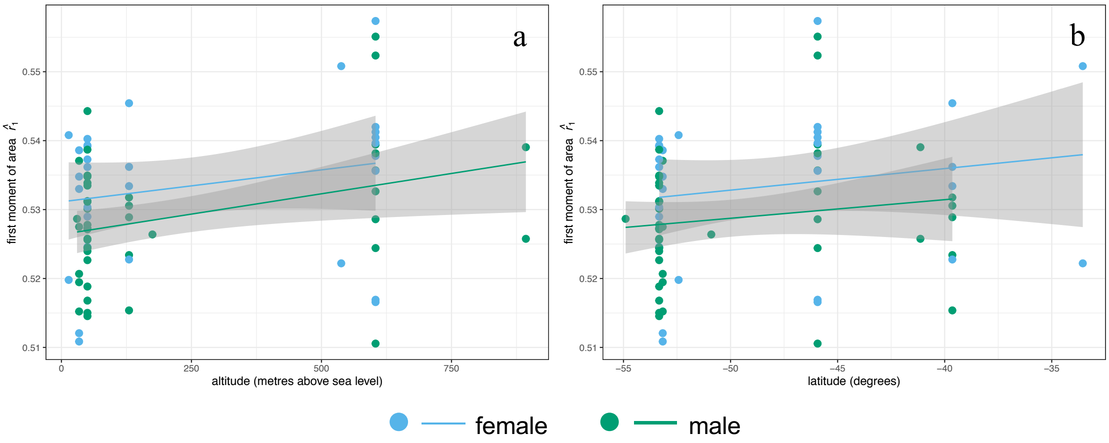

Figure 6.38 effect on forewing $\hat{r}_1$ of (a) elevation and (b) latitude in Latin American *Y. cytheris.* The grey areas represent the standard error of the fitted regression line after loess smoothing.

6.3.9 Effect of wind speed on forewings

Linear regressions for length, *AR* and $\hat{r}_1$ did not show any significant effects of wind speed at the butterflies' flying height at the four Falkland Island sites (Appendix Table C.24). Plots of the regressions (Figure 6.39), however, suggested further exploration with a larger data set might clarify the extent of an apparent inverse relationship between wind speed and forewing length.

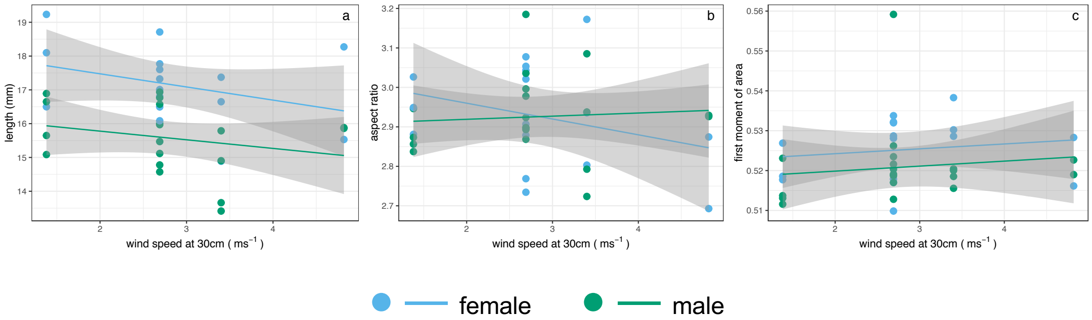

Figure 6.39 effect on (a) forewing length, (b) *AR*, and (c) $\hat{r}_1$ of wind speed on *Y. cytheris* at the four Falkland Island study sites. The grey areas represent the standard error of the fitted regression line after loess smoothing.

6.4 Discussion

6.4.1 Wing variation by sex and population

(i) **Findings**

Female forewings and hind wings were longer than male, with very large effect sizes. Female forewing $\hat{r}_1$ and hind wing *AR* were higher than male. Falkland Island butterflies were smaller than Latin American, with a higher forewing *AR*. The Falklands male had a lower $\hat{r}_1$ than the Latin American.

Variation was also apparent in the geometric morphometric analyses. Female forewings were proportionally narrower, with clear scalloping between the second medial and second cubital veins, although *AR* did not vary significantly between the sexes. Female hind wings narrowed towards the proximal end, with a marked lengthening in the second medial, producing a clear angle between the distal end and the distal ends of the first and third medials. This narrowing and lengthening was reflected by their higher *AR*. Latin American butterflies showed a more scalloped shape for both sexes in the forewing. Hindwing shapes reflected the difference in *AR*, with the Latin American population being narrower and longer.

(ii) **Discussion**

The larger wings of the female are consistent with sexual size dimorphism (Wiklund and Forsberg 1991, Teder and Tammaru 2005). Higher $\hat{r}_1$ and *AR* are consistent with slow, deliberate flight in seeking out oviposition locations, although the contrast between the sexes would be expected to be greater if the male adopted a perching strategy in seeking a mate (Dennis and Shreeve 1988, Jugovic et al. 2018). This was not the case in the Falklands, where no evidence was found of male territoriality, and it was not found possible to distinguish between the sexes by flight.

The contrast between shorter Falkland Islands forewings and hind wings, both female and male, than their Latin American counterparts provides some support for island species having shorter wings than mainland (Kotze 2008, Dennis and Hardy 2018). In the absence of comparative Latin American data it is not possible to establish whether the Falklands population has smaller wings because it is more sedentary (Sekar 2012) or because of a response to high winds (Dennis and Shreeve 1989, Dennis and Hardy 2018). The shorter forewings of *Y. cytheris* populations on the windiest islands, Bleaker and Sea Lion, suggested (albeit with a small sample size) that wind might be a factor, particularly as it was linked in both cases to a low $\hat{r}_1$. The response to wind of claw shape in these populations is considered further in Chapter 7.

Y. cytheris does not have a distinctive wing pattern, in the sense that one pattern element is prominent, either for mimicry or sexual signalling, therefore wing shape is not affected by the high, possibly exaggerated, $\hat{r}_1$ and *AR* which those traits would entail (Outomuro et al. 2013). No predation of butterflies was seen on the field visits, therefore no evidence was found of *Y. cytheris's* escape and evasion tactics which might be a factor in wing shape (Corcoran and Conner 2016). The ratio of hind wing area to total wing area was also consistent across sex and population, which did not suggest that any particular combination of the two might be subject to increased predation (Jantzen and Eisner 2008).

6.4.2 Wing variation at site level

(i) **Findings**

CVAs showed distinctive and significant groupings of sites in the Falklands and Latin America. In the Falklands, there was generally clear separation for male and female, forewing and hind wing, for Darwin, North Arm, Shallow Bay and Roy Cove, with Bleaker and the Frying Pan showing wider confidence ellipses. In Latin America, all CVA groupings for forewings were clearly separated, with the exception of Chubut and Panguipulli for females, and Chubut and Punta Arenas for males.

(ii) **Discussion**

Differentiation between sites was hampered by low sample size, but CVAs showed significant Mahalanobis distances between most. Traditional morphometrics proved to be less helpful in distinguishing between populations, although a number of Falkland sites, notably Shallow Bay, could be differentiated from others by wing length and $\hat{r}_1$, while in Latin America Rio McClelland was notable for male forewings which were long and had high aspect ratios. It is difficult to reach any conclusions, however tentative, which relate morphological development in wings to environmental factors at the site level. Because of the conservation listing of the butterfly, only a few samples could be taken from the study sites. Most specimens were from museums, with no clarity about where samples were taken, or the habitat in which they were caught. Even in the case of the Falklands, the sites described as Darwin, San Carlos and Shallow Bay could cover a variety of different habitats.

6.4.3 Wing variation attributable to environmental factors

(i) **Findings**

Latin American male wings varied in response to elevation and latitude. Males had shorter wings, higher *AR* and higher $\hat{r}_1$ at higher elevations and latitudes. Linear regression showed elevation to be the most significant factor, based on η_p^2 values. For females, only *AR* showed a significant effect, being higher at higher elevations.

(ii) **Discussion**

Analysis of morphometric response to wind was hampered by the small data set. Linear regressions showed no significant effects, although fitted regression lines on scatter plots showed potential negative correlation between wing size and wind speed at the butterflies' patrolling height in the Falklands, and positive correlation between $\hat{r}_1$ and wind speed. Shorter wings, with resultant lower dispersal power, might be expected from resident populations in windy areas. $\hat{r}_1$ is less clear: higher $\hat{r}_1$ is associated with dispersal, but for other factors, such as temperature, the evidence is contradictory.

6.4.4 Conservation implications

The significant size difference in wing size, of both sexes, between Falklands and Latin American populations is of potential importance in any restoration efforts. The evidence from Bleaker and Sea Lion suggests small wing size might be a response to strong wind; therefore a degree of caution in any reintroduction should be exercised, in accordance with IUCN guidelines (IUCN Wildlife Health Specialist Group 2013). Aardema et al. (2011) made clear the importance of ensuring that reintroductions to augment a declining population, or replace an extinct one, needed to take into account adaptations to local conditions. Schultz et al. (2009), in considering conservation measures for the Puget blue *Icaricia icarioides blackmorei*, noted that specialisation was common among endangered butterflies and often hindered restoration efforts. The nature of adaptation is important in this context: it is not possible, with the present data, to show whether the variation in wing shape is genetic or an example of phenotypic plasticity (Fusco and Minelli 2010, Kelly et al. 2012). As a precaution, in case of an urgent need of reintroduction, a Falklands donor population should be considered first. A captive breeding programme for Falkland butterflies, to ascertain whether, in windless conditions, their wings grow closer in length to the Latin American, would be informative.

6.4.5 Methodology

Wing length has long been used as a proxy for size (Dudley 1990, Kingsolver 1999, Sullivan and Miller 2007, Sekar 2012), particularly in investigating mobility and migration. As, however, Dennis and Hardy (2018) observed, in the context of establishing migration capacity and colonisation ability, comparisons of wing span were not enough. Suitable metrics for further work on wings were investigated in this chapter, with the exception of wing loading, for which sample size was too low.

Wing length was retained as it was an element of the derived metrics *AR* and $\hat{r}_1$ and as a readily understood way of comparing size. Length and width of wing base were strongly correlated and so were dropped. Wing angle and area were correlated with, but less informative than, *AR* and $\hat{r}_1$. The former were therefore dropped, and the latter retained. Traditional morphometrics focused on these three measurements. Comparative studies, important in exploring the underlying forces behind wing shape, were hampered by lack of agreement on how *AR* and $\hat{r}_1$ should be calculated, and by lack of clarity on landmarks used (Van Hook et al. 2012). The metrics used in this chapter were selected as being readily replicable and straightforward to analyse, and are recommended for future investigations.

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Chapter 7: Claw shape and size: variation and wind

7.1 Introduction

Duplouy and Hanski (2013), addressing the question of how the Glanville fritillary, *Melitaea cinxia*, survived on the windswept Baltic island of Pikku-Tytärsaari, found its claws were more sharply curved, and provided a better grip, than those on more sheltered mainland sites. To assess the extent to which claw shape might similarly answer Vallentin's question (6.1), *Yramea cytheris* claws from Falkland Island sites with varying mean wind speeds were compared with each other, and then compared with the contrasts provided by Duplouy and Hanski's *M. cinxia* data set. While claws from seven specimens from the Magallanes region of Chile were used for morphometric comparison between Falkland and Latin American specimens of *Y. cytheris*, the only data on their origins were place names. These were inadequate to link them to any usable wind data.

7.1.1 Claw studies: function, shape and size

Claw studies across taxa have sought to identify and define shapes associated with a range of activities such as climbing, grooming, extracting larvae from tree cavities and catching prey (Cartmill 1974, Milliken et al. 1991, Soligo and Müller 1999, Zani 2000, Birn-Jeffery et al. 2012, Fowler et al. 2009). The potential predictive ability of these studies has been used in palaeobiology, where bird claws were analysed to determine whether *Archaeopteryx* was a ground or tree dweller (Peters and Görgner 1992, Feduccia 1993), and mammal claws to determine the functions of claws in *Therizinosaurus* (Lautenschlager 2014).

A way of characterising claw shape was necessary to enable comparisons. Early studies were verbally rather than mathematically descriptive: thus Miller, in a paper delivered in 1916 (Brakefield and Frankino 2009), contrasted the "strongly curved claw" of a climbing bird with the "relatively straight claw" of a non-climbing bird. The question became how best to describe and define curvature, ideally by a single angle. Approaches included measuring the curve as part of a spiral (Richardson 1942); as part of a parabola (Peters and Görgner 1992), and, most simply, as the arc described by the inner edge of the claw, occasionally supplemented with a comparable measurement of the dorsal curve (Cartmill 1974, Zani 2000, Csermely and Rossi, 2006, Fowler et al. 2009, Burnham et al 2011, Csermely et al 2012). This latter approach was formalised by Feduccia (1993), most elegantly defined by Pike and Maitland (2004) as the angle subtended by radii extending to each end of the arc described by the inner edge of the claw.

Tinius and Russell (2017) compared the various approaches, finding a modified version of Feduccia's formula (Zani 2000) most powerful in describing the claw curve. They nevertheless concluded that the traditional morphometric approach, definable as the direct measurement of linear distance and angles (Adams et al. 2004, Mitteroecker and Gunz 2009), was not adequate for prediction. They advocated instead a geometric morphometric approach, analysing the relationship between sets of morphological landmarks (Rohlf and Marcus 1993); for a review, see Adams et al. (2013).

7.1.2 Insect claws: structure and function

The main areas of focus for insect claw studies have been predation and substrate grip. Predation has generally involved the development of foreclaws to aid seizing and holding prey in e.g. scorpion flies (Mecoptera) (Bornemissza 1966); water bugs (Nepomorpha) (Gorb 1995); the beetle *Philonthus marginatus* (Betz and Mumm 2001); and water bugs and mantises (Mantodea) (Petie and Muller 2007).

Substrate grip can involve the entire tarsal structure, including the arolium. Bräuer et al (2017), investigating the attachment of honey bees and greenbottle flies to petals when pollinating, noted the role of adhesive pads on the pretarsi of both study species; Gladun and Gorb (2007), considering insect attachment to thin stems from ten species across a range of orders (Orthoptera, Heteroptera, Coleoptera and Hymenoptera), again noted the varying use of adhesive pads as well as the flexibility of the arolium and flexion of tarsal claws. Zurek et al. (2017), investigating the beetle *Gastrophysa viridula* focused on the role of the arolium and adhesion, while Pattrick et al. (2018), investigating cockroaches, a stick insect and a leaf cutter ant, looked principally at claw sharpness, raising the question of the interrelation between claw sharpness and other factors in gripping, and the consequent effects of claw morphology on habitat or niche selection. Leaf surfaces, for example, affected tenacity for the bug *Dicyphus errans*, which demonstrated a better grip on hairy leaf surfaces than on smooth or waxy.

There have been few studies of form and function in claws of Lepidoptera. As butterflies and moths are not predatory, claws are likely to assist in the range of functions requiring substrate grip: as an aid to locomotion, for example the female moving on a leaf to identify an oviposition site, or either sex moving into the plant substrate for protection;

for basking, whether on plant, rock, gravel or sand substrate; or for attachment to plant substrate while nectaring or mating. The claw itself appears to be the provider of grip. Betz and Kölsch (2004) found no evidence of adhesive mechanisms in lepidopteral tarsal structures, although Al Bitar et al. (2009), in a study of the codling moth *Cydia pomonella,* found that its smooth and flexible arolium helped provide grip under experimental conditions. Duplouy and Hanski (2013) were the first to try to quantify grip by moving a source of air - in this case a hairdryer on the cold setting - towards the butterfly, recording when its grip failed. They showed that butterflies on a windswept island were able to grip longer than those from more sheltered locations and observed that their claws were more curved.

7.1.3 Research questions

RQ1: what are the key features of the claw morphology of *Y. cytheris*, and do they have implications for wider morphological analysis of butterflies?
RQ2: to what extent does claw morphology differ between populations of *Y. cytheris*?
RQ3: does *Y. cytheris* claw morphology support the hypothesis that claws are more curved in locations with high winds?
RQ4: what are the implications of claw morphology for conservation management of *Y. cytheris*?

7.2 Materials and methods

7.2.1 Study populations

The principal analyses were carried out on samples from the four Falkland Islands study sites: Bleaker Island, the Frying Pan, Roy Cove and Sea Lion Island, with additional material from Latin America (Table 7.1,Table 7.2, Figure 7.1).

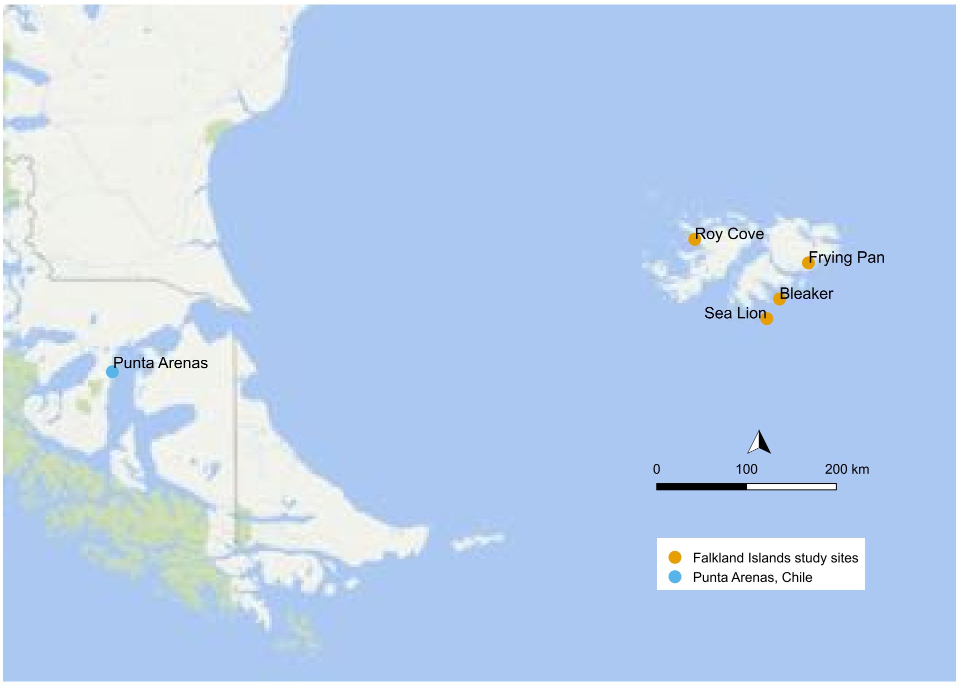

Figure 7.1 Falkland Islands study sites relative to Latin America. Punta Arenas, the source of claws used for the broad comparison of the continental samples of *Yramea cytheris* with the insular, is approximately 600 km from the nearest part of the Falklands archipelago; the shortest distance between Latin America (Isla de los Estados, Argentina) and the Falkland Islands is approximately 400 km.

7.2.2 Claw data sets

(i) ***Yramea cytheris***

The Falkland Islands Government's Conservation Committee allowed eight butterflies to be taken, with no more than two from an individual site, during the first field visit (January-February 2016), and twelve, again with no more than two from a site, during the second (December 2016- February 2017) and third (January -February 2018) visits respectively. The committee gave permission for the removal of one leg from any individual butterfly during the second and third visits to enable analysis of claw curvature and for subsequent DNA work.

Sample claws were also taken from five Latin American specimens in the British Museum of Natural History, London (BMNH). These were representative of the Magallanes region of Chile, centred on Punta Arenas, the nearest continental populations to the Falkland Islands. Two further specimens from Punta Arenas were kindly supplied by Alvaro Zúñiga-Reinoso from his own collection. A field visit to Punta Arenas to try to capture further samples, 3-8 February 2018, was unsuccessful. The combined data set comprised 112 claws (Table 7.1).

Table 7.1. Combined data set of *Y. cytheris* claws by population, sex and leg pair. Falkland Island samples from Bleaker Island, the Frying Pan, Roy Cove and Sea Lion Island were collected during the 2016-2018 field visits; North Arm and San Carlos specimens were from the BMNH. Punta Arenas (Chile) and other Magallanes specimens were from BMNH or were provided by Alvaro Zúñiga-Reinoso.

	female		male		
site	hind	middle	hind	middle	total
Bleaker	7	12	3	7	29
Darwin	0	0	1	1	2
Frying Pan	4	6	5	10	25
North Arm	1	1	1	2	5
Roy Cove	6	7	4	7	24
San Carlos	1	1	0	0	2
Sea Lion	3	3	2	2	10
Punta Arenas	2	3	1	2	8
other Magallanes	2	2	1	2	7
total	26	35	18	33	112

(ii) **Comparative data: *Melitaea cinxia***

Duplouy and Hanski's (2013) data set (Table 7.2) comprised claw surface area and width, together with chord length and curvature (the internal claw angle), both taken from the ventral side. This enabled a comparison of responses of *Y. cytheris* and *M. cinxia* claw shapes to site wind speeds.

Table 7.2. *M. Cinxia* samples, listed by site, in supplementary material to Duplouy and Hanski (2013). No differentiation was made between hind and middle leg pairs, or distal and proximal claws.

site	female	male	total
Åland	22	27	49
Pikku -Tytärsaari	21	21	42
Saaremaa	11	10	21
Uppland	7	8	15

7.2.3 Meteorological data sets

(i) **Falkland Islands**

Wind direction and speed data were taken from the WMO weather stations nearest to each of the four study sites (Table 7.3, Figure 7.2). The exception was Bleaker Island, which has a non-WMO weather station, a MetPak II (Gill Instruments, Lymington, Hants, UK) using PC200W software (Campbell Scientific, UT, USA). WMO weather station data were accessed from the OGIMET website (Valor and López 2017). Bleaker data were supplied by the landowner, Mike Rendell.

Data covered *Y. cytheris's* flying season, taken as the four summer months of November to February, covering the period from November 2013 to February 2018, with the exception of Mount Byron for which only the period from November 2014 to February 2018 was available.

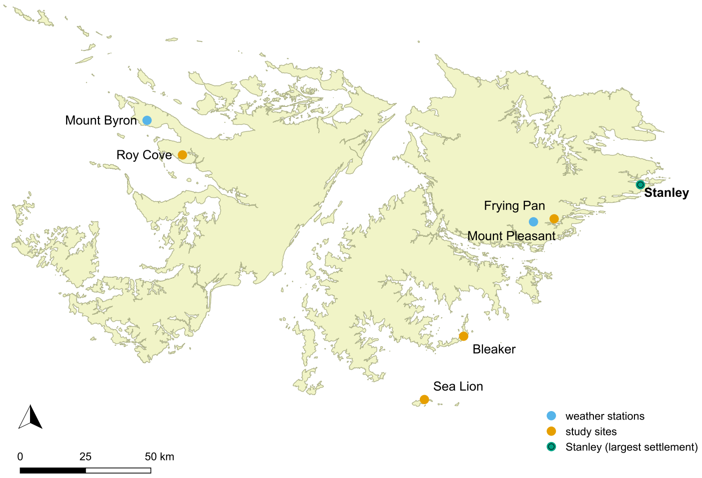

Figure 7.2 Map of the Falkland Islands showing the four *Y. cytheris* study sites and their associated weather stations. Meteorological data for the Frying Pan were taken from Mount Pleasant; Roy Cove data were taken from Mount Byron; Sea Lion and Bleaker Islands had their own weather stations, which were adjacent to the study sites.

Local wind and other environmental readings were taken during the three field visits with a Kestrel 4500 Pocket Weather Tracker (Nielsen-Kellerman, Boothwyn, PA, USA). There were no detailed location records for specimens from Punta Arenas or the surrounding Magallanes Region. As that meant that elevation and the distance between station and site could not be calculated, and that therefore data would be too coarse-grained, no wind data were used from the Punta Arenas weather station (WMO station ID 85934).

(ii) **Baltic sites**

Duplouy and Hanski (2013) correlated their claw measurement data with June wind speeds from Baltic weather stations, contrasting the mainland locations, Jomala Jomalaby and Kumlinge, with the isolated island sites of Lemland Nyhamn, Loviisa Orrengrund and Kotka Haapasaari. To enable comparisons to be made with Falkland Islands, data sets were drawn up from two of those stations. Jomala Jomalaby (WMO ID 02741) on Åland, represented the mainland and large island sites, and Kotka Haapasaari (WMO ID 02967), a small island, represented Pikku-Tytärsaari, which lies 54 km to its south. Data sets covered the period May to August over the five years from 2014 to 2018.

Table 7.3. Falkland Island sites for which *Y. cytheris* claw data were available, together with the nearest weather station. Weather station IDs are represented by WMO indices.

site	weather station	station ID	station location		elevation (m)		station /site (km)
			latitude	longitude	station	site	
Bleaker	Bleaker	NA	-52.20912	-58.85009	15	10	0.2
Frying Pan	Mount Pleasant	88889	-51.82000	-58.44806	74	10	7.9
Roy Cove	Mount Byron	88870	-51.42389	-60.56389	480	50	18.7
Sea Lion	Sea Lion	88897	-52.43333	-59.08333	15	10	0.5
Åland	Jomala Jomalaby	02741	60.17830	19.98640	14	NA	NA
P.Tytärsaari	Kotka Haapasaari	02967	60.28640	27.18500	4	5	54.5

(iii) **The relationship between weather station and site data**

The effect of wind gradient, the variation of wind speed with elevation, has to be taken into account when applying weather station data to study sites. A particular concern for Falkland sites was the 430m difference in elevation between Roy Cove and Mount Byron (Table 7.3) There is no wind speed equivalent of the lapse rate adjustment in temperature based on the International Civil Aviation Organization's standard atmosphere (International Civil Aviation Organization 1993). While logarithmic models have been used to model wind gradient over the sea in the South Atlantic (Sachs 2004, Wakefield 2009), they become more difficult to develop for land-based sites as wind shear is affected by terrain (Ray et al. 2006).

To investigate further the difference in wind speed between Roy Cove and Mount Byron data were recorded hourly using the Kestrel 4500 set up at Roy Cove from 1 to 13 and 22 to 26 January 2017. The data were compared with hourly data from Mount Byron. It was not possible to make a similar comparison between Frying Pan and Mount Pleasant, with a 64m difference, because of the risk of leaving the Kestrel unattended in an area of high public footfall.

(iv) **Meteorological data sets: study sites**

Wind speed measurements were taken at 150cm, approximating to the maximum observed flying height above ground level of *Y. cytheris*; 30cm, approximating to the observed patrolling height; and 3 cm, approximating to the mean height of oviposition locations. All measurements were taken when the wind was blowing consistently from the prevailing direction. These were the same measurements used in habitat analysis (4.2.3, 4.3.2) as the butterfly's flying area closely matched oviposition locations (3.2.3, 3.3.3).

An initial reading was taken at each study site, from a high central point with no topographic obstructions. The Kestrel 4500 was held at shoulder height until the measurement of mean wind speed stabilised. This was recorded as the site speed. The 150cm, 30 cm and 3cm measurements were taken at larval host-plant locations, and measurements were recorded as a proportion of the site speed. To enable comparisons between sites and locations, the data set was rebased to a site measurement of 8.11 ms^{-1}, the mean wind speed of the flying periods 2013-2018. Location proportions were applied to the normalised site measurement to produce a data set of derived wind speeds, assuming a linear relationship between site and location measurements.

7.2.4 Claw measurement

Both traditional and geometric morphometrics were used in this chapter. Traditional morphometrics enabled comparison between *Y. cytheris* and Duplouy and Hanski's (2013) *M. cinxia* data, for which no geometric data were available. They also allowed comparison by size, by definition excluded from geometric morphometrics, to be made.

(i) **Image acquisition**

To minimise the possibility of measurement error, all specimens were photographed, under identical conditions, with the built-in camera of a Keyence VHX-600 microscope at 200x magnification. Images were saved as lossless TIFF files. The process is described in more detail at Appendix D.1

(ii) **Image analysis: traditional morphometrics**

Three points were fixed on each claw: the tip, and the points on the dorsal and ventral curves at their tarsal end where there was a slight outward inflection in the curve (Figure 7.3). These subsequently served as the fixed landmarks in geometric morphometric analysis.

Lines were constructed between the fixed points, envisaged as chords of a circle. These are shown in Figure 7.3 as AB, the ventral chord, and BE, the dorsal. Chord length served as proxy for claw size. The angles which subtended these chords were then constructed following Feduccia (1993). These are shown as angles ACB (ventral) and EFB (dorsal). Duplouy and Hanski (2013) used mathematical variants of these, ADB EGB where, if Feduccia's angle is a° and Duplouy and Hanski's b°, then b=360-2a. Feduccia's angle was used in this chapter, and Duplouy and Hanski's converted accordingly.

The process is described in more detail at Appendix D.2.1.

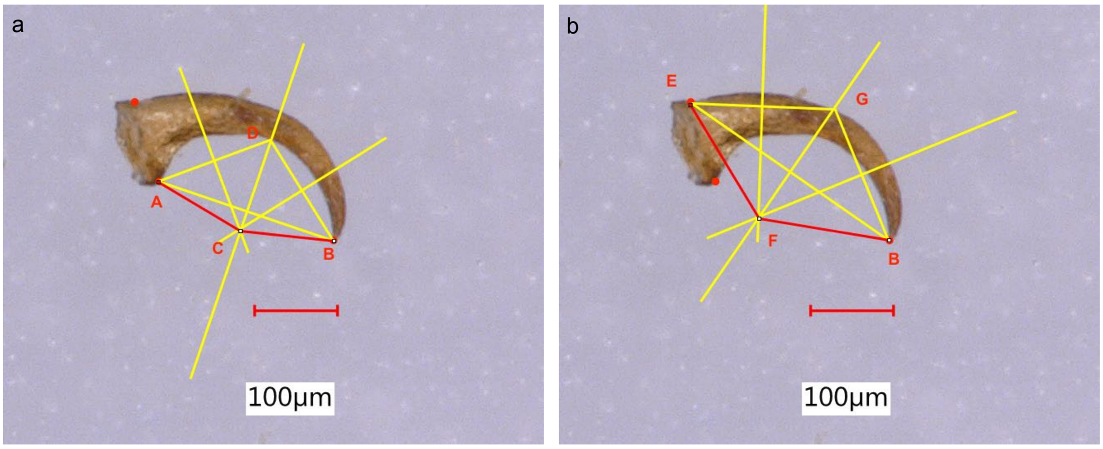

Figure 7.3. Methods of measuring ventral and dorsal angles and chords. (a) shows the ventral angle ACB and chord AB, and (b) the dorsal angle EFB and chord EB. The points A, B and E are the fixed landmarks used for geometric morphometric analysis. The angle subtending the ventral chord, the basis of Feduccia's (1993) shape analysis, is at ACB. The angle used by Duplouy and Hanski (2013) is at ADB. Photographs by the author

(iii) **Image analysis: geometric morphometrics**

The fixed landmarks used in traditional morphometrics (Figure 7.3) formed the basis for geometric morphometric analysis (Figure 7.4). No points on the claw conformed to Bookstein's (1991) preferred type 1 landmarks, that is the discrete juxtaposition of tissues (see also Zelditch et al. 2012). The three landmarks selected conformed with his type 2, locally defined, and part of geometric constructs, and served to anchor the shape of the claw.

Figure 7.4. Placement of landmarks on the claw. The large red points represent the fixed landmarks. The sliding landmarks applied to the dorsal curve, after resampling, are shown as small red points. The blue line without points follows the resampled sliding landmarks on the ventral curve. Photograph by the author.

Sliding landmarks were then added between the fixed landmarks of their respective tarsal junctions and the claw tip. The curve was then resampled, and the number of landmarks reduced to 30 equidistant from each other. Each data set was saved as a single set of landmarks. These were subsequently handled as if they were permanent landmarks (as in Tinius and Russell 2017), and combined into data sets of 59 landmarks (those at the tip from each data set being identical) to enable analysis of the overall shape of the claw. The process is described in more detail at Appendix D.2.2.

7.2.5 Y. cytheris claw morphology

RQs 2-4 (7.1.3) require analysis of study populations, in which, to avoid the pitfall of pseudoreplication, *sensu* Hurlbert (2013), the single experimental unit is the individual butterfly. It is therefore necessary to determine whether an individual can be represented by any one of its claws, the approach of Duplouy and Hanski (2013), or whether there are significant differences, relevant to RQ1, which require subsetting. In studying populations it is also necessary to test for significant sexual dimorphism.

Both sexes of *Y. cytheris* have four pairs of claws, two on each leg, on the hind and middle leg pairs. The front leg pair are much reduced, and clawless, hence the Nymphalidae's informal name of brush-footed butterflies (Ford 1957, Wolfe et al. 2011, Moreira et al. 2017). The claws on the pretarsus of the hind and middle legs of *Y. cytheris* are separated by the arolium (Figure 7.5), from which they slightly curve outwards.

Each claw pair comprises a distal claw, further from the butterfly's body on a given pretarsus, and a proximal, nearer to the body. Given that the middle leg pair points forwards and the rear backwards, it can be seen that, absent any information other than its curvature, the top claw in Figure 7.5 could be the right middle proximal (looking at the butterfly from the dorsal side), the left middle distal, the right hind distal or the left hind proximal.

Analysis of *Y. cytheris's* claw morphology explored differences attributable to sex, to leg pair and to position on the tarsus recognising that different claws might have different functions, for example in basking, feeding or mating, and that shapes might differ accordingly (Burnham et al. 2011). It did not consider bilateral symmetry as the sample size was inadequate.

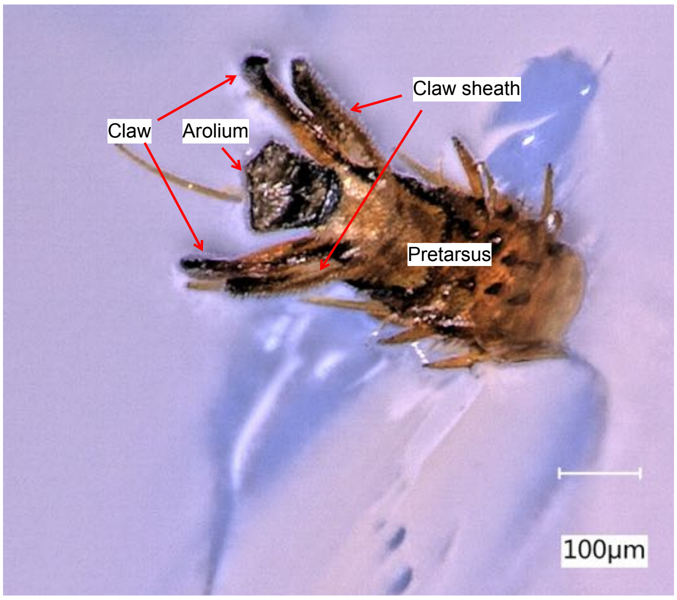

Figure 7.5 Structure of the pretarsus of *Y. cytheris* showing the arolium and the claws and claw sheaths either side of it. Photographed with the built-in camera of a Keyence VHX-600 microscope, using focus stacking. Photograph by the author.

To assess the various contrasts between claws a data subset was drawn up from those Falkland Island butterflies for which both distal and proximal claws in a tarsal pair were available. This comprised samples from 46 leg pairs: 25 female (11 hind, 14 middle) and 21 male (6 hind, 15 middle). It was used to test the effect of the independent variables of tarsal claw pair, leg pair and sex on the dependent variables of, respectively, chord (for size) and angle (for shape).

7.2.6 Comparative data: M. cinxia in the Baltic

Duplouy and Hanski's (2013) experimental approach to analysing *M. cinxia's* ability to grip (7.1.2) was not suitable for *Y. cytheris* for practical as well as legal reasons. Comparisons between *Y. cytheris* and *M. cinxia* were therefore restricted to claw data. *M. cinxia* claw angles and chords were first compared by site, then by Duplouy and Hanski's groupings of small and large islands. *Y. cytheris* data were grouped in the same way, to assess whether they supported a similar division. The small number of Latin American samples were considered at the same time. This helped address RQ2: to what extent does claw morphology differ between populations of *Y. cytheris*?

7.2.7 Claw shape, landscape and wind

The comparative data from the Baltic (Duplouy and Hanski 2013) and the Falklands were tested for correlation with site wind speeds. Wind speeds at three heights at oviposition sites were also compared for the Falklands. This helped address RQ3: does *Y. cytheris* claw morphology support the hypothesis that claws are more curved in locations with high winds?

7.2.8 Data analysis

Measurement error was assessed by calculating the intra-class correlation coefficient (Lessells and Boag 1987, Bailey and Byrnes 1990) in the R package *ICC* (Wolak et al. 2012). The scores were not low enough to give concerns about repeatability. Details are given in Appendix D.3.

TIFF images for angle and chord measurement were processed in *ImageJ* using the *FIJI* platform (Schindelin et al. 2012, Schneider et al. 2012), with the aid of the macro *bissect* [*sic*] (Burri 2016).

TIFF images for geometric morphometrics were processed in the *tps* software suite (Rohlf 2010, 2015). Initial grouping and importing were done in *tpsUtil*. *tpsDig2* was then used to produce landmark files. Subsequent processing of landmark files through Procrustes fits (Mitteroecker and Gunz 2009), the production of covariance matrices, PCAs and CVAs was undertaken in *MorphoJ* (Klingenberg 2011, 2013).

The majority of calculations were done in *R* (R Core Team 2018). The *tidyverse* package, in particular *ggplot2* and *dplyer* (Wickham 2017), provided the principal resource for visualisations, supplemented through *github* (Makiyama 2018). ANOVAs, t-tests, correlations and regressions were carried out using elements of *car* (Fox and Weisberg 2018); *corrplot* (Wei and Simko 2017); *effsize* (Torchiano 2017); *EnvStats* (Millard 2013); *Hmisc* (Harrell 2019); *lmerTest* (Kuznetsova et al. 2017); *lsr* (Navarro 2015); *multcomp* and *PerformanceAnalytics* (Hothorn et al. 2016); and *Psych* (Revelle 2018).

All mapping was done in *QGIS 3.4* (QGIS Development Team 2018).

7.3 Results

7.3.1 *Y. cytheris* claw morphology: chord length

There were significant contrasts in dorsal and ventral chord length between leg pairs, sexes and claw positions on the tarsus (Figure 7.6). As Shapiro-Wilk and Levene's tests showed no violation of the assumption of normality or unequal variances, these were investigated through three-way ANOVAs, with only two-way interactions tested, and dorsal and ventral chord lengths (Figure 7.3) considered separately.

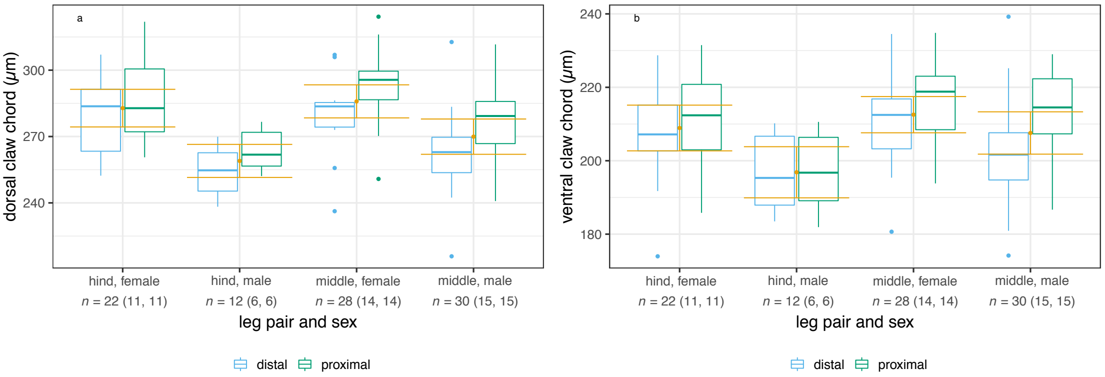

Figure 7.6 Comparison of (a) dorsal and (b) ventral chord length of *Y. cytheris* claws by leg pair, sex and tarsal position. Numbers in brackets refer to distal and proximal claws respectively. There were equal numbers of distal and proximal claws for each combination of claw pair and sex. The orange bars represent the 95% CI. Note the differing y-axis values.

(i) **Dorsal chords**

Female dorsal chords were significantly longer than the male, with a large effect size ($F = 21.07$, $p < 0.001$, $\eta_p^2 = 0.212$); the proximal claw dorsal chord was significantly longer than the distal, though with only a medium effect size ($F = 9.61$, $p = 0.003$, $\eta_p^2 = 0.1$). There was no significant difference in length attributable to claw pair ($F = 2.43$, $p = 0.122$, $\eta_p^2 = 0.028$) (Figure 7.6 (a), Appendix Table D.1 (a)).

(ii) **Ventral chords**

The patterns for ventral chords were similar to those for dorsal chords, although the effect size was, in all cases, small. Female ventral chords were significantly longer than male, ($F = 5.13$, $p = 0.026$, $\eta_p^2 = 0.074$); the proximal claw ventral chord was significantly longer than the distal, (*F* = 5.04, $p = 0.027$, $\eta_p^2 = 0.056$) and again there was no significant difference in length attributable to claw pair (*F* = 4.82, $p = 0.031$, $\eta_p^2 = 0.054$) (Appendix Table D.2 (b), Figure 7.6(b)).

(iii) **Correlation between chord length and forewing length**

The simplest explanation for the variation between male and female claw length - that bigger butterflies have bigger claws - was tested using forewing length (6.3.1(ii)) as proxy for butterfly size (Dudley 1990, Kingsolver 1999, Sullivan and Miller 2007). Female *Y. cytheris* forewings, (*M* = 17.77*, SD* = 1.38) were longer than male (*M* = 16.45*, SD* = 1.4), *t*(138) = 5.56, $p < 0.001$, 95% CI [0.85, 1.79], *d* = 0.94. Forewing length for both sexes was plotted against chord length to investigate any isometric relationship (Figure 7.7).

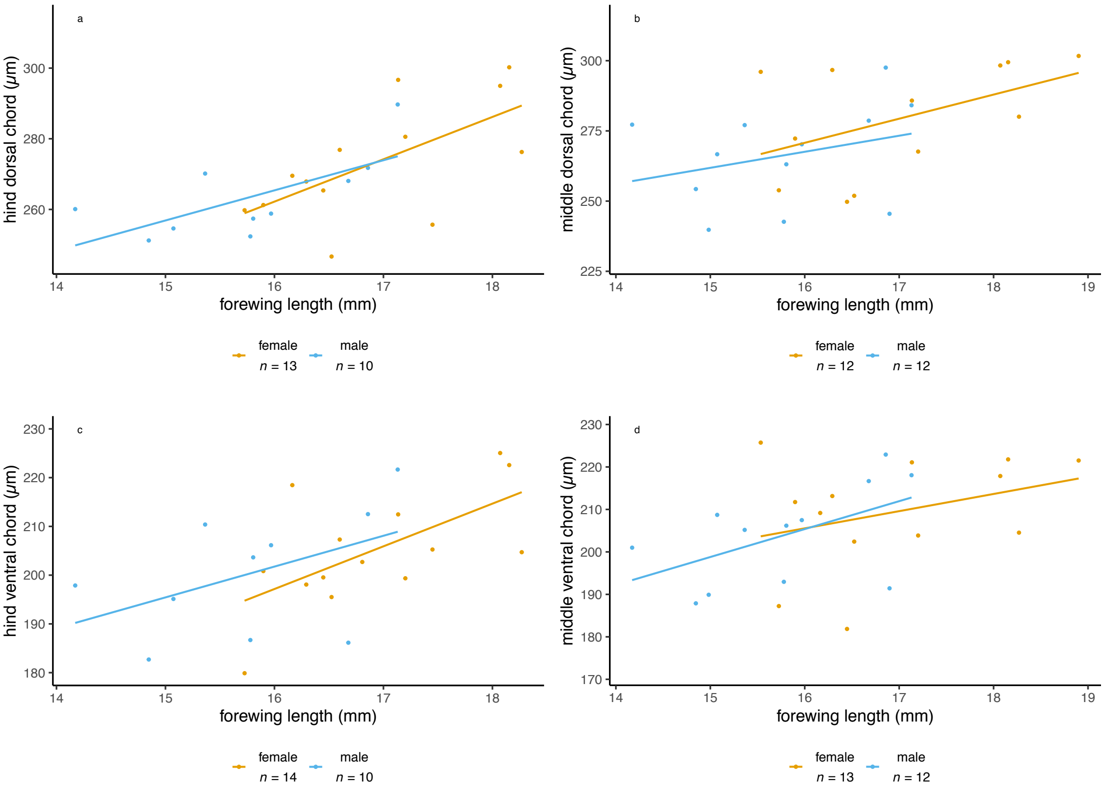

Figure 7.7 Correlation between forewing length and chord length. Forewing length was compared with the lengths of: (a) hind dorsal chord; (b) middle dorsal chord; (c) hind ventral chord; and (d) middle ventral chord for both females and males.

A Spearman rank order test showed forewing length was moderately correlated with ventral chords for both the hind leg pairs (ρ = 0.57, *S* = 988, $p = 0.004$), and the middle (ρ = 0.45, *S* = 1438, $p = 0.026$); and with dorsal chords for both the hind leg pairs (ρ = 0.66, *S* = 682, $p < 0.001$) and the middle (ρ = 0.52, *S* = 1098, $p = 0.01$). This suggested that chord length was correlated with butterfly size, as represented by forewing length, with no evidence that female claws were larger for the butterfly's size than male.

(iv) **Practical implications for later analyses**

Contrasts showing a significance at $p < 0.05$ suggested that any chord data should be subsetted by: sex (dorsal and ventral chords); tarsal pair (middle claw pair, dorsal and ventral chords, although middle female could be pooled); and leg pair (ventral chord, although the effect size was small).

The correlation between chord length and forewing size suggested that chord length could also be used as a proxy for butterfly size. In this case, however, the data set was small, and the need to subset for the female/male contrast diminished already low statistical power beyond utility. Chord length was therefore not considered further in addressing Falkland Island study sites. It was, however, used in comparisons with the *M. cinxia* data set in Duplouy and Hanski (2013), (7.3.5 (ii)) where no distinction was made between claw positions.

7.3.2 Y. cytheris claw morphology: angle

Visualisation of dorsal and ventral angles by leg pair, sex and claw position on the tarsus (Figure 7.8) suggested there were significant contrasts. Levene’s tests enabled homogeneity of variances to be assumed. A Shapiro-Wilk test showed a violation of the assumption of normality for the ventral angle ($W = 0.971$, $p = 0.038$). Mardia's tests subsequently measured skewness for the ventral angle at -0.59 and kurtosis at 0.028, which were moderate. As ANOVAs are robust to moderate skewness and kurtosis (Glass et al. 1972, Spencer et al. 2017), three-way ANOVAs were used in the same way as for chords.

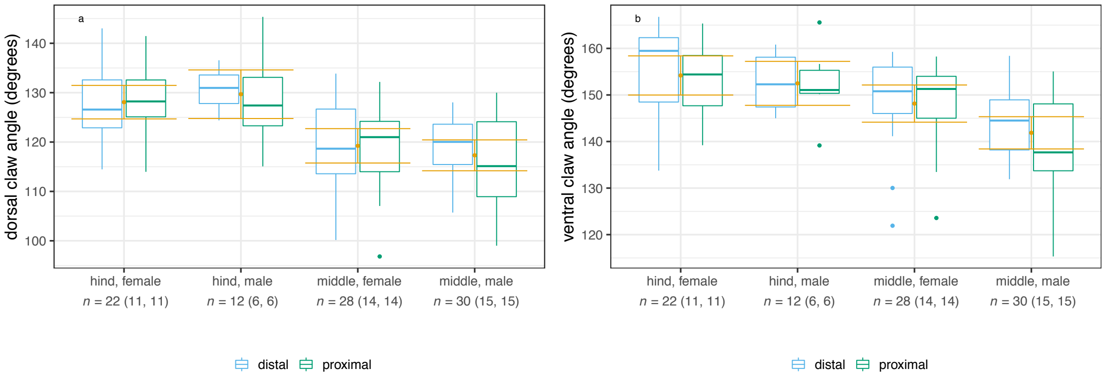

Figure 7.8 Comparison of (a) dorsal and (b) ventral claw angles by leg pair, sex and tarsal position. Numbers in brackets refer to distal and proximal claws respectively. There were equal numbers of distal and proximal claws for each combination of claw pair and sex. The orange bars represent the 95% CI. Note the differing y-axis values.

(i) **Dorsal angle**

Hind claw dorsal angles were significantly greater than middle, with a large effect size ($p < 0.001$, $\eta_p^2 = 0.27$). There was no significant difference in angle attributable to sex or claw position (Appendix Table D.2 (a)). This suggested that hind and middle claws should be treated separately in any subsequent analysis of dorsal angles.

(ii) **Ventral angle**

Hind claw ventral angles were, like dorsal angles, significantly greater than middle, with a large effect size ($p < 0.001$, $\eta_p^2 = 0.145$). Unlike the dorsal angles, however, female middle claw ventral angles were significantly greater than male, though with a small to medium effect size ($F = 8.78$, $p = 0.004$, $\eta_p^2 = 0.059$). There was no significant difference in ventral angle attributable to claw position on the tarsus (Appendix Table D.2 (b)).

A post-hoc Mann-Whitney U test, used because the assumption of normality had been violated for ventral angles, supported the ANOVA results ($p < 0.001$ for the leg pair and $p = 0.003$ for sex). A post-hoc Tukey HSD test showed significant contrasts between the middle male claw and (i) the hind female, $p < 0.001$, 95% CI [-19.27, -5.38], and (ii) the hind male, $p = 0.008$, 95% CI [-19.07, -2.17]. The contrast between middle male and middle female claw angles was not significant ($p = 0.062$, 95% CI [-12.78, 0.22]).

(iii) **Practical implications for subsequent analysis**

Contrasts showing a significance at $p < 0.05$ suggested that any angle data should be subsetted by sex (ventral angle, although the effect size was small) and leg pair (dorsal and ventral angles). The variation between proximal and distal claws was principally of size, as represented by chord length, rather than shape, as represented by angle, suggesting that claws developed isometrically. As the Procrustes fit removed consideration of size, no sub-setting based on claw position was necessary for geometric morphometric work.

7.3.3 Y. cytheris claw morphology: shape (geometric morphometrics)

(i) **Claw position**

PCs 1 and 2, contributing 69.4% to the PCA, showed no significant difference in shape between distal and proximal claws (Figure 7.9), with the two 95% confidence ellipses of means centred on the origin. The data sets for subsequent analyses were therefore adjusted so that, where data for both distal and proximal claws from the same tarsus were available, the leg was represented by the mean of the two.

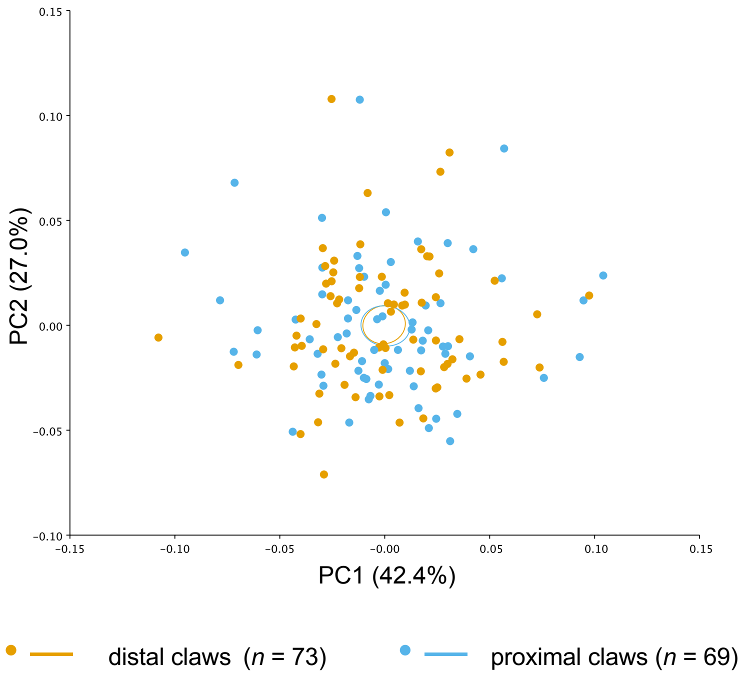

Figure 7.9 PCA of shape variation associated with distal and proximal claws. The percentage of the analysis contributed by each PC is recorded on the appropriate axis. 95% confidence ellipses of means are shown in the colour of their respective claw positions.

(ii) **Leg pair**

PCs 1 and 2, contributing 70.3% to the PCA, showed significant variation between hind and middle claw pairs (Figure 7.10). Middle claws tended towards the target shape of both PC1 (45.3%), a slight narrowing of the ventral surface and thinning towards the distal end of the claw, and PC2 (25.0%), an overall flatter shape, with a less curved ventral surface.

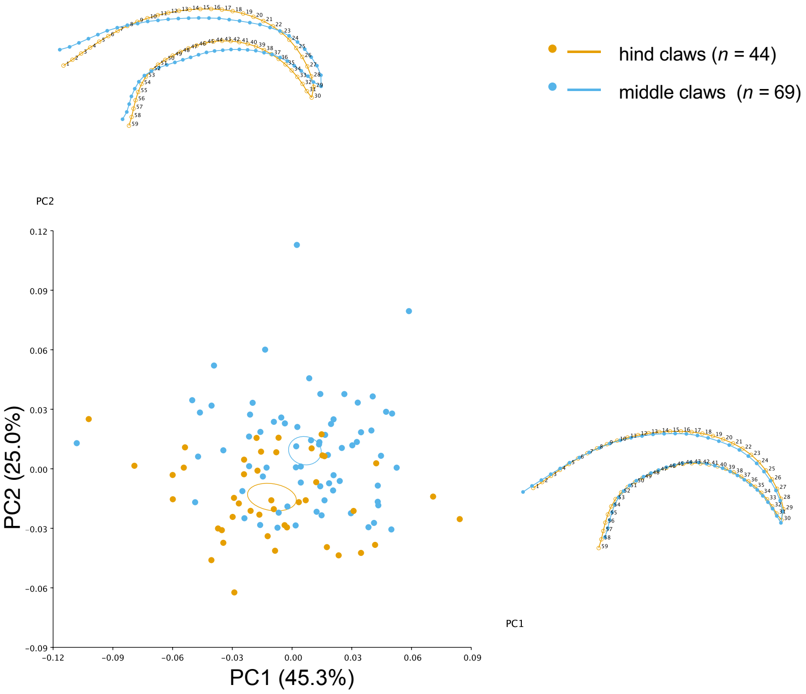

Figure 7.10 PCA of shape variation associated with leg pair (hind or middle). The percentage of the analysis contributed by each PC is recorded on the appropriate axis. Sex was pooled. 95% confidence ellipses of means are shown in the colour of their respective claw pairs. The wire frame diagrams of claws show, in blue, the target shape of the PC on each axis, compared with the mean shape, in orange.

(iii) **Sex**

PCs 1 and 2, contributing 71.7% to the PCA, showed no clear variation between female and male hind claws (Figure 7.11), with the two 95% confidence ellipses of means overlapping on the origin. There was a similar pattern for middle claws (Figure 7.12), with PCs 1 and 2, contributing 68.1% to the PCA, again showing no clear variation between male and female.

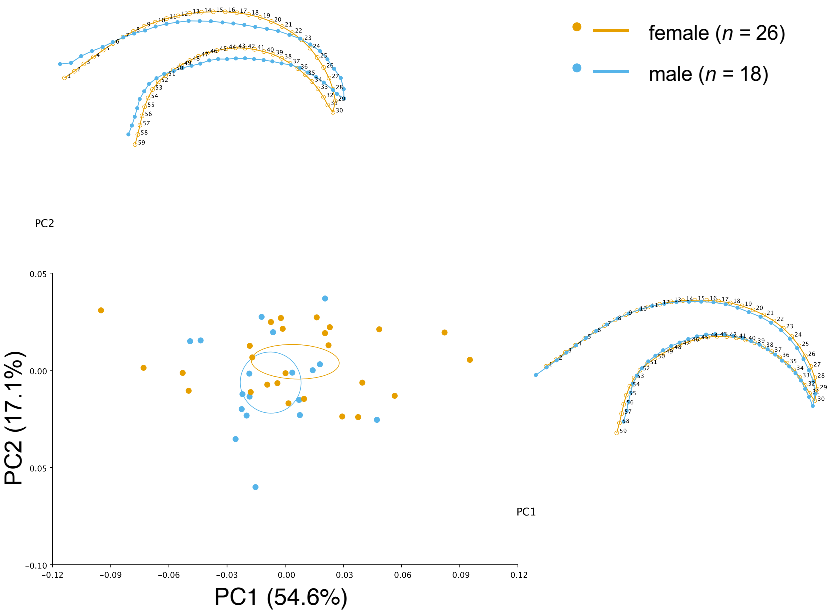

Figure 7.11 PCA of shape variation between female and male hind claws. The percentage of the analysis contributed by each PC is recorded on the appropriate axis. Claw position on the tarsus was pooled. 95% confidence ellipses of means are shown in the colour of their respective sexes. The wire frame diagrams of claws show, in blue, the target shape of the PC on each axis, compared with the mean shape, in orange.

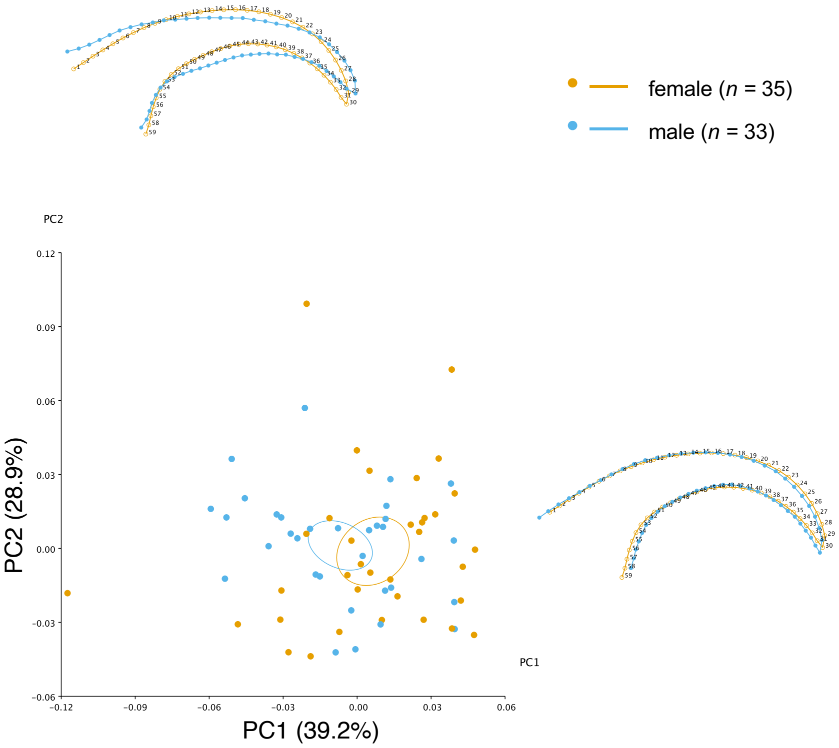

Figure 7.12 PCA of shape variation between female and male middle claws. The percentage of the analysis contributed by each PC is recorded on the appropriate axis. Claw position on the tarsus was pooled. 95% confidence ellipses of means are shown in the colour of their respective sexes. The wire frame diagrams of claws show, in blue, the target shape of the PC on each axis, compared with the mean shape, in orange.

(iv) **CVA of leg pair and sex**

To better understand the contrasts in shape attributable to sex and leg pair, the four possible combinations were subjected to a CVA. CVs 1 and 2, contributing 84.8% to the CVA, showed clear separation between the combinations (Figure 7.13). Male hind claws tended very strongly towards the target shape of CV1 (62.9%), a slightly more hooked shape with a raised dorsal curve, with the other three combinations tending away from the target shape towards being more open. Both female and male middle claws tended slightly towards the target shape of CV2 (21.9%), a more open claw without pronounced dorsal or ventral curves in the middle section; male hind claws tended strongly away from it. Mahalanobis distances between all combinations were significant at $p < 0.0001$, Procrustes distances were significant at $p < 0.05$ for all contrasts other than between female and male claws from either leg pair (Appendix Table D.3).

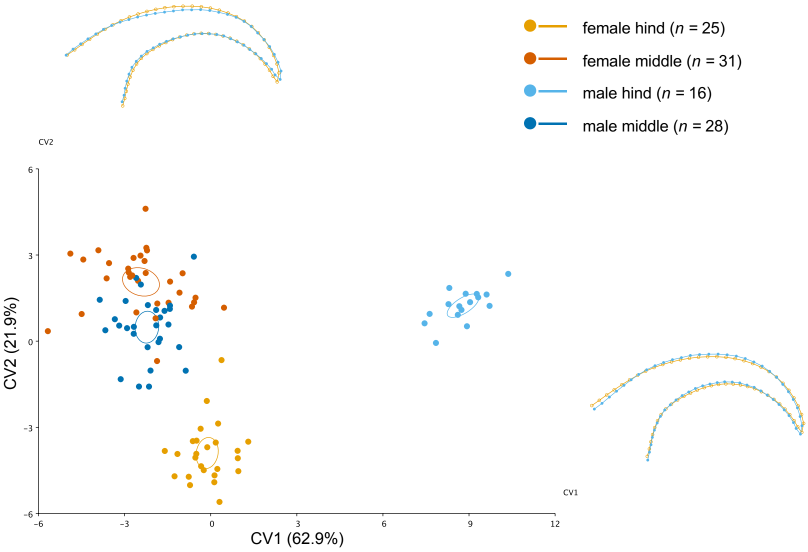

Figure 7.13 CVA of claw shape variation by sex and claw pair. The percentage of the analysis contributed by each CV is recorded on the appropriate axis. A mean was used in cases where two claws were from the same tarsus. 95% confidence ellipses of means are shown in the colour of their respective sexes. The wire frame diagrams of claws show, in blue, the target shape of the CV on each axis, compared with the mean shape, in orange.

7.3.4 Variation in Y. cytheris claws in Falkland Islands sites

(i) **Angles**

A between-sites analysis of both dorsal and ventral claw angles showed similar patterns (Figure 7.14 (a) and (b)). Male and female samples were pooled in both cases. The significant difference in middle claw ventral angles (7.3.2(ii)), albeit with a small effect size ($\eta_p^2 = 0.059$), means those results should be treated with caution.

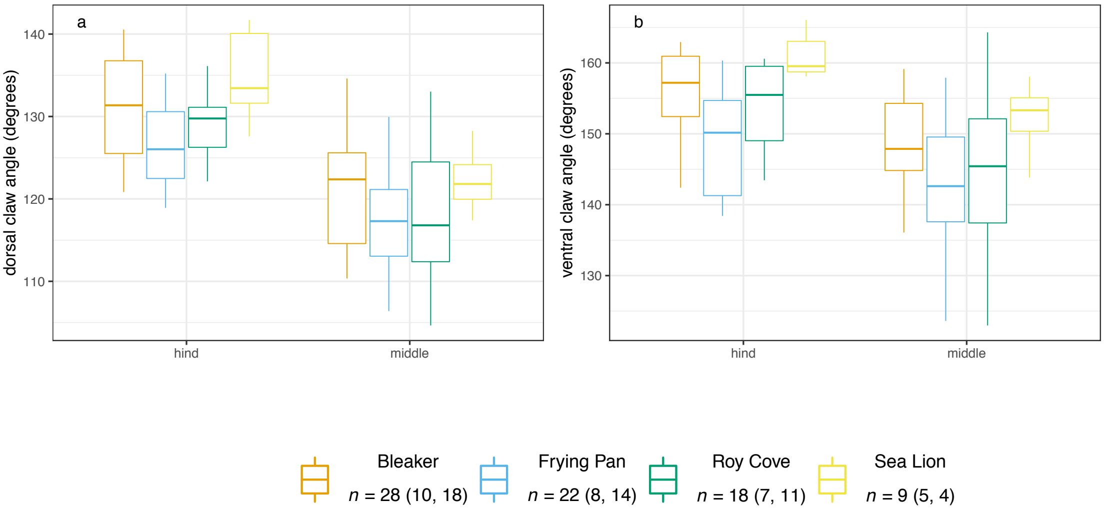

Figure 7.14 Claw angles of *Y. cytheris* in the Falkland Islands by site. For both dorsal (a) and ventral (b) angles, hind and middle claws were analysed separately, with females and males pooled. Numbers in brackets refer to hind and middle claws respectively. Note the differing y-axes.

One-way between-groups ANOVAs, however, showed that only one contrast was significant at $p < 0.05$. That was for the ventral angle of the hind claw, F (3, 27) = 3.85, $p = 0.02$, with a very large effect size, $\eta_p^2 = 0.299$. Post-hoc comparison using the Tukey HSD test showed that Sea Lion (161.09, 95% CI [156.09, 165.28]) had a greater angle than Frying Pan (148.75, 95% CI [141.92, 155.58]), $p = 0.014$.

(ii) **Geometric morphometrics**

CVAs of hind and middle claws showed clear separation between the four Falkland study sites.

Hind claw pair

Bleaker and Sea Lion tended towards the target shape for CV1 (Figure 7.15) a more inwardly-curved ventral surface, with the Sea Lion samples particularly advanced along that axis. Frying Pan and Roy Cove tended away from the target shape. Frying Pan tending towards the target shape, a flatter claw, in CV2.

Mahalanobis separations were significant at $p < 0.001$ (Tabachnick and Fidell 2013) between Sea Lion and each of the other sites, and at $p = 0.001$ between Bleaker and Frying Pan. No Procrustes distances were significant at $p < 0.05$ (Appendix Table D.4).

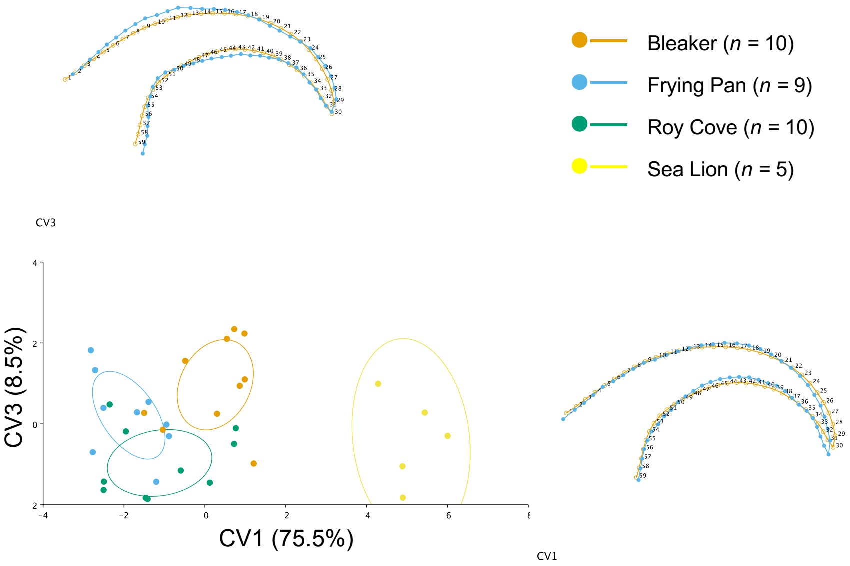

Figure 7.15 CVA results of hind claws from the four Falkland Islands study sites. Sex was pooled. 95% confidence ellipses of means are shown in the colour of their respective sites.

Middle claw pair

Sea Lion claws tended strongly towards the target shape of CV1 (Figure 7.16), a more hooked shape at the distal end, and a thickening at the proximal end. Bleaker claws tended away from the target shape of CV1, though, like Sea Lion claws, they tended towards the target shape of CV2 (28.5%), a very curved claw. Frying Pan claws were centred on the origin, while Roy Cove claws tended strongly away from the hooked shape of CV2. All Mahalanobis contrasts were significant at $p < 0.001$, although no Procrustes distances were significant at $p < 0.05$ (Appendix Table D.5).

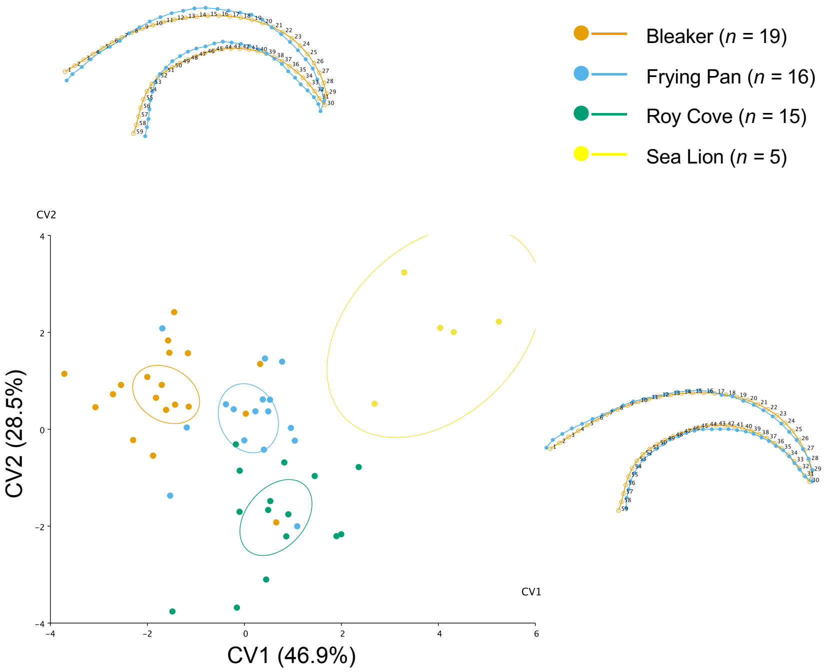

Figure 7.16 CVA results of middle claws from the four Falkland Islands study sites. Sex was pooled. 95% confidence ellipses of means are shown in the colour of their respective sites.

7.3.5 Baltic comparison: Melitaea cinxia

Duplouy and Hanski (2013) compared *M. cinxia* data from a small island population, Pikku-Tytärsaari, with three other populations from mainland or large island sites, Åland, Saaremaa and Uppland. They further compared Pikku-Tytärsaari, as an isolated island, with the data from the other three sites pooled as mainland sites. They drew no distinction between hind and middle leg pairs. They also drew no distinction between male and female, but their supplementary data, used in this analysis, allowed for separation by sex.

(i) ***M. cinxia* at the site level**

Size

Duplouy and Hanski (2013), considering the ventral chord length of a pooled male and female sample, found no significant difference between the populations.

A reanalysis of their data, taking into account sex, showed that the female ventral chord was, on average, significantly longer than male, with a medium effect size (*F* = 8.09, *p* = 0.005, η_p^2 = 0.067). This showed the same relationship with forewing length as did the Falkland samples: the mean forewing length for *M. cinxia* has been measured at 16.2 mm for a female, and 14.5 mm for a male (Breuker et al. 2007).The contrast for site was significant, with a large effect size (*F* = 6.3, *p* < 0.001, η_p^2 = 0.145) (Figure 7.17, Appendix Table D.6).

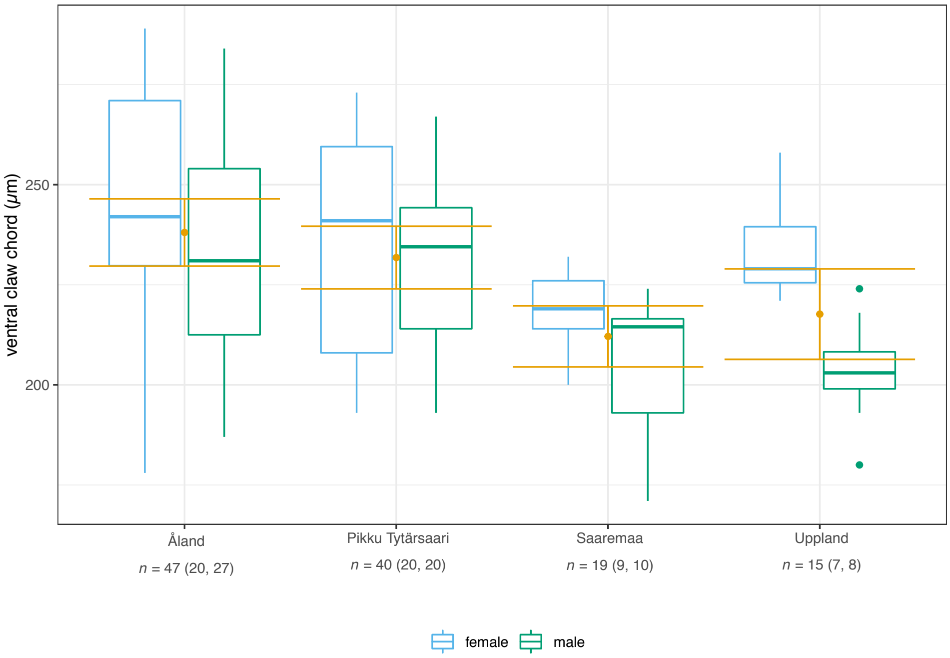

Figure 7.17 Comparison of ventral claw chord length of *M. cinxia* by sex and site. Data are from the supplementary material to Duplouy and Hanski (2013), and do not distinguish between hind and middle claws. The orange bars represent the 95% CI. Numbers in brackets refer to female and male claws respectively.

A Tukey HSD test showed significant differences between Saaremaa and Åland (*p* = 0.001); Uppland and Åland (*p* = 0.025) and Saaremaa and Pikku-Tytärsaari (*p* = 0.027). When the data was subset by sex to account for the observed difference in chord length, there was no significant difference at *p* < 0.05 between sites for female claws, but there was between males in the same pairings: Saaremaa and Åland (*p* = 0.009); Uppland and Åland (*p* = 0.009) and Saaremaa and Pikku-Tytärsaari (*p* = 0.041).

Angle

A plot of the ventral angle, redrawn from Duplouy and Hanski (2013), showed a significant variation in angle size, with medium effect, attributable to site (F = 3.89, p = 0.011, ηp2 = 0.097). There was no significant difference in angle attributable to sex or the interaction of site and sex (Appendix Table D.7). Post-hoc comparison using the Tukey HSD test showed a significant difference in the ventral angle between specimens from Pikku-Tytärsaari and Åland (p = 0.01) (Figure 7.18); no other differences were significant.

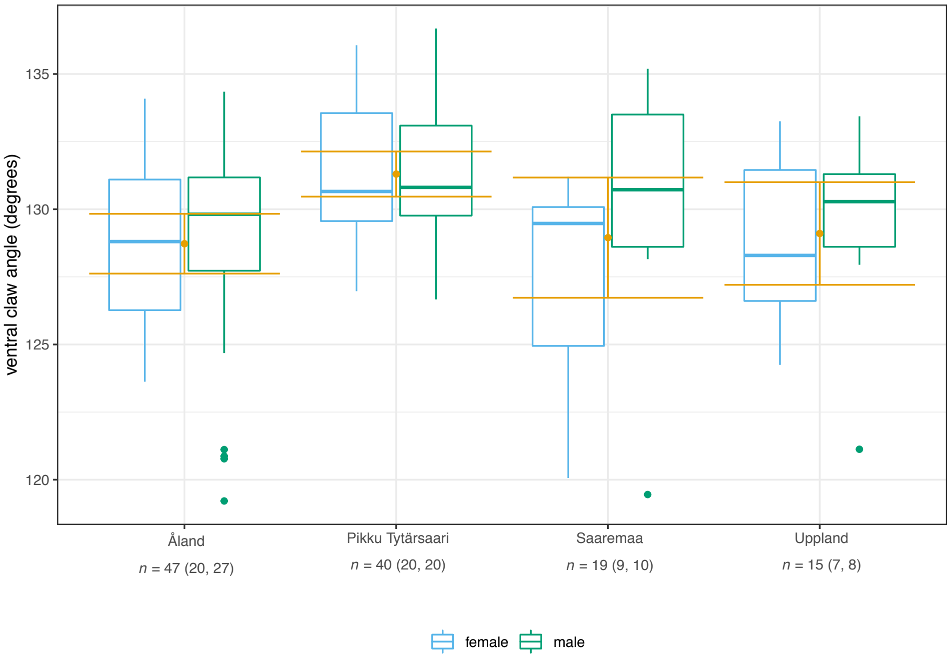

Figure 7.18 Ventral claw angles of *M. cinxia* by site and sex. Redrawn from Duplouy and Hanski (2013), following their pooling of hind and middle claws. Angle calculations follow Feduccia (1993). The orange bars represent the 95% CI. Numbers in brackets refer to female and male claws respectively.

(ii) **Claw morphology of *Y. cytheris* and *M. cinxia* compared at the landscape level**

Duplouy and Hanski (2013) distinguished between *M. cinxia* claws from small island sites, represented by Pikku-Tytärsaari, and mainland or large island sites, represented by Åland, Saaremaa and Uppland.

In this reanalysis *Y. cytheris* data were compared with *M. cinxia* data at the landscape level to assess their support for such a classification. Bleaker and Sea Lion were selected as small island sites, Frying Pan and Roy Cove as large (Chapter 2). The small number of samples from the Magallanes region were also included in case they showed a significant divergence: that not being the case, they are included in Figure 7.19 and Figure 7.20, but not considered further.

Size

The significant contrasts in the Falklands data set for each of sex, tarsal position and leg pair, combined with a limited sample size, made it difficult to draw inferences from chord size. While such contrasts might not apply to *M. cinxia*, they suggested at least a degree of caution, given that the Duplouy and Hanski data set did not take tarsal position or leg pair into account.

A one-way ANOVA showed that female *M. cinxia* chords (*M* = 236.3, *SD* = 26.2) were, on average, larger than male (*M* = 224.1, *SD* = 24.9); *F* (1, 119) = 6.88, *p* = 0.01, 95% CI [-21.42, -2.99], although with a small effect (*d* = 0.478), a similar pattern to that for *Y. cytheris* (Appendix Table D.10).

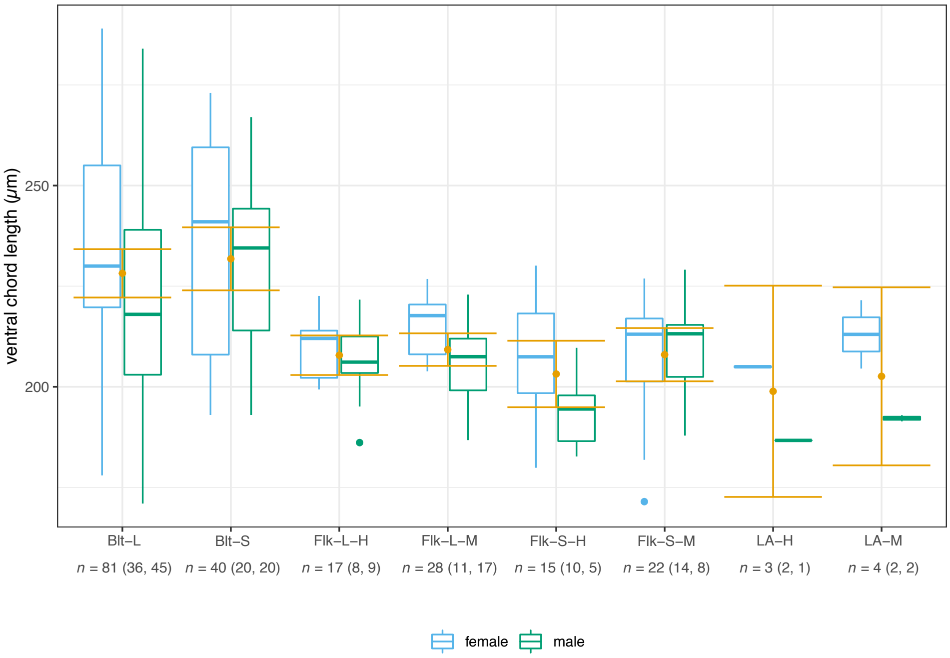

Figure 7.19. Comparison of ventral claw chords of *M. cinxia* in the Baltic with *Y. cytheris* in the Falklands and Latin America. Blt = Baltic populations of *M. cinxia*, Flk = Falkland Island populations of *Y. cytheris*, LA = Latin American (in this case Magallanes) population of *Y. cytheris.* L = mainland or large islands; S = isolated or small islands. H = hind leg pair, M = middle leg pair. Numbers in brackets refer to female and male claws respectively. Small islands in the Baltic were represented by Pikku-Tytärsaari, and in the Falklands by Bleaker and Sea Lion.

Leaving aside the statistically inconclusive Latin American evidence, the only significant contrast suggested by the visualisation (Figure 7.19) was that male hind claw chords on small islands in the Falklands were shorter than those on larger sites, although a subsequent ANOVA did not find the difference significant $F(1, 12) = 3.9$, $p = 0.072$, 95% CI [-24.48, 1.2].

Shape

Duplouy and Hanski found a significant difference between the ventral claw angles of small islands and mainland landscapes (Figure 7.20). A one-way ANOVA using their data showed that angles for Pikku-Tytärsaari samples ($M = 131.3$, $SD = 2.61$) were, on average, larger than those for the mainland ($M = 128.8$, $SD = 3.88$) at a high level of significance, $F(1, 119) = 13.06$, $p = 0.0004$, 95% CI [1.11, 3.8], though at only a medium effect size, $d = 0.698$ (Appendix Table D.9). Sex was pooled, as the distinction between male and female claws was not significant at $p < 0.05$.

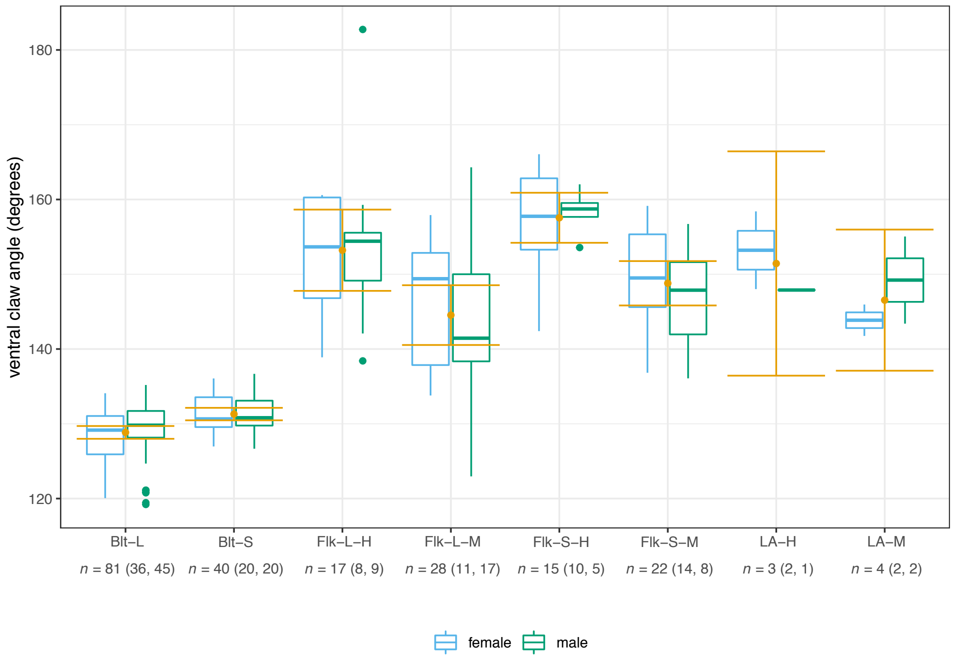

Figure 7.20. Comparison of ventral claw angles of *M. cinxia* in the Baltic with *Y. cytheris* on the Falklands and in Punta Arenas. Blt = Baltic populations of *M. cinxia*, Flk = Falkland Island populations of *Y. cytheris*, LA = Latin American (in this case Punta Arenas) specimens of *Y. cytheris.* L = mainland or large islands; S = isolated or small islands. H = hind leg pair, M = middle leg pair. Numbers in brackets refer to female and male claws respectively. Small islands in the Baltic were represented by Pikku-Tytärsaari, and in the Falklands by Bleaker and Sea Lion.

The distinction between small islands and the two larger islands in the Falklands was not significant at $p < 0.05$ for either hind or middle claws. While the data set for Latin

America was too small to be informative, it provided no evidence for anything unexpected.

7.3.6 Relationship between wind speeds and claw shape

(i) **Wind speeds: Falkland Island weather stations**

Wind speed data from Falkland Island weather stations (Figure 7.21(a)) showed considerable variation. Mean wind speeds over the November - February flying season were: Bleaker 6.85 ms^{-1} (*SD* = 2.13); Mount Byron 9.35 ms^{-1} (*SD* = 2.88), Mount Pleasant 8.38 ms^{-1} (*SD* = 2.44), Sea Lion 7.47 ms^{-1} (*SD* = 2.78).

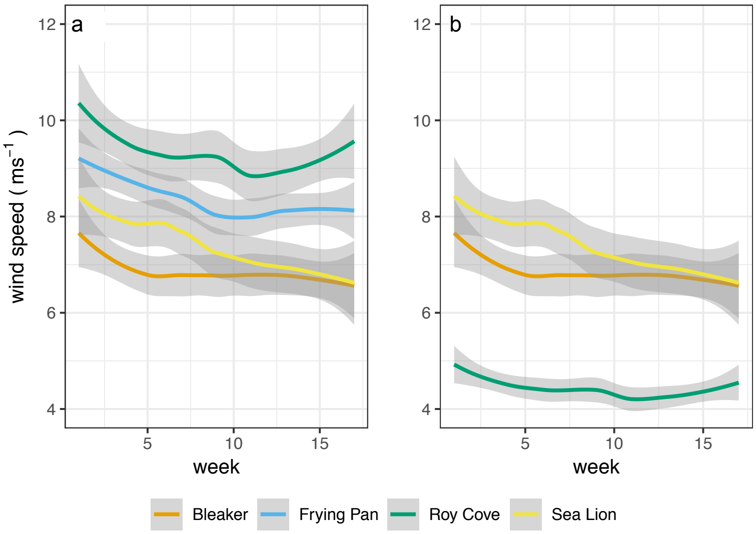

Figure 7.21 Mean weekly wind speeds during *Y. cytheris's* flying season. This was taken as November-February. Initial data (a) were from daily readings at 1200 from the four weather stations nearest to the Falkland Island study sites (Mount Pleasant for Frying Pan, Mount Byron for Roy Cove). Wind speeds were then adjusted (b). Mount Byron data were multiplied by 0.47, reflecting the wind shear effect on Roy Cove, 430m lower. Data from Mount Pleasant were omitted as the effect of wind shear on the Frying Pan could not be calculated. The grey shaded areas represent the standard error of the fitted regression line after loess smoothing.

These data did not take wind gradient into account. Hourly data for Roy Cove for 1-13 and 22-26 January 2017, was compared with equivalent data from Mount Byron. A Shapiro-Wilk test showed that in each case the assumption of normality was violated (Roy Cove W = 0.985, p = 0.002; Mount Byron W = 0.965, p < 0.001), therefore Spearman's rank order was used to test for correlation. Spearman's ρ, at 0.44, showed a medium correlation, p < 0.001. A comparison of medians, to take account of the non-parametric distribution, showed Mount Byron had a median wind speed of 8.75 ms^{-1} (*IQR* = 3.62), and Roy Cove 4.16 ms^{-1} (*IQR* = 2.32). Field data were not taken over a long enough time period to enable comparison of Frying Pan with Mount Pleasant.

To visualise adjusted wind speeds, (Figure 7.21 (b)), Mount Byron data were multiplied by 0.47 (the median of Roy Cove divided by the median of Mount Byron). Frying Pan and Mount Pleasant data were removed.

(ii) **Wind speeds: oviposition sites**

Visualisation of site wind speed data at the four Falkland Island sites, based on a site measurement of 8.11 ms^{-1} (7.2.3(iv)), showed a wide range of medians, with Bleaker and Sea Lion having particularly high winds (although Sea Lion's sample size was very small).

Levene's test showed violation of homogeneity of variance at 150cm. Accordingly, the Kruskal Wallis test was used to consider differences between sites. This showed significant variance at 150cm ($\chi^2 = 13.23$, *df* = 3, *p* = 0.004) and 30cm ($\chi^2 = 14.63$, *df* = 3, *p* = 0.002), although not at 3cm ($\chi^2 = 1.55$, *df* = 3, *p* = 0.67).

Given the uneven number of samples, Dunn's test was used post-hoc to identify significant between-site variation (Zar 2013), and Bonferroni's correction was applied to control the familywise error rate. Using the adjusted *p*-values, at 150cm the differences between Bleaker and Frying Pan were significant at *p* = 0.027, and between Sea Lion and Frying Pan at *p* = 0.014. At 30cm the differences between Bleaker and Frying Pan were significant at *p* = 0.008, and between Sea Lion and Frying Pan at *p* = 0.019.

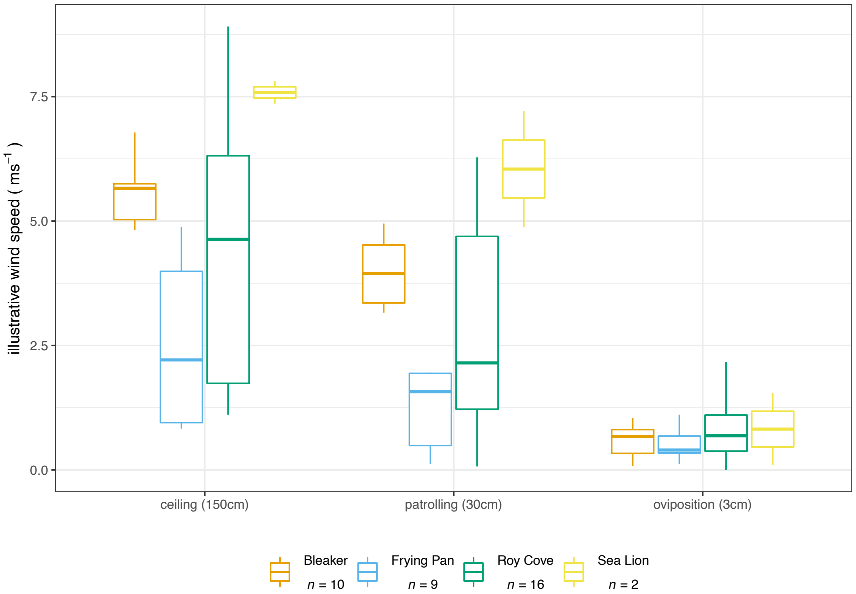

Figure 7.22 Wind speeds at oviposition locations, measured at the butterfly's perceived ceiling, patrolling and oviposition heights. Wind speeds were measured against a normalised site speed of 8.11 ms^{-1}, the mean noon wind speed for November-February for the four Falkland Island weather stations over the five flying seasons 2013 - 2018

(iii) **Relationship between claw angle and wind speed**

The mean claw angle at each of the four Falkland sites was compared with the mean wind speed for each of the parameters. A larger claw angle, that is to say a more curved claw, was positively correlated with higher wind speeds at ceiling and patrol level (Appendix Table D.8). For the ventral angle, the measurement used in Hanski and Duplouy (2013), the hind claw correlation at ceiling height was $t(2) = 15.10$, $r = 0.996$, 95% CI [0.80, 1.0], $p = 0.004$ and the middle claw $t(2) = 6.79$, $r = 0.979$, 95% CI [0.30, 1.0], $p = 0.021$. At patrolling height, the hind claw correlation was $t(2) = 13.71$, $r = 0.995$, 95% CI [0.76, 1.0], $p = 0.005$ and the middle claw $t(2) = 8.23$, $r = 0.98$, 95% CI [0.46, 1.0], $p = 0.014$. There was no correlation at 3 cm. A comparison between Figure 7.14 and Figure 7.22 shows the similarities more graphically.

(iv) **Wind speeds: comparison between Falkland and Baltic sites**

Wind speed data from Kotka Haapasaari, representing Pikku-Tytärsaari, and Jomala Jomalaby, representing mainland sites, were compared for the May to August flying period over 2014-2018. Data from both weather stations were used by Duplouy and Hanski (2013). Their respective daily means were plotted and compared with the mean midday daily wind speeds of all Falkland Island weather stations for the *Y. cytheris* November to February flying period from 2013 to 2018.

The Falklands had consistently higher wind speeds (8.11 ms^{-1}, *SD* = 2.72) than Kotka Haapasaari (5.43 ms^{-1}, *SD* = 2) (Figure 7.23). The Åland site of Jomala Jomalaby (3.49 ms^{-1}, *SD* = 1.18) had consistently lower wind speeds than Kotka Haapassari. These findings were comparable with those used by Duplouy and Hanski over a different period of time, of 5.61 ms^{-1} and 3.92 ms^{-1}.

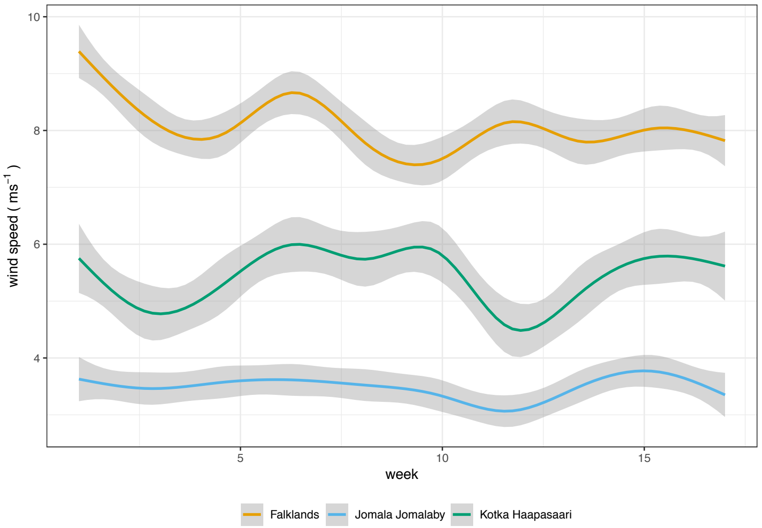

Figure 7.23 Comparison of daily wind speeds over a four month flying period for Falkland Island and Baltic sites. The flying period was taken as November - February for the Falklands, and May - August for the Baltic sites. The Falkland Islands were represented by the mean of daily readings at 1200 from Bleaker, Mount Byron, Mount Pleasant and Sea Lion from 2013 to 2018. The data sets for Jomala Jomalaby, on Åland, and Kotka Haapasaari, taken by Duplouy and Hanski as the nearest reference point for Pikku-Tytärsaari, cover 2014 to 2018.

7.4 Discussion

Yramea cytheris claws showed a number of significant variations. There were contrasts in size and shape related to sex, leg pair and position on the tarsus, which are considered here as functional aspects of the butterfly's structure. There were also contrasts in shape between comparable claws related to geographical origin, which are considered as a response to environmental conditions, specifically wind. It was not possible to say whether variation was genetic or based on phenotypic plasticity, but any restoration efforts which involve reintroductions need to consider its implications.

7.4.1 Claw variation based on function

(i) **Findings**

Female claws from both the hind and middle leg pairs were larger than male. Proximal claws were larger than distal in the tarsal pairs from both the hind and middle legs. Hind claws in both sexes were more sharply curved than middle claws, and female middle claws were more curved than male.

(ii) **Discussion**

No studies were found which described differences in claw shape or size in a single insect species based on claw position or the insect's sex. Duplouy and Hanski's (2013) investigation of claw grip in *Melitaea cinxia* was the first to consider variation in claw shape as a response to environmental conditions, in their case wind, although without considering sex and claw position. The two principal functions of insect claws, discussed in 7.1.2 are predation and substrate grip. As *Y. cytheris* is not predatory, investigation of variation focused on how individual claws might function in providing grip in whatever circumstances it was required.

The variation between female and male claw size, however, fell outside this approach, as it appeared to reflect sexual size dimorphism (SSD) rather than a differing requirement for grip (other than that bigger butterflies might benefit from bigger claws). Females, taking forewing length as proxy for body size (Dudley 1990, Kingsolver 1999, Sullivan and Miller 2007), were larger than males, something Teder and Tammaru (2005), in their investigation of SDD in insects, noted in over 80% of the species they studied. The correlation between claw and forewing size suggested that claws grew isometrically with the butterfly, irrespective of sex. SSD is considered more widely, in connection with wings, in Chapter 6.

Analyses of other contrasts are less straightforward. Claw function is an aspect of the function of legs as a whole; these are an integral part of the insect's flight mechanism, as shown by the termination of some flight muscles within the first and second leg segments (Dudley 2002). The development of legs and wings as an aspect of a butterfly's *Bauplan* (Gould and Lewontin 1979) was explored by Heers and Dial (2015), who showed that there was a developmental trade-off between legs and wings in birds in response to different environments. So, for example, ground dwelling pheasant pigeons had stronger legs, but weaker wings, than long-distance flying rock doves. Their findings, that in circumstances where trade-offs could be harmful they could be offset by cooperative use of legs and wings, suggest a way in to the analysis of butterfly claws.

Stoneflies and mayflies have been shown to use legs and wings cooperatively in their flight pattern, the surface skimming of water (Marden and Kramer 1994, Marden et al. 2000). But even the process of getting airborne, with legs forcing the insect into the air while wings gradually took over, requires cooperation, as shown in the study by Bimbard et al. (2013). of the butterfly *Pieris rapae*. The opposite challenge, that of avoiding getting airborne inadvertently, is equally likely to require legs and wings to be used cooperatively. Laminar air flow over wings can be expected to help keep butterflies on the substrate when facing into the wind. Facing away from the wind, airflow over the trailing edges of wings is likely to make the butterfly more unstable. Therefore, the finding that hind claws, which would help anchor a butterfly in a tailwind, were more curved than middle claws, would be a predictable aspect of a butterfly Bauplan, with the potential for adaptation in response to windier environments (Shreeve et al. 2009, Van Dyck and Windig 2009). This might be investigated further by considering the role of the leg as a whole in providing stability, as grip is provided by the entire tarsal (Dai et al. 2002) and leg (Spilman 1966) structure, rather than just the claw. The identification of potential key points on legs, and then tracking them on camera (Bimbard et al. 2013) would be a helpful first step.

Understanding of the remaining contrasts would also benefit from video recording. The larger size (although with the same shape) of the proximal claw on the tarsus suggests it has a greater role in gripping than the distal, but how it is deployed is unknown. Contrasts based on sex, here the more curved nature of the female claw, suggest that even with a focus solely on substrate grip, limbs might have varied functions based on the requirements of different activities such as basking, feeding or mating (Burnham et al. 2011). The major differences in sexual behaviour in butterflies relate to mate-seeking, mating and ovipositing (Shreeve 1987, Berwaerts et al. 2002, Dudley 2002), although as both male and female *Y. cytheris* are patrollers (see Chapter 3), morphological variation based on a male perching strategy does not apply. It might be that the process of ovipositing requires a greater grip from the middle claws than is necessary for other activities which females and males have in common, but evidence is lacking.

Two points emerge from this consideration of form and function. The first is procedural: it is unsafe to make comparisons based on random claws. Data sets need to be drawn up which recognise differences between claw shape and size based on sex and position. This means, assuming non-lethal sampling, identifying and collecting the single leg best able to address a particular hypothesis. The second is that the evolutionary and developmental linkages between wings and legs, and their role in butterfly locomotion, deserve more attention than they have so far been given.

7.4.2 Claw variation attributable to environmental factors

(i) **Findings**

Y. cytheris claws, like those of *M. cinxia*, were more sharply curved at windier sites. Wind data from weather stations in the Falklands were, however, at too coarse a level of detail to investigate this and the pattern only became clear when wind readings were taken at the study sites at *Y. cytheris's* patrolling and maximum flying heights.

(ii) **Discussion**

The analysis of *Y. cytheris* claws was motivated by Duplouy and Hanski's (2013) ground-breaking analysis, before which there had been little consideration of butterfly tarsal claws and their function. On the basis of mean wind speeds at the nearest weather stations, they found that claws at the windiest site, Pikku-Tytärsaari, were more curved than at other sites.

Falkland Island sites could not be investigated on the same basis, as the nearest weather stations to the mainland sites were situated at higher elevations than the sites. This was particularly the case for Roy Cove, with the study site at 50 m, compared with the weather station, Mount Byron, at 480 m. Wind speeds, due to wind gradient, are generally higher at altitude, but, unlike the lapse rate for temperatures, there is no standard method of deriving data at one altitude from those at another. Weather stations in the Baltic were at elevations of < 20 m, comparable with the elevation of the study sites, which made comparisons more reliable. The important point, however, is the same as that in Chapter 4: meaningful metrics for butterflies need to be taken at a microhabitat scale. If there is claw adaptation, it will be because of wind speeds at the flying height of butterflies at the habitat in question, not because of wind speeds at the most open available position at a different altitude.

Data from field measurements at 30 cm and 150 cm, representing the butterfly's observed patrolling and ceiling heights, showed that *Y. cytheris* claws, in line with the *M. cinxia* findings, were more curved at those sites with higher wind speeds. A comparison of the profiles of the wind speeds shown at Figure 7.22 with the claw angles at Figure 7.14, enables a visualisation of the very strong correlations at site level. Both Bleaker and Sea Lion showed greater claw angles than Frying Pan and Roy Cove, although only one contrast, between the ventral angles of hind claws from Sea Lion and Roy Cove, was significant at $p < 0.05$. CVAs showed the extent to which Sea Lion claws, both middle and hind, differed from those of other sites, with a much more curved shape, particularly visible in CVs 1 and 2 for middle claws.

These findings suggest adaptation in *Y.cytheris* in response to a major environmental challenge, coping with wind, although caution is necessary, as there may be confounding factors. The difference in substrate, for example, between the stony raised beach of Bleaker compared with the sand, bog and penguin burrows of Sea Lion, suggests that shape might not be simply a response to wind alone but to surface. The possibility of other factors was recognised by Duplouy and Hanski (2013), who suggested that the improved grip of *M. cinxia* on Pikku-Tytärsaari might be a response not simply to reducing emigration loss, but also to the demands of nectar feeding in wind conditions. It might be envisaged that sites where nectaring mainly occurred on groundsel, *Senecio vulgaris*, in loose stands 40 cm high, would pose different gripping challenges from those with the much more tightly packed and low-lying Christmas bush *Baccharis magellanica*. There is considerable scope for research in this area: understanding of variation in claw shape requires much more detailed analysis of substrate use, particularly in varying wind conditions.

There is also scope, not simply for investigation into conspecific variation in claw length, but much more widely. Figure 7.20 shows that *M. cinxia* claws are less curved than

Y. cytheris. Pooling all claws, *M. cinxia* shows a mean angle of (degrees) 129.7 ±3.7, compared with *Y. cytheris's* 149.0 ±4.1. It would be instructive to understand the extent to which claw angles varied to investigate the reasons across butterfly species.

7.4.3 Conservation implications

The distinct variation in claw curvature seen in *Y. cytheris* should be taken into account in any planning for reintroduction, in accordance with IUCN guidelines (IUCN Wildlife Health Specialist Group 2013). As with wing size (Chapter 6) the nature of adaptation is important: it is not possible, with the present data, to show whether the variation in claw shape is genetic or an example of phenotypic plasticity. Further investigation of claw use in varied substrates, for different activities, in varying weather conditions, is also necessary before a clear linkage between claw shape and wind can be made. The evidence, however, which supports the findings by Duplouy and Hanski (2013), suggests that in exposed, small island sites such as Bleaker and Sea Lion, any reintroduction should be of butterflies with sharply curved claws. For other sites, even Latin American specimens might be introduced successfully, were it not for the significant differences in wing size. This is another example of the benefits that an investigative captive breeding programme would bring.

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Chapter 8: General discussion

8.1 Thesis overview

It is particularly challenging to work on a conservation plan for a butterfly, the Falkland fritillary, *Yramea cytheris*, which everyone in the Falklands knows about, but which few have seen, and about which very little is known. The collection of very basic information has to go hand in hand with the necessary fine-grain detail of genetic and ecological work on which planning and recommendations need to be based.

This thesis builds up a picture of a butterfly living in small colonies, with generally fewer than ten adults flying on a given day, coextensive with a habitat patch of *Viola* spp, usually the common violet, *Viola maculata*. It does not fly far - the average recorded flight was 26 m - and probably does not, as a rule, move from its own colony. It has a very long flying season, from mid-November to early March. Females have an apparent survival of 5.5 days , with a daily apparent survival probability (φ) of 0.78; for males it is 3.3 days, with a φ of 0.68 (Chapter 3).

A typical habitat patch comprises a mosaic of grass, bare ground and dwarf shrub heath, with *Viola* scattered in small clusters. It prefers a north-east facing slope, which shelters it from the strong prevailing westerly winds, and enables it to benefit from the morning sun, which is important as it is most active in the morning. Eggs are found singly, usually on the underside of the warmer leaves of medium-sized *Viola* plants. The oviposition plants are in warmer parts of the patch than general, often with higher chlorophyll levels (Chapter 4).

The butterfly has two described subspecies, *Y. c cytheris* in the Falkland Islands, and *Y. c.siga* in Latin America. They could not readily be separated through analysis of *COI, EF-1α* or *wingless* genes. Although, from a low number of samples, particularly from Latin America, there were haplotypes which were only found in Falklands specimens, and others only in Latin American, they differed by a maximum of four base pairs, whereas 85% of samples analysed, whether from the Falklands or Latin America, represented one haplotype (Chapter 5).

The Falklands butterfly has shown some adaptation to a windy climate. It can find suitable patches in a variety of environments, as long as there are *Viola* plants. The study sites represent the range of vegetation and topographies occupied, from a raised beach through cliffs and a sandy island to grazed slopes (Chapters 2 and 4). It has also adapted morphologically, showing more sharply curved claws in windier locations (Chapter 7). Its wing size, much smaller than that of the Latin American subspecies, might also reflect an adaptation to wind, making it easier to withstand being dislodged from the substrate, although other factors, such as diminished mobility as part of an island population, need to be considered. (Chapter 6).

This chapter considers the implications of this thesis's findings for conservation planning; it considers what further knowledge gaps need to be addressed; it considers the importance of the butterfly in the Falklands, and how clarity about its life cycle and its taxonomic status might help conservation efforts; and it makes recommendations about approaching conservation in the current economic climate, which include a draft Species Action Plan.

8.2 Discussion

8.2.1 Implications of thesis findings

There are many positive points about the status of *Y. cytheris* in the Falklands. The populations have a wide geographic spread, as do their larval host plants, *Viola* spp. They have coped with the strong winds of the Falklands by adaptations in claw shape and wing size and shape. A variety of sites, from exposed raised beaches to grazed pasture, are able to produce the conditions which meet its habitat requirements.The Latin American population, indistinguishable genetically, is widespread, with a 1700 km range in latitude, and is found from sea level to 890 m, at sites with maximum temperatures ranging from 15º C to 29º C. But it is instructive to remember that the passenger pigeon *Ectopistes migratorius* (Bucher 1992) and the Rocky Mountain locust *Melanoplus spretus* (Lockwood 2010) went from being two of the most common species in the world to extinction within a few decades, and to look for danger signs.

The danger signs are there. The sub-Antarctic is particularly sensitive to climate change (Pendlebury and Barnes-Keoghan 2007, Terauds et al. 2012, Chown and Convey 2016), with a predicted rise in mean temperature on the islands of 1.8°C between 1990 and 2080 (Jones et al. 2013). Maritime warming threatens a change in the Antarctic

Circumpolar Current, leading to a rise in sea level and an increase in storms (Pendlebury and Barnes-Keoghan 2007). There is a risk of tsunamis (Regnauld et al. 2008, Nicholson et al. 2020). All of these threaten a species with oviposition sites near to the shoreline.

While these threats are not capable of local mitigation, threats caused by changing land use are. *Y. cytheris's* habitat preferences appear now to be most commonly met by a grazed environment, which affords the mosaic of grass, bare patches, dwarf shrub heath and *Viola* in which most oviposition sites are found, even if this is only a product of the last two hundred years (4.1.1 (ii)). Any proposals for changed land use should ensure that these mosaics continue to exist, whether through grazing or through land management: the choking of *Viola* by rank grass, with observable (though uninvestigated) reduction in *Viola* and butterfly numbers seen on Sea Lion a cause for concern.

Y. cytheris, while having distinct habitat preferences, is able to have these met by a wider range of environments than grazed dwarf shrub heath and grazed pasture mosaics, as the variation in the study sites demonstrates. These give it scope to respond to climate change by moving to cooler microhabitats, whether at higher elevations or more sheltered from solar radiation. The maintenance of this range of environments, through ensuring a range of vegetation types and management regimes, to enable it to meet its resource and microclimate needs, is likely to be the key to conserving the species.

8.2.2 Knowledge gaps

(i) ***Y. cytheris***

The history of the *Y. cytheris* population in the Falkland Islands, and its relationship with the Latin American populations, is still to be clarified. The processes underlying island colonisation have been intensively studied, particularly within the field of island biogeography, as have the relationships between genetic and geographical distance (Slatkin 1993, Nève 2009). The ability to model the processes has developed in parallel (Matzke 2014, for a review see Fenderson et al. 2020). But the investigation is at an early stage with *Yramea cytheris*, and further molecular analysis, with wider sampling, will be necessary before it can be determined whether the Falklands populations are a product of a single founder event or several, or for that matter, regular migrations.

There is still much to be understood about *Y. cytheris's* autecology: the timings of the larva's instars; the stage at which the diapause is entered into; diapause duration; process, duration and location of pupation; and overall phenology, with clarity about the number of generations over the four month flying period. No work has been done on biological threats to the butterfly, whether from predators such as birds and spiders, from parasitoids, or from bacteria such as *Wolbachia* (Salunkhe et al. 2014: *Y. cytheris* was not tested, although there was no evidence of male-female balance in the Falklands).

Y. cytheris's distribution on the islands is still not well enough known: there is no real base line, therefore, for assessing its conservation status. Where it has been found, while apparent survival probability and probability of capture rates have been calculated, population sizes remain unclear. If there is to be meaningful monitoring of populations through, for example, transect walking (Pollard and Yates 1994), the relationship between numbers seen and estimated population numbers needs to be established (Harker and Shreeve 2008). The extent to which populations of patches on the same site are connected is also unknown. There is inadequate evidence at present for or against mobility between patches, and therefore for or against a group of patches functioning as a metapopulation (Hanski 1991, 2010, Gyllenberg and Hanski 1992), but this is an area which would benefit from further investigation, both in terms of butterfly movement and of genetics (5.4.3).

The final set of known unknowns relate to the Latin American population, which has not been studied in any depth. An investigation would provide valuable comparative material, particularly in terms of robustness and adaptability, and, perhaps more importantly, help identify suitable donor populations in the event of widespread extinctions in the Falklands (Seddon et al. 2007).

(ii) ***Viola* spp.**

For *Y. cytheris* to survive on the Falklands, it is necessary to ensure that *Viola spp.*, particularly *V. maculata*, continue to flourish. The distribution of *Viola* has been recorded by Falklands Conservation, but it needs to be updated, to ensure a baseline for monitoring the overall population health exists. The tolerance of *Viola spp.* for a range of temperatures and potential habitats is not known, but, as with the butterfly, is fundamental in predicting response to climate change.

The impact of changes in land use on *Viola* have also not been analysed, particularly of decreased grazing, or even the removal of grazing. The mechanism by which *Viola* spread is also unknown, including whether sheep have a role to play through endozoochorous dispersal.

8.2.3 A question of identity

(i) **The importance of species**

One of the overall Research Questions (1.6) asked whether *Y. c. cytheris* and *Y. c. siga* were subspecies of the same species. This opens up a longstanding debate. Agapow et al. (2004) observed that species were the currency of biology, at least in the way that discussion of conservation priorities operated. Hence the research question was not simply a matter of classification: if the Falklands butterfly proved to be an endemic species, for example, even if only cryptic, the discovery might have attracted considerable attention as happened with the discovery of a cryptic species of wood white butterfly, *Leptidea reali*, in 1988 (Réal 1988, cited in Cupedo and Hoen 2006) which subsequently became a model for the study of speciation (Dincă et al. 2011).

Considerable attention, when misplaced, can be disruptive: witness the need for secrecy about the sites used for the reintroduction of the large blue, *Maculinea arion*, to the UK (Thomas et al. 2009). But publicity can be an enabler in conservation, the downside of which Dennis (1997) saw as a trend to promote local populations to species status, which, while giving publicity to the taxon concerned, raised the global conservation load, particularly if rarity and endemism were equated with threat of extinction.

(ii) **Subspecies, evolutionarily sigificant units and a sense of place**

Although the International Code of Zoological Nomenclature (International Commission on Zoological Nomenclature 1999) set out the basic naming principles, the subject remained contentious, particularly at the level of subspecies. The question of circularity, particularly on the relationship between subspeciation and geographical limitation, became prominent. Wilson and Brown (1953), in describing the subspecies concept as "the most critical and disorderly area of modern systematic theory", added, "in its unassumed function as a formal means of registering geographical variation within the species it tends to be both illusory and superfluous".

Braby et al. (2012) shared Wilson's and Brown's reservations. They found the subspecies concept hampered by inconsistencies in its conceptual definition, lacking objective criteria or properties to delimit its boundaries, and frequently failing to reflect distinct evolutionary units according to population genetic structure. They proposed a clear definition: "Subspecies comprise evolving populations that represent partially isolated lineages of a species that are allopatric, phenotypically distinct, have at least one fixed diagnosable character state, and that these character differences are, or are assumed to be, correlated with evolutionary independence according to population genetic structure".

At the same time a parallel proposal was emerging, for the Evolutionarily Significant Unit (ESU) (Ryder 1986). This similarly attracted a variety of definitions, summarised by Funk et al. (2012), but had the clear practical aim of identifying populations that warranted separate management, or priority for conservation, because of their high genetic and ecological distinctiveness (Fraser and Bernatchez 2001). The practical intent was recognised by governments, including those of USA, Canada and Australia, recognising intraspecific units as a basis for legal protection.

The emphasis on geographical limitations in both subspecies and ESU debates is not surprising. A sense of place (Tuan 1979, Stedman 2002) is an important factor in conservation (Masterson et al. 2017), as local ownership of a particular taxon is a major driver to conserving it. This was seen, for example, in community engagement with the Eltham copper, *Paralucia pyrodiscus lucida* (Roitman et al. 2017) and the Karner blue, *Lycaeides melissa samuelis* (Oberhauser and Guiney 2009). As community structures develop, the role of the threatened species can move on: *P. p. lucida* became a symbol of welcome to refugees who were being settled in the Melbourne suburb of Eltham (Calligeros 2016); *L. m. samuelis* became a focal point for wider conservation awareness, including a summer festival, in Black Fall Rivers, Wisconsin (Oberhauser and Guiney 2009). The butterflies, while remaining a conservation challenge, had become more than that.

(iii) **What should the status of the Falkland fritillary be?**

This thesis has shown that it is not possible to allocate a specimen of *Y. cytheris* to either *Y. c. cytheris* or *Y. c. siga* on the basis of its *COI, EF-1α* or *wingless* genes, or a concatenation of all three, thus it fails one test for subspecies (Braby et al. 2012) and ESU status (Fraser and Bernatchez 2001). But its isolation, 400 km east of the nearest

Latin American population, and evidence for local adaptations in wing size and shape, together with claw curvature, argue for its inclusion in both categories, as measurement of forewing length and evaluation of forewing shape, should enable correct classification. The original split between subspecies, made on the basis of wing colour (Watkins 1924), might also be determinative. It was not pursued in this thesis, as the majority of samples were museum specimens over 100 years old, and no Latin American specimen was more recent than 2003, which made it difficult to assess levels of fading or deterioration in pigmentation.

In a sense, neither of these classifications is particularly important. *Y. cytheris* is the only resident butterfly on a group of islands 400 km away from the Latin American coast. That defines its status, and, in a sentence, the importance of its conservation.

8.2.4 Flagship species

(i) **The role of flagship species**

The position of *Y. cytheris* as the only resident butterfly in the Falkland Islands suggests that it could be a candidate for a flagship species (Smith and Sutton 2008, Barua et al. 2011 and Jepson and Barua 2015). Flagship species serve to raise public awareness, support interventions, and to raise funding, principally for their own conservation (Caro et al. 2004). They tend to be popular and charismatic: a flagship invertebrate, therefore, is more likely to be a butterfly than a slug (Barua et al. 2012). This is a potential problem: as Small (2011) observed, most of the world's species at risk of extinction are neither particularly attractive nor obviously useful, and consequently lack conservation support. Veríssimo et al. (2011) defined a flagship species as "the focus of a broader conservation marketing campaign based on its possession of one or more traits that appeal to the target audience". This moved the concept on, with the explicit mention of, and indeed language of, marketing, and its need to identify the target audience. The examples of *P. p. lucida* and *L. m. samuelis* show that charisma is not dependent on whether a taxon is a species or a subspecies. There is a *prima facie* case for *Y. c. cytheris's* filling a similar role in the Falkland Islands.

(ii) **The Falkland fritillary as a potential flagship species**

The Falkland Islands are rich in popular and charismatic species, particularly birds, including five species of penguin and six species of albatross, which attract many tourists to the islands. It is a crowded field, and introducing a butterfly as the islands' first formal flagship species may appear counterintuitive. But there are potential advantages. The islands' birds are already well known and heavily studied, whereas their insects are not, with only Jones and Lewington's (2004) guide as an introduction. The guide was the outcome of a three-year project to study the islands' terrestrial vertebrates, which was well received and introduced the study of insects to a large number of people on the islands, although it ultimately failed to find traction. With the growing in strength of Falklands Conservation, and the establishment of SAERI, in the intervening years, the conservation landscape has improved considerably.

The butterfly benefits from being distinctive, as a bright orange insect flying across dull green heathland, and relatively easy to photograph. It is the nominate subspecies, and meets the criteria for both sub-species and ESU. Making it a flagship species would raise awareness, of the butterfly itself, and provide a basis for getting support, including financial, for steps to secure its continued existence on the Falklands. Producing a species action plan to engage stakeholders in the process, would be the starting point. It might then be used to reenergise work on Falkland terrestrial invertebrates in general. Jones and Lewington (2004), for example, identify endemic species of Noctuid moth (*Pareuxonia falklandica*), tussac moth (*Borkhausenia falklandensis*), water beetle (*Lancetes falklandicus*) and camel cricket (*Parudenus falklandicus*), none of which has been studied. Nor have any insects been assessed for IUCN Red List status. *Y.c. cytheris* would also serve as a flagship for the dwarf shrub heath habitat, possible threats to the existence of which were noted in Chapter 4.

There are various audiences for a flagship project in the Falklands: landowners; other Falkland Islanders, especially children; visitors, particularly wildlife tourists; academic researchers; conservationists; and bodies with an interest, actual or potential, in Falklands conservation work, including possible partners in the United Kingdom. The Islanders themselves are the primary audience, however, as the butterfly relies on their stewardship. They have pride in the name Falkland fritillary: there is even some preference for the term Queen of the Falklands fritillary, reflecting the Islands' relationship with the UK Crown. Getting their backing for a Species Action Plan would be a valuable step in starting the conservation process and engaging further stakeholders. A suggested Species Action Plan, for discussion with stakeholders, is at Appendix E.

8.2.5 The political context: a difficult time for conservation

Conservation does not take place in a vacuum. As this paragraph is being finalised, the world is on the verge of a recession owing to the impact of COVID-19 on the global economy (McKibbin and Fernando 2020). While the economic and social value of biodiversity might be accepted internationally (Pearce and Moran 1994, Seddon et al. 2016), a case for the conservation of a single species not in immediate danger of extinction is a difficult one to make at a time of intense competition amongst priorities for scarce resources (Ando and Langpap 2018, Carwardine et al. 2019).

The responsibility, in terms of international law, for environmental issues in UK Overseas Territories, such as the Falkland Islands, lies with the United Kingdom (House of Commons Environmental Audit Committee 2013), although in practice it is devolved to the territories themselves (DEFRA 2012, Foreign and Commonwealth Office 2012). The Falkland Islands Government (FIG) have a strong commitment to managing the Islands' environment and wildlife (FIG Environmental Planning Department 2018a, 2018b), working closely with two NGOs, the South Atlantic Environmental Research Institute and Falklands Conservation. Their approach is prioritisation of key species and habitats: at this stage, neither *Y. cytheris* nor *V. maculata*, their protected status notwithstanding, is seen as a priority, and dwarf shrub heath is not seen as a key habitat. With no funding from the UK, and a difficult financial environment for the Falkland Islands, the case for direct FIG action on the Falkland fritillary does not seem compelling. This is perhaps the strongest reason to look at community action, based on a Species Action Plan.

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APPENDICES

Appendix A Supplementary material for chapter 3, population dynamics and behavioural patterns

A.1 Mark, release and recapture data

Appendix Table A.1 Numbers of *Y. cytheris* caught at the four Falkland study sites over the period 2016-2019

			all captures			marked		recaptures	
visit	days	date	total	F	M	F	M	F	M
SL01	1	16/01/2016	6	4	2	4	2	0	0
BL01	2	17/01/2016	10	5	5	5	5	0	0
		20/01/2016	21	18	3	18	3	0	0
RC01	1	23/01/2016	12	7	5	7	5	0	0
FP01	3	29/01/2016	0	0	0	0	0	0	0
		01/02/2016	0	0	0	0	0	0	0
		02/02/2016	2	2	0	2	0	0	0
SL11	4	14/12/2016	19	4	15	4	15	0	0
		15/12/2016	23	12	11	11	8	1	3
		16/12/2016	28	11	17	8	13	3	4
		17/12/2016	8	6	2	4	1	2	1
BL11	4	18/12/2016	22	7	15	7	15	0	0
		19/12/2016	37	11	26	8	23	3	3
		20/12/2016	44	21	23	17	11	4	12
		22/12/2016	28	17	11	10	7	7	4
FP11	1	27/12/2016	5	0	5	0	5	0	0
RC11	6	01/01/2017	12	7	5	7	5	0	0
		02/01/2017	8	8	0	6	0	2	0
		03/01/2017	1	0	1	0	1	0	0
		04/01/2017	9	9	0	3	0	6	0
		05/01/2017	5	5	0	2	0	3	0
		07/01/2017	9	6	3	5	2	1	1
RC12	1	13/01/2017	7	1	6	1	6	0	0
BL12	2	19/01/2017	13	9	4	9	4	0	0
		20/01/2017	12	8	4	3	3	5	1
RC13	3	22/01/2017	2	2	0	2	0	0	0
		25/01/2017	6	5	1	5	1	0	0
		26/01/2017	2	2	0	2	0	0	0
FP12	1	01/02/2017	2	2	0	2	0	0	0
RC21	7	09/01/2018	5	4	1	4	1	0	0
		10/01/2018	2	0	2	0	2	0	0
		11/01/2018	2	0	2	0	1	0	1
		13/01/2018	1	1	0	1	0	0	0
		14/01/2018	1	0	1	0	0	0	1
		15/01/2018	1	0	1	0	1	0	0
		16/01/2018	2	1	1	1	0	0	1
SL21	2	19/01/2018	0	0	0	0	0	0	0
		20/01/2018	0	0	0	0	0	0	0
BL21	7	21/01/2018	6	2	4	2	4	0	0
		22/01/2018	5	1	4	1	2	0	2
		23/01/2018	5	2	3	0	1	2	2
		24/01/2018	1	0	1	0	0	0	1
		25/01/2018	3	2	1	0	0	2	1
		26/01/2018	1	0	1	0	1	0	0
		27/01/2018	2	2	0	1	0	1	0
FP21	4	28/01/2018	4	3	1	3	1	0	0
		30/01/2018	1	1	0	0	0	1	0
		01/02/2018	1	1	0	0	0	1	0
		02/02/2018	7	1	6	1	6	0	0
totals	49		403	210	193	166	155	44	38

BL = Bleaker, FP = Frying Pan, RC = Roy Cove and SL = Sea Lion. The following digits combine the season number (0 = 2015-2016, 1 = 2016-2017 and 2 = 2017-2018) and the visit number within that season (1-3). Days = the number of days on which MRR was undertaken. Marked = the number of individuals marked for the first time in a visit; recaptures = the number of individuals recaptured on subsequent days in total, irrespective of the number of times any individual was recaptured. F and M refer to female and male.

Appendix Table A.2 Jolly-Seber analysis of MRR data from seven events of four days or more.

		M		*n*			φ			*pent*		
site	*i*	*M*	*SE*	*N*	*SE*	95% CI	φ	*SE*	95% CI	*B*	*SE*	95% CI
BL1	1						0.70	0.21	0.29, 1.12			
	2	15.5	4.2	84.1	31.8	21.8, 146	0.81	0.30	0.21, 1.40	37.6	31	-23.2,98.4
	3	37.5	13.4	105.5	40.7	25.6, 185	0.16					
(5)	4	11.0		11.0								
BL3	1						0.58	0.28	0.04, 1.13			
	2	3.5	1.1	7.0	2.6	1.8, 2.2	0.61	0.22	0.19, 1.04	0.7	1.4	-2.0, 3.4
	3	4.0	0.0	5.0	0.0	5.0, 5.0	0.60	0.22	0.17, 1.03	0.0	0.0	0.0, 0.0
	4	3.0	0.0	3.0	0.0	3.0, 3.0	1.00	0.00	1.00, 1.00	0.0	0.0	0.0, 0.0
	5	3.0	0.0	3.0	0.0	3.0, 3.0	0.67					
(7)	6	2.0		4.0								
FP1	1						0.25					
	2	1.0		1.0			1.00			0.0		
	3	1.0	0.0	1.0	0.0	1.0, 1.0	1.00			0.0		
	4	1.0		1.0			1.00			0.0		
(6)	5	1.0		1.0						0.0		
RC1	1						0.22	0.12	0.00, 0.45			
	2	3.1	0.5	8.4	2.1	4.3, 12.5	0.86			-0.2		
	3	7.0		7.0			1.21			3.6		
	4	8.5	2.5	12.1	3.7	5.0, 19.3	0.52	0.42	-0.31,1.35	2.7	3.0	-3.1, 8.5
	5	6.0	4.4	9.0	6.5	-3.8, 21.8	0.25			-0.2		
(7)	6	2.0		2.0								
RC2	1						0.0					
	2	0.0		0.0								
	3	0.0		0.0								
(5)	4	0.0		3.0								
RC3	1						0.00					
	2	0.0	0.0	3.0			0.33	0.27	-0.2, 0.87	0.0		
	3	1.0	0.0	1.0	0.0	1.0, 1.0	1.00			0.0		
	4	1.0		1.0			2.00			2.0		
	5	2.0		4.0			0.33			-0.3		
	6	1.0	0.0	1.0	0.0	1.0, 1.0	2.00			2.0		
(8)	7	2.0		4.0								
SL1	1						0.63	0.35	-0.47,1.31			
	2	12.0	6.2	57.6	34.6	-10.3,125	0.22	0.09	0.05, 0.40	15.0	6.7	1.9, 28.1
(4)	3	7.0	0.0	28.0	0.0	28.0, 28.0						

The bracketed numbers in the site column show the total number of days over which MRR was conducted, the last day of which is not included in the Jolly-Seber analysis. φ represents the probability of survival from one day to the next, *pent* the probability of entry into the population, including eclosion, between the sampling periods.

Appendix B Supplementary material for chapter 4, habitat requirements

B.1 Habitat factors in the selection of oviposition locations

Appendix Table B.1 Oviposition in relation to wind shade at each site.

site	oviposition	*n*	*M*	*SD*	*df*	*t*	*p*-value	95% CI	*d*
Bleaker	no	1	5.00	NA	3	-0.58	0.602	-52.28, 36.16	0.65
	yes	4	13.06	12.43					
Frying Pan	no	5	24.44	20.09	22	-1.55	0.135	-35.33, 5.09	0.78
	yes	19	39.56	4.82					
Roy Cove	no	32	17.76	7.93	48	-1.43	0.160	-10.08, -1.71	0.42
	yes	18	21.95	12.85					
Sea Lion	no	4	29.72	10.48	4	0.12	0.910	-40.19, 43.82	0.10
	yes	2	27.91	29.86					

Units are percentages, per circular image, of black pixels in the four azimuth bins centred on the prevailing wind direction. There was no significance at $p < 0.05$, or large effect size at $d > 0.8$

Appendix Table B.2 Oviposition in relation to openness at each site.

site	ov	*n*	*M*	*SD*	*df*	*t*	*p*-value	95% CI	*d*
Bleaker	no	1	95.00	NA	3	0.75	0.507	-18.87, 30.53	**0.84**
	yes	4	89.17	6.94					
Frying Pan	no	5	80.16	7.69	22	1.19	0.247	-3.84, 14.16	0.60
	yes	19	74.99	8.83					
Roy Cove	no	32	81.38	4.83	48	-1.43	0.055	-0.07, 6.54	0.58
	yes	18	78.14	6.73					
Sea Lion	no	4	69.81	6.05	4	0.78	0.480	-9.17, 16.31	0.67
	yes	2	66.24	1.63					

Units are percentages of white pixels per circular image. ov = oviposition site. There was no significance at $p < 0.05$, Large effect size (while not informative, given the high *p*-value) at $d > 0.8$ is shown in bold.

Appendix Table B.3 Oviposition in relation to direct radiation at each site.

site	oviposition	n	*M*	*SD*	*df*	*t*	*p*-value	95% CI	*d*
Bleaker	no	1	23.10	NA	3	0.45	0.685	-0.05, 0.06	0.50
	yes	4	23.09	0.02					
Frying Pan	no	5	22.33	1.44	22	0.69	0.490	-1.12, 2.25	0.35
	yes	19	21.77	1.65					
Roy Cove	no	32	22.99	0.33	48	1.50	0.140	-0.7, 0.47	0.44
	yes	18	22.79	0.64					
Sea Lion	no	4	19.22	4.73	4	0.20	0.852	-9.86, 11.38	0.17
	yes	2	18.45	3.29					

Units are mean moles per square metre per day over the November - February flying season. There was no significance at $p < 0.05$, or large effect size at $d > 0.8$

Appendix Table B.4 Mean wind speeds at oviposition and non-oviposition locations. *t*-tests of wind speeds at three heights, 150cm (ceiling: the highest observed flight), 30cm (flight: the observed flying height) and 3cm (oviposition: oviposition height). Wind speeds were recorded as proportions of the wind speed at a site when measurements were taken, and applied to a base 8.11 ms^{-1}, the mean wind speed of the weather stations nearest to the four study sites over November-February, 2013-2018.

height	oviposition	*n*	*M*	*SD*	*df*	*t*	*p*-value	95% CI	*d*
150 cm	no	61	5.81	2.19	96	2.96	**0.004**	0.45, 2.30	0.62
(ceiling)	yes	37	4.44	2.29					
30 cm	no	61	4.04	1.74	96	3.05	**0.003**	0.40, 1.89	0.63
(flight)	yes	37	2.90	1.90					
3 cm	no	61	1.08	0.70	96	2.87	**0.005**	0.12, 0.65	0.60
(oviposition)	yes	37	0.70	0.53					

Significance at $p < 0.05$ is shown in bold. Wind speed is measured in ms^{-1}.

Appendix Table B.5 Comparison of illustrative wind speeds at oviposition and non-oviposition locations at the four study sites. Welch two sample *t*-tests. Ceiling (150cm) and flight (30cm) heights are compared; there was no clear variation at oviposition level. Wind speeds were recorded as proportions of the wind speed at a site when measurements were taken, and applied to a base 8.11 ms^{-1}, the mean wind speed of the weather stations nearest to the four study sites over November-February, 2013-2018.

height	site	ov	*n*	*M*	*SD*	*df*	*t*	*p*-value	95% CI	*d*
ceiling	Bleaker	N	7	5.62	0.71	12.63	0.007	0.994	-0.74, 0.75	0.003
		Y	10	5.62	0.68					
	Frying Pan	N	6	4.65	2.72	7.48	1.73	0.125	-0.75, 5.03	**1.01**
		Y	9	2.51	1.65					
	Roy Cove	N	44	5.92	2.29	24.45	2.13	**0.044**	0.05, 3.02	0.65
		Y	16	4.39	2.53					
	Sea Lion	N	4	6.73	1.93	3.30	-0.86	0.446	-3.58, 2.14	0.51
		Y	2	7.58	0.32					
flight	Bleaker	N	7	4.17	0.83	11.11	0.53	0.607	-0.63, 1.03	0.27
		Y	10	3.97	0.67					
	Frying Pan	N	6	2.36	2.19	5.89	1.29	0.244	-1.09, 3.50	**0.81**
		Y	9	1.15	0.8					
	Roy Cove	N	44	4.24	1.73	23.68	2.53	**0.018**	0.26, 2.59	0.79
		Y	16	2.81	2.00					
	Sea Lion	N	4	4.18	1.37	1.74	-1.38	0.318	-8.60, 4.86	**1.29**
		Y	2	6.04	1.65					

Significance at $p < 0.05$ and large effect size at $d > 0.8$ are shown in bold. ov = oviposition site: N = no, Y = yes.

Appendix Table B.6 Temperatures at oviposition and non-oviposition locations. Temperatures from data loggers covering 24hrs, day (1000 - 1600) and night (2200 - 0400) were recorded as proportions of the mean temperature of each site. Those proportions were transformed by mean combined temperatures for 24 hrs (14.93º), day (21.35º) and night (9.55º) to enable comparisons of data taken from different sites at different times. Comparisons are *t*-tests.

time	oviposition	*n*	*M*	*SD*	*df*	*t*	*p*-value	95% CI	*d*
24 hrs	no	30	14.37	1.44	50	-3.32	**0.002**	-2.13, -0.52	**0.93**
	yes	22	15.70	1.41					
day	no	30	20.29	2.70	50	-3.17	**0.003**	-4.07, -0.91	**0.89**
	yes	22	22.79	2.93					
night	no	30	9.48	1.03	50	-0.68	0.501	-0.73, 0.36	0.19
	yes	22	9.66	0.86					

Significance at $p < 0.05$ and large effect size at $d > 0.8$ are shown in bold. Temperature is measured in ºC.

Appendix Table B.7 Comparison of illustrative temperatures at oviposition and non-oviposition locations at the four study sites. Welch two sample *t*-tests . Temperatures from data loggers covering 24hrs, day (1000-1600) and night (2200-0400) were recorded as proportions of the mean temperature of each site. Those proportions were transformed by mean combined temperatures for 24 hrs (14.93º), day (21.35º) and night (9.55º) to enable comparisons of data taken from different sites at different times.

time	site	ov	*n*	*M*	*SD*	*df*	*t*	*p*-value	95% CI	*d*
day	Bleaker	N	3	19.00	3.01	3.45	-1.67	0.182	-9.03, 2.60	**1.22**
		Y	7	22.35	2.67					
	Frying Pan	N	2	20.79	4.62	2.36	-0.21	0.847	-16.93, 15.08	0.19
		Y	3	21.72	4.84					
	Roy Cove	N	22	20.27	2.74	19.9	-3.17	**0.005**	-5.36, -1.10	**1.18**
		Y	11	23.51	2.77					
	Sea Lion	N	3	21.44	1.62	NA	NA	NA	NA	NA
		Y	1	21.11	NA					
24 hr	Bleaker	N	3	14.20	0.81	6.39	-1.52	0.176	-2.70, 0.61	**0.86**
		Y	7	15.24	1.33					
	Frying Pan	N	2	14.44	1.42	2.73	-0.55	0.621	-5.69, 4.08	0.48
		Y	3	15.25	1.81					
	Roy Cove	N	22	14.43	1.57	22.9	-2.86	**0.009**	-2.61, -0.42	**1.00**
		Y	11	15.94	1.36					
	Sea Lion	N	3	14.05	1.49	NA	NA	NA	NA	NA
		Y	1	17.60	NA					
night	Bleaker	N	3	9.82	0.65	3.08	0.92	0.424	-0.93, 1.70	0.71
		Y	7	9.43	0.50					
	Frying Pan	N	2	9.23	0.05	2.02	-1.15	0.367	-2.53, 1.45	**0.81**
		Y	3	9.77	0.81					
	Roy Cove	N	22	9.42	1.08	20.15	-1.04	0.310	-1.24, 0.41	0.38
		Y	11	9.83	1.08					
	Sea Lion	N	3	9.73	1.55	NA	NA	NA	NA	NA
		Y	1	9.02	NA					

Significance at $p < 0.05$ and large effect size at $d > 0.8$ are shown in bold.

Appendix Table B.8 The contrast between non-oviposition and oviposition locations for six types of ground cover. Analysis by *t*-test was based on percentage cover in 50x50 cm quadrats, and covered the four main study sites.

cover	ov	*n*	*M*	*SD*	*df*	*t*	*p*-value	95% CI	*d*
Viola	N	105	6.94	10.87	129.20	-1.96	0.052	-6.82, 0.02	0.31
	Y	62	10.34	10.75					
bare ground	N	105	14.40	15.92	93.96	-3.67	**<0.001**	-19.16, -5.72	0.65
	Y	62	26.84	23.69					
litter	N	105	11.33	13.46	93.72	-3.83	**<0.001**	-16.69, -5.29	0.68
	Y	62	22.32	20.10					
dwarf shrub	N	105	30.95	31.71	160.56	3.88	**<0.001**	7.95, 24.40	0.57
	Y	62	14.76	22.03					
grass	N	105	18.64	23.37	146.53	0.97	0.333	-3.38, 9.92	0.15
	Y	62	15.37	19.50					
other	N	105	17.73	21.44	164.44	2.73	**0.007**	2.04, 12.69	0.39
	Y	62	10.37	13.39					

Significance at $p < 0.05$ is shown in bold. Levene's test showed equal variances could not be assumed, therefore the Welch two sample *t*-test was used. ov = oviposition location, values are Y = yes, N = no.

Appendix C Supplementary material for chapter 6, wings: function, shape and size

C.1 Sources of *Y. cytheris* wing samples

Appendix Table C.1 Sources of *Y. cytheris* wing samples analysed in this chapter.

land mass	site label (attribution)	source	year collected	forewings F	forewings M	hind wings F	hind wings M
FK	Bleaker Island	field visit	2016, 2017, 2018	3	4	4	4
FK	Darwin	field visit	2017	0	1	0	1
FK	(Darwin)	BMNH, Reid	1909	2	1	2	1
FK	Darwin	OUMNH,Reid	1908-1909	3	10	3	10
FK	Frying Pan	field visit	2016, 2017, 2018	3	4	3	4
FK	(North Arm)	BMNH, Elliott	1934	5	5	5	5
FK	San Carlos	BMNH, Bonner	1935	3	1	3	1
FK	Sea Lion Island	field visit	2016, 2017	2	2	2	2
FK	Roy Cove	field visit	2016, 2017, 2018	3	5	3	5
FK	(Roy Cove)	BMNH, Vallentin	1904	4	5	4	5
FK	Shallow Bay	OUMNH,Vallentin	1910-1911	17	16	4	5
LA	Bariloche, Chile	BMNH, Edwards	1926	0	2	0	0
LA	Isla Isabel, Chile	BMNH, Coppinger	1879	2	0	2	0
LA	Panguipulli, Chile	BMNH,Fay	1928	4	5	0	0
LA	Pto. Williams, Chile	Zúñiga collection	2002	0	1	0	1
LA	Punta Arenas, Chile	BMNH, Walker	1915	4	3	4	3
LA	Punta Arenas, Chile	BMNH, Nicoll	1903	0	1	0	1
LA	Punta Arenas, Chile	Zúñiga collection	2002	1	0	1	0
LA	Rio McClelland, Chile	BMNH, Crawshay	1904	7	11	0	1
LA	Rio McClelland, Chile	OUMNH, Crawshay	1904	1	14	1	14
LA	Torres del Paine, Chile	Zúñiga collection	2003	0	1	0	1
LA	Santiago, Chile	Zúñiga collection	2000	2	0	2	0
LA	Chubut, Argentina	BMNH	NK	5	5	0	0
LA	Chubut, Argentina	OUMNH, Rosenberg	before 1904	5	4	5	4

FK = Falkland Islands, LA = Latin America; OUMNH = Oxford University Museum of Natural History, BMNH = British Museum of Natural History; F = female, M = male. Attribution: to Darwin of BMNH specimens collected by Reid is Boyson (1924), and by association with the OUMNH specimens; to Roy Cove of those collected by Vallentin is Vallentin (1904); to North Arm of those collected by Elliott is Elliott (1927). The date of the BMNH Chubut specimens, marked NK, is not known.

C.2 Image acquisition and handling

C.2.1 Images acquired using predetermined protocols

Images were recorded using a Nikon D800 single lens reflex camera and Nikon 105mm AF Micro Nikkor lens. They were stored in TIFF files, a lossless digital format usable by most morphometric software. File size in each instance was approximately 110MB.

Butterflies taken in the field were dissected in the laboratory. Their wings were removed and placed between microscope slides which were then cemented together. This eliminated possible measurement error due to wing curvature or angle, and allowed them to be photographed from both the ventral and dorsal sides. The camera was mounted on a tripod, with the specimen at a distance of 33.5 cm from the focal plane. This enabled an entire butterfly to be photographed in a single frame. Exposures were in diffused natural light with an aperture of f32 at ISO 640 for 1/2 second. A ruler marked in 1mm divisions was included in each photograph in the same plane as the wing to enable measurements to be included in the digitisation.

Specimens from museum collections were photographed individually on their original pins. To enable specimens' undersides (whether dorsal or ventral, depending on how the specimen was mounted) to be photographed, the pins were inverted and their heads pushed into mounting putty. Unlike slide-mounted wings, those of museum specimens can show curvature or be angled, so careful positioning was necessary to ensure they appeared in the same plane as a measuring gauge marked in 0.5 mm divisions (BMNH) or a reference length of 10mm (OUMNH). This was checked by ensuring both wings and gauges were in focus at the open aperture of f2.8, which gives a working depth of field of 1.2 mm at 33.5 cm (Greenleaf 1950).

Exposures were made at varying distances between 30 cm and 35 cm with an aperture of f32, giving a depth of field range of 1.02 cm at 30 cm and 2.06 cm at 35 cm (Greenleaf 1950). This allowed much of the butterfly other than the wings to be in focus, which meant the images could be of wider use.

Exposures were at ISO 640 for 1/4 -1/5 second (BMNH) and 1/25 second (OUMNH), using integrated lightbox and camera stands supplied by the museums.

C.2.2 Images supplied by others

An additional set of forewing dorsal images of Latin American *Yramea cytheris* was supplied by Geoff Martin of the BMNH. These had been photographed using the SatScan tray scanner system (Blagoderov et al. 2010), a method that Johnson et al (2013) had found to be as accurate as photographing pinned specimens. As some of the supplied images had also been photographed under the preceding protocols, it was possible to cross-refer and make comparisons which showed close correspondence of measurements. Accordingly the samples were used alongside others.

A limitation of the whole tray scan was that it was not possible to use hind wings, as they were partially covered by forewings in setting, and ventral images were not available.

C.2.3 Image processing

TIFF files were initially processed in the TPS suite of software (Rohlf 2015). They were first read into a TPS file in tpsUtil 1.70x64 . The resulting files were transferred to tpsDig ver.2.26, where the scale was set in accordance with the measurement gauge included in the images.

C.2.4 Wing structure and landmark selection

Landmarks were selected for digitisation on both fore and hind wings. Sites chosen were either the intersections of veins, or the points where a vein met the edge of a wing.

Forewing landmarks were generally registered on the dorsal surface. Hindwing venation was less clearly visible on the dorsal surface of hindwings, in which cases the ventral surface was used, with the dorsal only used when landmarks were obscured, for example by legs. If necessary, the image was adjusted using the left/right flip in tpsDig to ensure all images were oriented in the same way.

The TPS files were read into MorphoJ 1.06d (Klingenberg 2011). The landmark coordinates were then subjected to Procrustes superimposition (Klingenberg and McIntyre 1998).

C.2.5 Warped outline drawings

Shape changes were visualised in warped outline drawings. An outline file was made from a wing image with its landmarks marked. Lines were then constructed using a series of intermediate landmarks to show wing outlines and the major veins. The file was then imported into MorphoJ as a basis for warped outline drawings. These show initial and target shapes of a shape variation using the information provided by the landmarks.

It should be noted that the warped outline drawing is an aid to visualisation only. While the original file follows a semi-landmark structure, none of the subsequent images does. Therefore, while the landmarks carry biological information, the warped outline drawings (as is also true of deformation grids) carry no biological information.

C.3 Additional statistical material

C.3.1 The effects of land mass and sex on wing metrics

Appendix Table C.2 The effect on forewing length of land mass and sex.

Factor	*df*	*SS*	*MS*	*F*-ratio	*p*-value	η_p^2
land mass	1	130.8	130.8	94.48	**<0.001**	**0.355**
sex	1	114.7	114.7	82.83	**<0.001**	**0.325**
land mass: sex	1	0.3	0.3	0.23	0.632	0.001
residuals	172	238.1	1.4			

Two-way ANOVA using type II sum of squares. Significance at $p < 0.05$ and large effect size at $\eta_p^2 > 0.14$, are shown in bold.

Appendix Table C.3 The effect on forewing angle of land mass and sex.

factor	*df*	*SS*	*MS*	*F*-ratio	*p*-value	η_p^2
land mass	1	30.1	30.5	10.49	**0.001**	0.057
sex	1	0.1	0.1	0.04	0.840	<0.001
land mass: sex	1	0.1	0.1	0.02	0.887	<0.001
residuals	172	493.2	2.9			

Two-way ANOVA using type II sum of squares. Significance at $p < 0.05$ is shown in bold.

Appendix Table C.4 The effect on forewing AR of land mass and sex.

factor	*df*	*SS*	*MS*	*F*-ratio	*p*-value	η_p^2
land mass	1	0.2	0.2	14.97	**< 0.001**	0.080
sex	1	0.0	0.0	0.70	0.404	0.004
land mass: sex	1	0.0	0.0	0.04	0.843	<0.001
residuals	172	2.4	0.0			

Two-way ANOVA using type II sum of squares. Significance at $p < 0.05$ is shown in bold.

Appendix Table C.5 The effect on forewing $\hat{r}_1$ of land mass and sex

factor	*df*	*SS*	*MS*	*F*-ratio	*p*-value	η_p^2
land mass	1	<0.01	<0.01	15.82	**<0.001**	0.098
sex	1	<0.01	<0.01	22.43	**<0.001**	0.115
land mass: sex	1	<0.01	<0.01	0.24	0.237	0.008
residuals	172	0.01	<0.01			

Two-way ANOVA using type II sum of squares. Significance at < 0.05 is shown in bold.

Appendix Table C.6 The effect on hind wing length of land mass and sex.

factor	*df*	*SS*	*MS*	*F*-ratio	*p*-value	η_p^2
land mass	1	34.6	29.5	71.00	**<0.001**	**0.346**
sex	1	41.8	41.8	85.59	**<0.001**	**0.390**
land mass: sex	1	1.4	1.4	2.84	0.094	0.021
residuals	134	65.4	0.5			

Two-way ANOVA using type II sum of squares. Significance at $p < 0.05$ and large effect size at $\eta_p^2 > 0.14$, are shown in bold.

Appendix Table C.7 The effect on hind wing angle of land mass and sex.

factor	*df*	*SS*	*MS*	*F*-ratio	*p*-value	η_p^2
land mass	1	5.9	5.9	0.84	0.361	0.006
sex	1	18.3	18.3	2.60	0.109	0.019
land mass: sex	1	0.3	0.3	0.04	0.834	<0.001
residuals	134	940.5	7.0			

Two-way ANOVA using type II sum of squares. There was no significance at $p < 0.05$, or large effect size at $\eta_p^2 > 0.14$

Appendix Table C.8 The effect on hind wing AR of land mass and sex.

factor	*df*	*SS*	*MS*	*F*-ratio	*p*-value	η_p^2
land mass	1	<0.01	<0.01	0.31	0.580	0.002
sex	1	0.05	0.05	18.66	**<0.001**	0.122
land mass: sex	1	<0.01	<0.01	<0.01	0.976	<0.001
residuals	136	0.38	<0.01			

Two-way ANOVA using type II sum of squares. Significance at $p < 0.05$ is shown in bold.

Appendix Table C.9 The effect on hind wing $\hat{r}_1$ of land mass and sex

factor	*df*	*SS*	*MS*	*F*-ratio	*p*-value	η_p^2
land mass	1	<0.01	<0.01	0.009	0.923	<0.001
sex	1	<0.01	<0.01	0.001	0.970	<0.001
land mass: sex	1	<0.01	<0.01	3.001	0.085	0.021
residuals	134	0.09	<0.01			

Two-way ANOVA using type II sum of squares. There was no significance at $p < 0.05$, or large effect size at $\eta_p^2 > 0.14$

C.3.2 Geometric morphometric analysis of differences in wing shapes between sites

Appendix Table C.10 Mahalanobis and Procrustes distances between female and male, Falklands and Latin America forewing shapes following CVA.

	n	Falklands, female	Falklands, male	Latin America, female	Latin America, male	
Falklands, female	48	-	3.31 **(<0.0001)**	2.50 **(<0.0001)**	4.17 **(<0.0001)**	Mahalanobis distance (*p*-values)
Falklands, male	52	0.031 **(<0.0001)**	-	3.74 **(<0.0001)**	2.84 **(<0.0001)**	
Latin America, female	30	0.019 **(0.0001)**	0.039 **(<0.0001)**	-	3.30 **(<0.0001)**	
Latin America, male	48	0.030 **(<0.0001)**	0.022 **(<0.0001)**	0.027 **(<0.0001)**	-	
		Procrustes distance (*p*-values)				

Mahalanobis distances significant at $p < 0.001$, and Procrustes distances significant at $p < 0.05$ are in bold.

Appendix Table C.11 Mahalanobis and Procrustes distances between female forewings from eight Falkland Island sites following CVA.

site	*n*	BL	DA	FP	NO	RC	SC	SL	SW	
BL	4	-	5.21 (0.002)	6.4 (0.025)	5.02 (0.018)	5.07 **(0.001)**	4.72 (0.036)	8.6 **(0.0005)**	4.71 **(<.0001)**	Mahala-nobis distance (*p*-values)
DA	6	0.026 (0.348)	-	5.34 (0.010)	5.41 (0.010)	3.91 (0.002)	5.11 **(0.0004)**	8.42 **(0.0005)**	4.73 **(<.0001)**	
FP	3	0.033 (0.117)	0.024 (0.544)	-	6.51 (0.007)	4.37 (0.036)	6.75 (0.085)	9.98 (0.099)	4.68 **(<.0001)**	
NO	4	0.031 (0.257)	0.022 (0.700)	0.033 (0.082)	-	5.49 **(0.0005)**	6.53 (0.005)	10.57 **(<.0001)**	4.74 **(0.0001)**	
RC	8	0.033 (0.038)	0.019 (0.551)	0.021 (0.549)	0.029 (0.118)	-	4.92 (0.002)	9.21 (0.004)	3.14 **(<.0001)**	
SC	3	0.034 (0.153)	0.031 (0.142)	0.028 **(<.0001)**	0.034 (0.164)	0.022 (0.604)	-	8.3 **(<.0001)**	5.27 **(0.0001)**	
SL	3	0.030 (0.470)	0.026 (0.653)	0.027 (0.703)	0.034 (0.324)	0.028 (0.239)	0.029 (0.605)	-	10.37 **(0.0004)**	
SW	17	0.025 (0.066)	0.022 (0.072)	0.020 (0.412)	0.031 **(0.003)**	0.019 (0.067)	0.027 (0.071)	0.031 (0.027)	-	
		Procrustes distance (*p*-values)								

BL = Bleaker; DA = Darwin; FP = Frying Pan; NO = North Arm; RC = Roy Cove; SC = San Carlos; SL = Sea Lion; SW = Shallow Bay. Mahalanobis distances significant at $p < 0.001$, and Procrustes distances significant at $p < 0.05$ are shown in bold.

Appendix Table C.12 Mahalanobis and Procrustes distances between male forewings from eight Falkland Island sites following CVA.

site	*n*	BL	DA	FP	NO	RC	SC	SL	SW	
BL	4	-	7.21 (0.0006)	5.31 (0.024)	8.38 (0.003)	5.17 **(0.0005)**	11.39 (0.069)	10.12 (0.0831)	6.60 **(0.0001)**	
DA	11	0.027 (0.076)	-	5.28 **(<.0001)**	6.21 **(<.0001)**	4.65 **(<.0001)**	10.37 (0.023)	11.71 (0.061)	3.52 **(<.0001)**	
FP	4	0.022 (0.500)	0.026 (0.131)	-	7.68 (0.0009)	2.83 (0.871)	11.63 (0.085)	10.49 (0.137)	4.74 **(0.0001)**	
NO	6	0.028 (0.277)	0.020 (0.427)	0.033 (0.121)	-	6.46 **(0.0001)**	8.72 (0.097)	13.82 (0.081)	4.43 **(<.0001)**	Mahalanobis distance (*p*-values)
RC	9	0.021 (0.621)	0.017 (0.470)	0.017 (0.809)	0.021 (0.478)	-	10.71 (0.056)	10.64 (0.040)	3.55 (0.015)	
SC	1	0.054 (0.153)	0.054 (0.035)	0.063 (0.192)	0.052 (0.296)	0.056 (0.082)	-	17.12 (0.063)	9.53 (0.054)	
SL	1	0.040 (0.067)	0.040 (0.428)	0.039 (0.282)	0.048 (0.283)	0.040 (0.452)	0.063 (1.000)	-	11.74 (0.037)	
SW	16	0.026 (0.060)	0.023 (0.806)	0.023 (0.156)	0.023 (0.093)	0.015 (0.404)	0.054 **(0.021)**	0.037 (0.249)	-	

Procrustes distance (*p*-values)

BL = Bleaker; DA = Darwin; FP = Frying Pan; NO = North Arm; RC = Roy Cove; SC = San Carlos; SL = Sea Lion; SW = Shallow Bay. Mahalanobis distances significant at $p < 0.001$, and Procrustes distances significant at $p < 0.05$ are shown in bold.

Appendix Table C.13 Mahalanobis and Procrustes distances between female forewings from four Latin American sites following CVA.

	n	Chubut	Punta Arenas	Panguipulli	Rio McClelland	
Chubut	10	-	9.48 (<0.0001)	5.07 (0.0003)	9.37 (<0.0001)	
Punta Arenas	5	0.026 (0.354)	-	10.55 (0.002)	6.73 (0.0002)	Mahalanobis distance (*p*-values)
Panguipulli	4	0.028 (0.253)	0.035 (0.153)	-	10.28 (0.002)	
Rio McClelland	7	0.029 (0.060)	0.023 (0.540)	0.039 (0.031)	-	

Procrustes distance (*p*-values)

Mahalanobis distances significant at $p < 0.001$, and Procrustes distances significant at $p < 0.05$ are shown in bold.

Appendix Table C.14 Mahalanobis and Procrustes distances between male forewings from five Latin American sites following CVA.

	n	Bariloche	Chubut	Punta Arenas	Panguipulli	Rio McClelland	
Bariloche	2	-	11.5 (0.008)	12.75 (0.012)	12.70 (0.013)	13.02 **(0.001)**	Mahalanobis distance (*p*-values)
Chubut	9	0.037 (0.298)	-	7.19 **(0.0004)**	6.93 **(0.0004)**	4.24 **(<0.0001)**	
Punta Arenas	4	0.031 (0.891)	0.021 (0.832)	-	8.94 (0.006)	6.19 **(0.0001)**	
Panguipulli	5	0.047 (0.341)	0.034 (0.094)	0.037 (0.325)	-	7.00 **(0.0001)**	
Rio McClelland	26	0.042 **(0.019)**	0.018 (0.159)	0.026 (0.139)	0.040 **(0.0001)**	-	
		Procrustes distance (*p*-values)					

Mahalanobis distances significant at $p < 0.001$, and Procrustes distances significant at $p < 0.05$ are in bold font.

Appendix Table C.15 Mahalanobis and Procrustes distances between Falklands and Latin American female and male hind wing shapes following CVA.

	n	Falklands, female	Falklands, male	Latin America, female	Latin America, male	
Falklands, female	48	-	2.47 **(<0.0001)**	2.62 **(<0.0001)**	4.14 **(<0.0001)**	Mahalanobis distance (*p*-values)
Falklands, male	52	0.027 **(<0.0001)**	-	3.75 **(<0.0001)**	3.22 **(<0.0001)**	
Latin America, female	30	0.021 (0.0099)	0.040 **(<0.0001)**	-	4.04 **(<0.0001)**	
Latin America, male	48	0.034 **(<0.0001)**	0.028 **(<0.0001)**	0.038 **(<0.0001)**	-	
		Procrustes distance (*p*-values)				

Mahalanobis distances significant at $p < 0.001$, and Procrustes distances significant at $p < 0.05$ are in bold.

Appendix Table C.16 Mahalanobis and Procrustes distances between female hind wings from eight Falkland Island sites following CVA.

site	*n*	BL	DA	FP	NO	RC	SC	SL	SW	
BL	4	-	6.40 (0.003)	7.20 (0.017)	8.64 (0.002)	6.99 **(0.001)**	9.62 (0.023)	7.69 (0.051)	6.56 **(<.0001)**	
DA	5	0.031 (0.590)	-	8.74 (0.007)	7.79 **(0.0002)**	4.33 (0.004)	8.41 (0.016)	6.89 **(0.0005)**	5.16 **(<.0001)**	
FP	3	0.021 (0.978)	0.035 (0.401)	-	7.87 (0.004)	8.56 (0.005)	11.01 (0.081)	6.92 (0.035)	9.72 **(<.0001)**	
NO	5	0.030 (0.517)	0.037 (0.086)	0.023 (0.858)	-	6.01 **(0.001)**	9.00 (0.019)	9.42 (0.005)	7.17 **(<.0001)**	Mahalanobis distance (*p*-values)
RC	8	0.032 (0.224)	0.023 (0.437)	0.029 (0.294)	0.026 (0.108)	-	6.41 (0.004)	7.99 (0.022)	4.90 **(<.0001)**	
SC	3	0.043 (0.251)	0.055 **(0.037)**	0.035 (0.428)	0.030 (0.327)	0.043 **(0.010)**	-	10.73 (0.098)	8.90 **(0.0006)**	
SL	2	0.032 (0.795)	0.041 (0.257)	0.030 (0.899)	0.035 (0.155)	0.036 (0.081)	0.036 (0.164)	-	9.94 **(0.0001)**	
SW	17	0.031 (0.102)	0.016 (0.728)	0.033 (0.081)	0.034 **(0.004)**	0.021 (0.070)	0.053 **(0.0002)**	0.044 **(0.011)**	-	

Procrustes distance (*p*-values)

BL = Bleaker; DA = Darwin; FP = Frying Pan; NO = North Arm; RC = Roy Cove; SC = San Carlos; SL = Sea Lion; SW = Shallow Bay. Mahalanobis distances significant at $p < 0.001$, and Procrustes distances significant at $p < 0.05$ are in bold.

Appendix Table C.17 Mahalanobis and Procrustes distances between male hind wings from eight Falkland Island sites following CVA.

site	*n*	BL	DA	FP	NO	RC	SC	SL	SW	
BL	4	-	7.25 **(0.0009)**	5.06 (0.016)	6.82 (0.005)	5.35 **(0.0007)**	11.80 (0.136)	7.60 (0.037)	6.71 **(<.0001)**	
DA	11	0.033 (0.300)	-	7.85 **(0.0006)**	8.62 **(0.0002)**	4.71 **(<.0001)**	9.74 (0.012)	9.16 (0.0092)	3.75 **(<.0001)**	
FP	4	0.040 (0.053)	0.023 (0.699)	-	8.58 (0.003)	6.56 **(0.0002)**	13.77 (0.134)	8.05 (0.046)	7.10 **(<.0001)**	
NO	5	0.035 (0.595)	0.053 **(0.014)**	0.063 **(0.018)**	-	6.77 **(0.0005)**	10.44 (0.014)	7.44 (0.006)	8.27 **(<.0001)**	Mahalanobis distance (*p*-values)
RC	10	0.030 (0.314)	0.021 (0.478)	0.032 (0.182)	0.050 **(0.011)**	-	11.26 (0.008)	7.77 **(0.0005)**	4.59 **(<.0001)**	
SC	1	0.051 (0.518)	0.049 (0.446)	0.061 (0.203)	0.047 (0.841)	0.052 (0.402)	-	12.96 (0.331)	9.95 **(0.035)**	
SL	2	0.041 (0.384)	0.042 (0.372)	0.048 (0.068)	0.050 (0.390)	0.043 (0.253)	0.053 (1.000)	-	7.51 **(0.003)**	
SW	16	0.039 **(0.017)**	0.017 (0.536)	0.025 (0.278)	0.059 **(0.0005)**	0.022 (0.165)	0.051 (0.225)	0.044 (0.094)	-	

Procrustes distance (*p*-values)

BL = Bleaker; DA = Darwin; FP = Frying Pan; NO = North Arm; RC = Roy Cove; SC = San Carlos; SL = Sea Lion; SW = Shallow Bay. Mahalanobis distances significant at $p < 0.001$, and Procrustes distances significant at $p < 0.05$ are in bold font.

C.3.3 The effects of elevation and altitude on wing shape

Appendix Table C.18 The effect on female forewing length of elevation and latitude, and the interaction of the two.

factor	*df*	*SS*	*MS*	*F*-ratio	*p*-value	η_p^2
elevation	1	0.66	0.66	0.24	0.629	0.009
latitude	1	0.19	0.19	0.07	0.793	0.003
elevation:latitude	1	0.02	0.02	0.01	0.924	<0.001
residuals	26	71.50	2.75			

Linear model with Type II sums of squares

Appendix Table C.19 The effect on male forewing length of elevation and latitude, and the interaction of the two.

factor	*df*	*SS*	*MS*	*F*-ratio	*p*-value	η_p^2
elevation	1	7.51	7.51	6.66	**0.013**	0.134
latitude	1	0.70	0.70	0.62	0.435	0.014
elevation:latitude	1	2.66	2.66	2.36	0.131	0.052
residuals	43	48.46	1.13			

Linear model with Type II sums of squares. Significance at < 0.05 is shown in bold.

Appendix Table C.20 The effect on female forewing AR of elevation and latitude, and the interaction between the two.

factor	*df*	*SS*	*MS*	*F*-ratio	*p*-value	η_p^2
elevation	1	0.06	0.06	5.51	**0.027**	**0.174**
latitude	1	0.04	0.04	4.10	0.053	0.136
elevation:latitude	1	<0.01	<0.01	0.27	0.606	0.272
residuals	26	0.27	0.01			

Linear model with Type II sums of squares. Significance at $p < 0.05$ and large effect size at $\eta_p^2 > 0.14$, are shown in bold.

Appendix Table C.21 The effect on male forewing AR of elevation and latitude, and the interaction between the two.

factor	*df*	*SS*	*MS*	*F*-ratio	*p*-value	η_p^2
elevation	1	0.12	0.12	10.09	**0.003**	**0.190**
latitude	1	0.01	0.01	0.90	0.348	0.020
elevation:latitude	1	0.01	0.01	0.77	0.384	0.018
residuals	43	0.53	0.01			

Linear model with Type II sums of squares. Significance at $p < 0.05$ and large effect size at $\eta_p^2 > 0.14$, are shown in bold.

Appendix Table C.22 The effect on female forewing $\hat{r}1$ of elevation and latitude, and the interaction between the two.

factor	*df*	*SS*	*MS*	*F*-ratio	*p*-value	η_p^2
elevation	1	<0.01	<0.01	0.59	0.450	0.022
latitude	1	<0.01	<0.01	0.11	0.744	0.004
elevation:latitude	1	<0.01	<0.01	0.04	0.834	0.002
residuals	26	<0.01	<0.01			

Linear model with Type II sums of squares

Appendix Table C.23 The effect on male forewing $\hat{r}_1$ of elevation and latitude, and the interaction between the two.

factor	*df*	*SS*	*MS*	*F*-ratio	*p*-value	η_p^2
elevation	1	<0.01	<0.01	4.93	**0.032**	0.102
latitude	1	<0.01	<0.01	0.36	0.552	0.008
elevation:latitude	1	<0.01	<0.01	0.75	0.391	0.017
residuals	43	<0.01	<0.01			

Linear model with Type II sums of squares. Significance at < 0.05 is shown in bold.

Appendix Table C.24 The effect of wind speed at 30cm on forewing length, AR and $\hat{r}_1$

factor	*df*	*SS*	*MS*	*F*-ratio	*p*-value
length, female	1,14	2.323	2.323	1.87	0.193
length, male	1,17	1.162	1.161	1.13	0.302
AR, female	1,14	0.025	0.025	1.40	0.257
AR, male	1,17	0.001	0.001	0.10	0.756
$\hat{r}_1$,female	1,14	<0.001	<0.001	0.35	0.566
$\hat{r}_1$, male	1,17	<0.001	<0.001	0.26	0.614

Linear model with Type II sums of squares

Appendix D Supplementary material for chapter 7, claw shape and size: variation and wind

D.1 Image acquisition

Legs were removed in the field using forceps and were stored in Eppendorf tubes marked with identification numbers, leg side (left or right) and leg pair (hind or middle). hypertension Whole butterflies were initially stored in glassine envelopes. Their legs were subsequently removed in the laboratory and stored the same way as those in the field. Samples from the BMNH were removed at the museum, and stored in the marked tubes which the museum provided.

Claws were separated from the tarsus under a Leica S8 Apo microscope. They were placed on single cavity microscope slides, to avoid crushing, and covered with coverslips. The butterfly's ID and sex were recorded, together with the side of the body the leg was taken from and the leg pair, whether middle or hind.

Each claw was marked either A or B, depending on whether it was the left or right claw looking at the face of the arolium. As hind legs were backward-facing, and middle legs forward-facing, the distal claw on the middle right leg was, for example, initially recorded as A, and on the hind right leg as B.

The majority of legs collected were from the right side of the butterfly as seen from the dorsal side. There was not enough material to enable direct comparison between the left and right sides, although it is conventional to assume bilateral symmetry in butterflies. Thus no distinction was made between left and right legs within a leg pair, other than to ensure the correct identification of distal and proximal claws.

Each claw was photographed from both sides of the slide through a Keyence VHX-600 microscope at 200x magnification, using the microscope's inbuilt camera and 100 µm scale. Images were saved in the lossless format TIFF.

To minimise the possibility of measurement error, all photographs were taken under identical conditions.

D.2 Image processing

D.2.1 Traditional morphometrics

The TIFF images were processed in ImageJ using the FIJI platform (Schindelin et al. 2012, Schneider et al. 2012).

Three points were fixed on each claw. These subsequently served as the fixed landmarks in geometric morphometric analysis.

Lines were constructed between the fixed points, from the claw tip to, respectively, the ventral and dorsal inflections. These were envisaged as chords of a circle.

Chords were bisected using the macro “bissect” (sic) (Burri 2016). The chords from which the two angles were constructed were also measured to determine variation in claw size between populations.

The angles which were subtended by these chords were then constructed and measured, following the approach set out in Feduccia (1993). Duplouy and Hanski (2013) used a directly mathematically related variant of this where, if Feduccia's angle is a° and Duplouy and Hanski's b°, then b=360-2a. For comparisons, Feduccia's angle was used, and Duplouy and Hanski's converted accordingly.

The greater the Feduccia angle, the more sharply curved was the claw. While the angle measurement this produced might appear counter-intuitive, it was retained here in accordance with past practice.

D.2.2 Geometric morphometrics

TIFF images were processed in the tps software suite (Rohlf 2010, 2015).

Images were grouped and imported into tpsUtil, where files were created for processing in tpsDig2. Images were flipped in tpsDig2 where necessary to ensure all images had the same orientation. Three fixed landmarks were then applied to each image, at the point where the dorsal curve met the tarsus; at the claw’s tip; and at the point where the ventral curve met the tarsus.

Sliding landmarks were then added on dorsal and ventral curves between the fixed landmarks of their respective tarsal junctions and the claw tip.

As a first step, as many landmarks were applied (typically 30-40), using the curve tool, as would give the most accurate rendition of the curve. The curve was then resampled, and the number of landmarks reduced to 30, by length: that is to say, they were aligned equidistant from each other.

Each data set was saved as a single set of landmarks, which were subsequently handled as if they were permanent landmarks (as in Tinius and Russell 2017). The data sets were then combined into data sets of 59 landmarks (those at the tip from each data set being identical) to enable analysis of the overall shape of the claw.

The tps files were processed in MorphoJ (Klingenberg 2011, 2013). They were subjected to a Procrustes fit, followed by the generation of covariance matrices. On the basis of these, two analyses were undertaken:

- Principal Component Analysis (PCA), to investigate the differences between individuals, and to visualise the main components of claw shape in individuals;
- Canonical Variate Analysis (CVA), to investigate the relationship between data from pre-assigned groups, based on both Mahalanobis and Procrustes distances.

D.3 Measurement error

Measurement error was assessed through a subset of 17 claws, 6 from the middle pair and 11 from the hind pair, collected during the first two field trips. Each surface of each claw was measured five times. It was calculated by the intra-class correlation coefficient represented by the equation:

$$\text{ICC} = \frac{S^2_A}{S^2_A + S^2_W}$$

where S^2_A is the variance between groups, and S^2_W the variance within groups (Lessells and Boag 1987, Bailey and Byrnes 1990, Wolak et al. 2012).

The ICC scores were not low enough to give concerns about repeatability:
hind claw ventral angle: ICC 0.86, 95% CI[0.71 - 0.95], $V_W = 7.12$, $V_A = 42.28$; middle claw ventral angle: ICC 0.96 95% CI[0.88 - 0.99], $V_W = 7.62$, $V_A = 176.61$; hind claw ventral chord: ICC 0.96, 95% CI[0.91 - 0.99], $V_W = 10.18$, $V_A = 241.45$; middle claw ventral chord: ICC 0.98, 95% CI[0.95 - 1.00], $V_W = 3.71$, $V_A = 227.80$.

D.4 Additional statistical material on claw size and shape

Appendix Table D.1 The effect of sex (female, male), leg pair (hind, middle) and tarsal claw position (distal, proximal) on chord length. Three-way ANOVA, allowing only two-way interactions.

(a) dorsal chords

factor	*df*	*SS*	*MS*	*F*-ratio	*p*-value	η_p^2
sex	1	7188.3	7188.3	21.07	**<0.001**	**0.212**
pair	1	830.8	830.8	2.43	0.122	0.028
position	1	3280.6	3280.6	9.61	**0.003**	0.102
sex:pair	1	315.5	315.5	0.92	0.339	0.011
sex:position	1	51.7	51.7	0.70	0.698	0.001
pair:position	1	170.3	170.3	0.50	0.499	0.006
residuals	85	29001.7	70.4			

(b) ventral chords

factor	*df*	*SS*	*MS*	*F*-ratio	*p*-value	η_p^2
sex	1	949.4	949.4	5.16	**0.026**	0.073
pair	1	890.7	890.7	4.84	**0.030**	0.053
position	1	932.6	932.6	5.07	**0.027**	0.056
sex:pair	1	252.6	252.6	1.37	0.244	0.015
sex:position	1	40.4	40.4	0.22	0.641	0.001
pair:position	1	140.0	140.0	0.76	0.385	0.009
residuals	85	15637.8	184.0			

Significance at $p < 0.05$ and large effect size, $\eta_p^2 > 0.14$ are shown in bold.

Appendix Table D.2 The effect of sex (female, male), leg pair (hind, middle) and claw position (distal, proximal) on claw angle. A three-way ANOVA was used, allowing only two-way interactions.

(a) dorsal angles

factor	*df*	*SS*	*MS*	*F* - ratio	*p* -value	η_p^2
sex	1	118.3	118.3	1.68	0.198	0.002
pair	1	2213.4	2213.4	31.45	**<0.001**	**0.270**
position	1	43.4	43.4	0.62	0.435	0.007
sex:pair	1	62.9	62.9	0.89	0.347	0.010
sex:position	1	51.6	51.6	0.73	0.394	0.007
pair:position	1	24.4	24.4	0.35	0.557	0.004
residuals	85	5981.1	70.4			

(b) ventral angles

factor	*df*	*SS*	*MS*	*F* - ratio	*p* -value	η_p^2
sex	1	795.2	795.2	8.78	**0.004**	0.059
pair	1	1311.8	1311.8	14.49	**<0.001**	**0.145**
position	1	112.1	112.1	1.24	0.269	0.014
sex:pair	1	106.0	106.0	1.17	0.282	0.013
sex:position	1	39.3	39.3	0.43	0.511	<0.004
pair:position	1	13.8	13.8	0.15	0.696	<0.002
residuals	85	7693.3	90.5			

Significance at $p < 0.05$ and large effect size, $\eta_p^2 > 0.14$, are shown in bold.

Appendix Table D.3 Mahalanobis and Procrustes distances between female and male, hind and middle claw shapes following CVA.

	n	female hind	female middle	male hind	male middle	
female hind	25	-	6.49 **(<0.0001)**	10.34 **(<0.0001)**	6.14 **(<0.0001)**	Mahalanobis distance (*p*-values)
female middle	31	0.028 (**0.016**)	-	11.46 **(<0.0001)**	5.13 **(<0.0001)**	
male hind	16	0.020 (0.176)	0.041 (**<0.001**)	-	11.36 **(<0.0001)**	
male middle	28	0.025 (**0.029**)	0.019 (0.110)	0.038 **(<0.001)**	-	
		Procrustes distance (*p*-values)				

Significance at $p < 0.001$ for Mahalanobis distances, and $p < 0.05$ for Procrustes distances, are shown in bold. *p*-values are derived from 10,000 round permutation tests.

Appendix Table D.4 Mahalanobis and Procrustes distances between the four Falkland Islands study sites following a CVA of hind claws.

	n	Bleaker	Frying Pan	Roy Cove	Sea Lion	
Bleaker	10	-	3.31 (0.001)	2.38 (0.810)	5.29 **(0.0005)**	Mahalanobis distance (*p*-values)
Frying Pan	9	0.029 (0.247)	-	2.66 (0.093)	7.05 **(0.0005)**	
Roy Cove	10	0.014 (0.764)	0.032 (0.113)	-	6.43 **(0.0002)**	
Sea Lion	5	0.024 (0.454)	0.042 (0.105)	0.024 (0.255)	-	
		Procrustes distance (*p*-values)				

Significance at $p < 0.001$ for Mahalanobis distances, and $p < 0.05$ for Procrustes distances, are shown in bold. *p*-values are derived from 10,000 round permutation tests.

Appendix Table D.5 Mahalanobis and Procrustes distances between the four Falkland Islands study sites following a CVA of middle claws. All Mahalanobis distances were significant at $p < 0.001$.

	n	Bleaker	Frying Pan	Roy Cove	Sea Lion	
Bleaker	19	-	3.07 **(< 0.0001)**	3.41 **(< 0.0001)**	5.69 **(0.0001)**	Mahalanobis distance (*p*-values)
Frying Pan	16	0.020 (0.141)	-	3.12 **(0.0001)**	5.07 **(<0.0001)**	
Roy Cove	15	0.021 (0.226)	0.011 (0.852)	-	5.07 **(0.0004)**	
Sea Lion	5	0.037 (0.056)	0.030 (0.098)	0.041 (0.090)	-	
		Procrustes distance (*p*-values)				

Significance at $p < 0.001$ for Mahalanobis distances, and $p < 0.05$ for Procrustes distances, are shown in bold. *p*-values are derived from 10,000 round permutation tests.

Appendix Table D.6 The effect of sex and site on ventral chord length for *M. cinxia* in the Baltic. Two way ANOVA.

factor	*df*	*SS*	*MS*	*F*-ratio	*p*-value	η_p^2
site	3	10827	3609	6.30	**<0.001**	**0.145**
sex	1	4635	4635	8.09	**0.005**	0.067
site:sex	3	1841	614	1.07	0.364	0.028
residuals	113	64709	573			

Significance at $p < 0.05$ and large effect size, $\eta_p^2 > 0.14$ are shown in bold.

Appendix Table D.7 The effect of sex and site on ventral angles for *M. cinxia* in the Baltic. Two-way ANOVA

factor	*df*	*SS*	*MS*	*F*-ratio	*p*-value	η_p^2
site	3	2383	794	3.89	**0.011**	0.097
sex	1	227	227	1.11	0.294	0.010
site:sex	3	632	210	1.03	0.382	0.027
residuals	113	23093	204			

Significance at $p < 0.05$ is shown in bold.

Appendix Table D.8 Correlation between claw angles and wind speeds at the four Falkland Island study sites (Pearson's *r*)

wind speeds at:	claw angle			
	hind, dorsal	middle, dorsal	hind, ventral	middle, ventral
ceiling (150 cm)	1.00	0.95	1.00	0.98
patrol (30 cm)	1.00	0.95	0.99	0.99
oviposition (3cm)	0.56	0.31	0.67	0.46

Appendix Table D.9 Comparison of claw angles between small island (Bleaker and Sea Lion) and large island (Frying Pan and Roy Cove) sites.

a	p	s	l	*n*	*M*	*SD*	*df*	*SS*	*MS*	*F*	*p*	95% CI	*d*
d	h		i	15	132.3	6.68	1, 28	165.5	165.5	4.56	**0.042**	-9.21,-0.19	0.78
			m	15	127.7	5.30							
d	m		i	22	121.2	6.63	1, 45	156.9	156.9	3.18	0.081	-7.79, 0.48	0.52
			m	25	117.6	7.36							
d	h	f	i	10	130.3	6.65	1, 15	38.4	38.4	1.08	0.314	-9.30, 3.19	0.51
			m	7	127.3	4.69							
d	h	m	i	5	136.4	5.01	1, 11	219.2	219.2	6.72	**0.025**	-15.61,-1.27	**1.48**
			m	8	128.0	6.08							
d	m	f	i	14	121.3	6.53	1, 21	139.2	139.2	2.23	0.150	-12.05, 1.97	0.63
			m	9	116.3	9.71							
d	m	m	i	8	121.0	7.25	1, 22	39.9	39.9	0.99	0.332	-8.45, 2.98	0.42
			m	16	118.3	5.9							
v	h	f	i	10	157.2	7.21	1, 16	103.7	103.7	1.73	0.207	-12.61, 2.95	0.62
			m	8	152.3	8.36							
v	h	m	i	5	158.3	3.10	1, 11	193.0	193.0	5.37	**0.041**	-15.44,-0.40	**1.32**
			m	8	150.4	7.14							
v	m	f	i	14	149.9	6.47	1, 23	87.1	87.1	1.45	0.241	-10.22, 2.70	0.48
			m	11	146.2	9.15							
v	m	m	i	8	146.8	7.02	1, 22	99.8	99.8	1.07	0.312	-13.00, 4.35	0.45
			m	16	142.5	10.67							

a = angle, p = claw pair, s = sex, l = landscape; d = dorsal, v = ventral; h = hind, m = middle; f = female, m = male; i = small Falklands, m = other Falklands. Significance at $p < 0.05$ and $d > 0.8$ are shown in bold.

Appendix Table D.10 Comparison of claw chords between small islands (Bleaker and Sea Lion) and other Falklands (Frying Pan and Roy Cove) sites.

ch	p	s	c	l	*n*	*M*	*SD*	*df*	*SS*	*MS*	*F*	*p*	95% CI	*d*
d	h	f	d	i	7	284.8	22.95	1, 10	1.0	0.5	0.001	0.973	-26.51, 27.35	0.02
				m	5	285.2	16.58							
d	h	f	p	i	7	274.1	23.13	1, 11	103.0	103.0	0.26	0.620	-13.09, 18.84	0.38
				m	6	279.7	15.12							
d	h	m	d	i	4	261.2	9.30	1, 6	16.5	16.5	0.19	0.675	-13.10, 18.84	0.31
				m	4	264.1	9.16							
d	h	m	p	i	4	257.5	22.39	1, 9	419.6	419.6	1.21	0.299	-13.51, 39.19	0.69
				m	7	270.3	16.36							
d	m	f	d	i	8	280.1	22.36	1, 15	41.0	41.1	0.12	0.736	-16.26, 22.49	0.17
				m	9	283.2	14.79							
d	m	f	p	i	10	278.4	30.07	1, 16	362.0	361.5	0.53	0.476	-17.16, 35.20	0.35
				m	8	287.4	19.67							
d	m	m	d	i	6	266.5	24.80	1, 17	39.0	38.6	0.09	0.765	-24.35, 18.22	0.15
				m	13	263.4	18.32							
d	m	m	p	i	8	278.1	9.83	1, 18	58.0	58.2	0.15	0.703	-22.37, 5.41	0.18
				m	12	274.6	23.95							
v	h	f	d	i	7	209.8	15.32	1, 10	0.5	0.5	0.003	0.961	-18.20, 19.04	0.03
				m	5	210.2	12.54							
v	h	f	p	i	7	203.7	18.20	1, 11	98.3	98.3	0.49	0.498	-11.82, 22.85	0.38
				m	6	209.2	6.60							
v	h	m	d	i	4	190.7	7.64	1, 6	446.0	446.0	9.43	**0.022**	3.03, 26.83	**2.17**
				m	4	205.6	6.02							
v	h	m	p	i	4	193.0	11.75	1, 10	636.5	636.5	6.84	**0.026**	2.28, 28.62	**1.60**
				m	8	208.5	8.60							
v	m	f	d	i	8	208.4	14.36	1, 15	86.5	86.5	0.50	0.489	-9.06, 18.09	0.34
				m	9	212.9	11.91							
v	m	f	p	i	10	204.6	17.59	1, 17	721.0	721.2	3.04	0.099	-2.59, 27.27	**0.80**
				m	9	217.0	12.49							
v	m	m	d	i	6	207.0	21.35	1, 17	198.0	197.7	0.85	0.370	-22.82, 8.89	0.45
				m	13	200.1	11.82							
v	m	m	p	i	8	211.0	8.36	1, 18	2.2	2.2	0.01	0.906	-11.16, 12.52	0.05
				m	12	211.7	14.32							

ch= chord, p = claw pair, s = sex, c = claw, ld = landscape d = dorsal, v = ventral; h = hind, m = middle; f = female, m = male; d = distal, p = proximal; i = small island, m = other Falklands. Significance at $p < 0.05$ and $d > 0.8$ are shown in bold.

Appendix E Supplementary material for chapter 8: draft species action plan

Draft Species Action Plan

FALKLAND FRITILLARY

Yramea cytheris cytheris

April 2020

compiled by:
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This species action plan has been written to meet the requirements of the Falkland Islands Biodiversity Strategy (Falkland Islands Government 2008) for basic action plans for a number of species identified in the Falkland Islands State of the Environment Report (Otley et al. 2008). It follows the format adopted by Butterfly Conservation, the UK butterfly and moth conservation organisation.

CONTENTS

Part 1: Overview

Part 2: Biological Assessment

Part 3: Actions and Work Programme

Summary

- The Falkland fritillary, *Yramea cytheris cytheris*, also known as the Queen of the Falklands fritillary, is the Falkland Islands' only resident butterfly. It is listed by the Falkland Islands Government as a potentially threatened species and is protected under the Conservation of Wildlife and Nature Bill 1999.
- The implementation of this plan is given a **medium** priority, to ensure the continued presence of the Falkland fritillary in the islands.
- the Falkland fritillary is found on mosaics of grassland, dwarf shrub heath and bare ground which hold the larval host plant, *Viola* spp, principally *Viola maculata.*
- The main threats to the Falkland fritillary are climate change, stochastic events such as the inundation of oviposition sites, and changing land use. Broader conservation projects should take into account possible benefits to the butterfly of sites grazed by sheep, and nectaring plants with long flowering seasons, such as the invasive groundsel *Senecio vulgaris.*
- The main objective of the plan is to ensure a broad network of sites around the islands where the often small and isolated populations can flourish. It identifies the need for further research on the butterfly's life cycle; its population structures, including investigation of possible metapopulations; an investigation of the relationship between sheep grazing and host plant density, including sheep as a possible dispersal mechanisms; and of the genetic relationship of the various populations, including the Latin American sub-species.
- The objectives of the plan will only be met with wider awareness of the butterfly's requirements through stakeholder engagement, including government, conservation organisations and landowners.
- In the longer term, plans should be drawn up to identify donor populations to address local extinctions, and consideration should be given to developing captive-breeding expertise on the islands in case of wider extinctions.
- The Falkland fritillary's future would be more assured through greater public awareness beyond the Falklands. The elaboration of joint projects with Latin American researchers would be highly desirable, particularly in the context of donor populations. More widely, *Yramea cytheris,* through the Latin American sub-species, is a strong contender for the world's most southerly butterfly, a

potentially valuable study species in itself, but also helpful for comparative work with fritillary model species such as the Granville fritillary *Melitaea cinxia* and Edith's checkerspot, *Euphydryas editha.*

- The action plan covers the next ten years. It will be reviewed annually, and amended as necessary.

Part 1: Overview

1.1 PRIORITY STATEMENT

The Falkland Islands State of the Environment Report (Otley et al 2008) included the Falkland fritillary as a potential threatened species due to its apparent rarity in the islands. Subsequent research suggests that the butterfly is distributed throughout the Falklands, but in small, isolated populations, which are at risk from both climate change and changing land use. Based on current knowledge, a **medium** priority should be afforded to conservation action to protect and increase the number of Falkland fritillary colonies in the Falklands.

1.2 BROAD OBJECTIVES

The broad objectives of this plan are to:

- maintain a viable network of populations throughout the Falkland Islands;
- conduct further research on the life cycle, ecology and distribution of the species to help develop conservation policies;
- develop a system for monitoring population sizes to identify threats of local extinction;
- ensure plans for changing land use, including development of conservation areas, takes into account potential impact on the butterfly's habitat;
- develop stakeholder engagement on conservation, both in the islands and more widely, particularly in Latin America.

1.3 LEGAL STATUS

The Falkland fritillary is protected under The Conservation of Wildlife and Nature Bill 1999 (Falkland Islands Government 1999), as is its larval food plant *Viola maculata*.

1.4 STATUS AND LEVEL OF BIOLOGICAL KNOWLEDGE

Population:	size	Promising. Individuals and colonies have been found throughout the islands, although overall numbers, and the number and size of colonies, are unknown.
	trend: numbers	Unknown. Baselines and protocols for monitoring are urgently needed
	trend: range	Unknown. Early records were mainly from West Falkland, but sightings now come from both West and East, as well as outer islands. It is difficult to know whether this represents an increase in range. Reports are anecdotal, from sparsely populated areas, and lack of any systematic records hampers assessment.
Knowledge of:	status	No data. Location and size of colonies need to be established.
	trends	No data.
	conservation requirements	Improving. Habitat preferences, including larval host plants, are known. Further work is needed to establish: life cycle, particularly of pupal stage; population sizes; mobility between colonies; and appropriate monitoring methods.

Part 2: Biological Assessment

2.1 INTRODUCTION

Yramea cytheris comprises two subspecies, *Y. c. cytheris*, the Falkland fritillary (Figure 1), and *Y.c.siga*, known in South America as ana del sur. The initial distinction between the two was based on colour (Watkins 1924), with *Y. c. cytheris* females having pink and purple underwing tones compared with the ochre and brown of *Y. c. siga* , and both sexes having whiter underwing markings.

The Falklands butterfly is smaller, the female *Y. c. cytheris* has a mean forewing length of 17.3 mm, compared with *Y. c. siga's* 19.1 mm, while the male *Y. c. cytheris* has a mean forewing length of 15.7 mm, compared with *Y. c. siga's* 17.4 mm. The two subspecies are, however, genetically indistinguishable, with Latin America sharing the most common Falklands haplotype.

Figure 1: The Falkland fritillary, *Yramea cytheris cytheris*, on a patch of wild celery, *Apium australe* on Bleaker Island.

Yramea .cytheris is sexually dimorphic. Females are bigger than males, with a striking difference between the undersides of their respective hind wings (Figure 2).

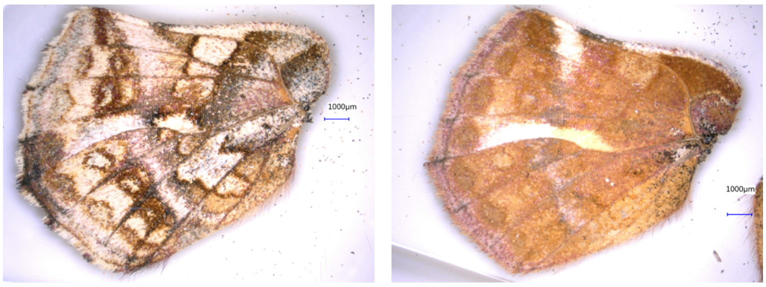

Figure 2: Ventral surfaces of the right hind wing of *Yramea cytheris cytheris* showing sexual dimorphism. The female is on the left, the male on the right.

The Falklands fritillary is found in sunny, sheltered areas, particularly on grassland and dwarf shrub heath, where its larval host plants, *Viola* spp., especially the common violet, *Viola maculata*, occur in small patches.

2.2 ECOLOGY

2.2.1 Life Cycle

Yramea cytheris is on the wing from November to the beginning of March, with most records from December and January. The adult stage is of four or five days for the female, slightly less for the male. Butterflies are usually only seen in ones or twos, the exception being when there is an abundance of nectaring plants in flower, particularly the native Christmas bush *Baccharis magellanica* and the introduced groundsel *Senecio vulgaris.* Both females and males show little evidence of mobility, restricting themselves to a home patch and adopting an apparently aimless patrolling pattern, making flights of a few metres between pauses for nectaring, basking or resting.

Eggs are laid on the leaves of *Viola* spp. (Figure 3), usually singly on the underside of the leaf. On the rare occasions that more than one egg is found on a leaf there is no clustering. The main larval host plant is the common violet, *V. maculata*, although *V. magellanica*, is utilised on Sea Lion Island. Benyamini (Benyamini in preparation) recorded that eggs had been found in Stanley on *V. tricolor*, and that in Latin America, the main larval food plant for *Y. c. siga* was *V. reichei*.

Figure 3: *Y.c. cytheris* egg on underside of *V. maculata* leaf

The larvae feed on both sides of the *Viola* leaf, in a distinctive pattern which effectively strips the flesh on each side from the skeleton (Figure 4). It is probable that, like many fritillaries, it enters diapause as a larva, although no records have been found of either larvae in diapause or pupae.

Figure 4: Larval feeding pattern. The larva eats the fleshy part of the leaf from each side, giving the leaf a diagnostic filleted appearance.

2.2.2 Habitat

The key habitat requirement for *Y. cytheris* is the presence of *Viola*, usually the common violet, *V. maculata*, which is widespread throughout the islands (Figure 5), generally on dry coastal heathland or dry sandy slopes (Broughton and McAdam 2005, Liddle 2007). Unpublished records held by Falklands Conservation show that most *Viola* sites are coastal, although they have been found at elevations of up to 600 m. Patches of *Viola* are most commonly found in a mosaic of acid grassland, bare ground and dwarf shrub heath, although this is not invariable: *V. magellanica* on Sea Lion Island grows amongst often dense grass on boggy ground; *V. maculata* at the Frying Pan grows in small clusters on gravel, as well as in thick stands of Christmas bush *Baccharis magellanica;* and at Bleaker Island on a raised beach on amongst large patches of flat stones. Each of these sites has colonies of Falkland fritillary.

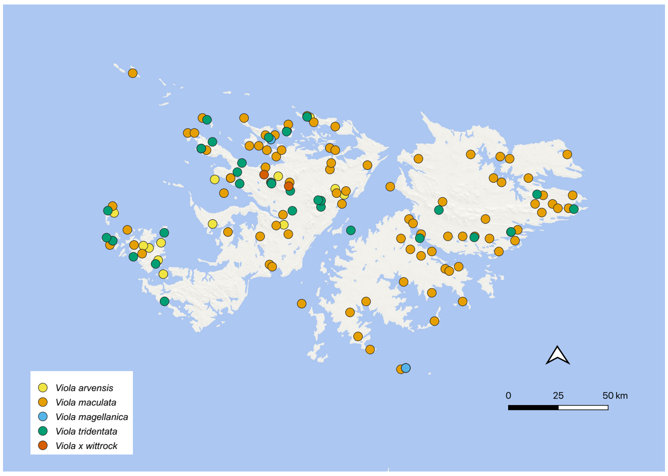

Figure 5: Map of Falkland Island sites for V*iola* spp. Data supplied by Rebecca Upson of Falklands Conservation. Most of the sites were around the coast, but all five species were found at elevations of up to 600m.

Y. cytheris shows a preference for medium-sized *Viola*, with a higher level of chlorophyll than neighbouring plants, for oviposition. It favours warm, sheltered sites particularly on north-east facing slopes which are protected from the prevailing winds and get the morning sun, helping speed eclosion.

2.3 DISTRIBUTION AND POPULATION

Carstairs (1990) concluded that the Falkland fritillary had been reliably reported from 12 locations in ten 10 km squares. A reappraisal of historical records, together with later observations, suggest a further nine sites hold, or have held, the Falkland fritillary (Figure 4).

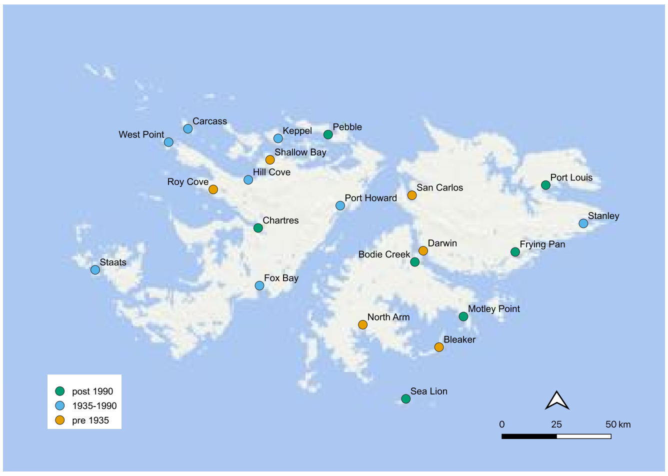

Figure 4: Map of Falkland Island records of *Yramea cytheris.* Records included museum specimens; the results of literature searches; personal communications and personal observations. They were divided into those found before 1935; between 1935 and 1990, drawing on Carstairs; and after 1990

There has been no systematic attempt to map colonies. In most years there are reports of sightings at a number of locations. Bleaker and Sea Lion feature prominently, but they have long tourist seasons with visitors who mostly have an interest in natural history, so that might be expected.

There is no long term data on population size. Mark-release-recapture studies suggest that, at most sites, fewer than ten adults are on the wing at any one time. When there are a lot of nectar plants in flower, particularly Christmas bush, *Baccharis magellanica*, and groundsel, Senecio vulgaris, up to 100 individuals have been recorded in a single day. There is an urgent need of a long-term count at one or two sites, on the basis of which a formula can be drawn up to allow population size to be estimated from a transect count.

2.4 LIMITING FACTORS

2.4.1 Historical

Since the Islands were permanently settled in 1833, the mixture of dwarf shrub heath, bare ground and grass which provides suitable habitat for *Viola*, *Y. cytheris's* larval host plant, has been the product of grazing, mainly by sheep. While no study of the relationship between sheep and *Viola* has been undertaken, it is noticeable that plants in grazed, sheltered areas are more likely to have *Y. cytheris* eggs laid on them than those in more overgrown environments. Any change in grazing patterns, or the cessation of grazing altogether, risks altering the nature of *Viola* growth, and thence the nature of the butterfly's present habitat.

2.4.1 Current and Future Limiting Factors

Possible concerns are any changes in farming patterns; industrialisation and increased population in camp and climate change. Work on conservation activity, such as the restoration of tussac, needs to take account of possible implications for both *Y. cytheris* and its larval host plant.

2.5 CONSERVATION TO DATE

The Conservation of Wildlife and Nature Bill 1999 (Falkland Islands Government 1999) lists as protected animals under Schedule 2 Part I “All species of butterflies (*Rhopalocera*)”. This affords *Y. cytheris* a considerable degree of protection. Part II Section 4 of the Bill makes illegal deliberately to capture or kill animals protected under this Schedule; to take or destroy their eggs; or to damage or destroy their breeding sites or resting places. It furthermore applies to all stages of life of those protected. The Bill makes clear, however, that a licence can be granted by the Governor, which would allow otherwise prohibited actions in certain cases, such as scientific or educational purposes, or conservation.

The Falkland fritillary’s larval food plant, *V. maculata*, was also given protected status “not because it is rare or endangered, but because it is thought to be the larval food plant of the Queen-of-the-Falklands Fritillary (*Issoria cytheris*) a nationally rare butterfly and protected wild animal” (Rendell n.d.)

Beyond legal protection, however, little has been done to conserve *Y. cytheris*. There have been attempts to collect data on distribution through appeals in the media, but these have produced few tangible results. Work, effectively, needs to start from scratch.

Part 3: Actions and Work Programme

Actions are given a low, medium or high priority. Lead organisations for each action are suggested.

	LEAD
3.1 POLICY AND LEGISLATIVE	
Action 1 PRIORITY: MEDIUM Consider potential damage to Falkland fritillary habitat when addressing planning and land use issues, and seek to mitigate.	FIG
3.2 SITE SAFEGUARD AND ACQUISITION	
Action 2 PRIORITY: MEDIUM Designate two or three sites, ideally already conservation areas, such as Bleaker and Sea Lion, as centres for appropriate research and management.	FIG, FC, Landowners
3.3 LAND MANAGEMENT	
Action 3 PRIORITY: HIGH Ensure management plans for any protected areas with Falkland fritillary colonies incorporate appropriate conservation measures, identifying and resolving conflicting priorities (see action 6).	FIG, FC
3.4 SPECIES PROTECTION AND LICENSING	
Action 4 PRIORITY: MEDIUM Instate captive breeding programme both for research purposes and to provide stock for reintroduction.	FIG, FC, SAERI
Action 5 PRIORITY: MEDIUM Conduct trial introductions into networks of suitable habitat, and monitor.	FC, SAERI

3.5 ADVISORY

Action 6 PRIORITY: MEDIUM Advise conservation organisations and landowners on practical management for the Falkland fritillary, and how to fit this in with other priorities.	FIG, FC, SAERI
Action 7 PRIORITY: MEDIUM Produce brief, practical guide on habitat management for the Falkland fritillary, aimed at landowners, coordinating this with management of other species.	FIG, FC, SAERI

3.6 INTERNATIONAL

Action 8 PRIORITY: MEDIUM Form strategic partnership with Chilean institutions to coordinate and encourage research on the two sub-species of *Y. cytheris.*	FC, SAERI
Action 9 PRIORITY: HIGH Open informal discussions with potential NGO and academic partners to identify areas of possible cooperation in research and practical conservation.	FC, SAERI

3.7 FUTURE RESEARCH, SURVEY AND MONITORING

Action 10 PRIORITY: HIGH Collate all records of Falkland fritillary and *Viola* spp.; create national distribution map; determine sites for further investigation.	FC, SAERI
Action 11 PRIORITY URGENT Investigate autecology of *Viola maculata*, with particular attention to impact of sheep grazing, and possible role of sheep in dispersal.	FC, SAERI
Action 12 PRIORITY: HIGH Identify colonies, particularly in proximity with each other, to form a network of study sites for future fieldwork.	FC, SAERI

ACTION 13 PRIORITY: HIGH FC, SAERI

Undertake wider MRR work over a full flying season to establish size of colonies. Devise methodology for assessing size of colony through indices drawn up from monitoring data.

ACTION 14 PRIORITY: HIGH FC, SAERI

Devise and implement method for calculating annual index to compare trends on selected sites.

ACTION 15 PRIORITY LOW

Conduct genetic research on historical museum specimens to clarify pattern of colonisation, whether a single founder event, or a series of immigrations.

ACTION 16 PRIORITY: MEDIUM FC, SAERI

Investigate genetic variation between sites to help establish existence and extent of dispersals.

ACTION 17 PRIORITY: HIGH FC, SAERI

Establish life-cycle of Falkland fritillary through combination of captive breeding and on-site investigation.

ACTION 18 PRIORITY: MEDIUM FC, SAERI

Identify areas of suitable, but unpopulated, habitat with the potential for (re-)introductions.

ACTION 19 PRIORITY: MEDIUM FC, SAERI

Establish phenology of Falkland fritillary as part of annual monitoring process. Seek to establish causes of any variation..

ACTION 20 PRIORITY: HIGH FC, SAERI

Establish population viability index, or other appropriate measure of identifying a colony in terminal decline.

ACTION 21 PRIORITY: MEDIUM Conduct research on threats to *Y. cytheris* from predation, parasitoids and bacteria such as *Wolbachia.*	FC, SAERI

3.8 COMMUNICATIONS AND PUBLICITY

ACTION 22 PRIORITY: HIGH Consult widely on this action plan; draft a full action plan, to be agreed by the Environmental Committee and publicise it, ideally as a published document.	FC, SAERI, FIG
ACTION 23 PRIORITY: MEDIUM Plan steps needed to determine IUCN Red List status, implement and publicise.	FC, SAERI

3.9 REVIEW

Action 24 PRIORITY: HIGH Review this action plan (or its successor) annually, and rewrite in five years.	FC, SAERI, FIG

Key to abbreviations

FC = Falklands Conservation
FIG = Falkland Islands Government
SAERI = South Atlantic Environmental Research Institute

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