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Reinfection by soil-transmitted helminths in Sumbawa Island, Indonesia six or seven months after albendazole therapy

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インドネシア国スンバワ島における土壌媒介性線虫の再感染　－アルベンダゾール投与後6-7ヶ月後の調査－　宇賀昭二[1], シバ K ライ[2], 胡　立志[1], マフーズ[3], ユース P ダッハラン[3]（[1]神戸大学医学部保健学科病態解析学講座, [2]ネパール医科大学細菌学講座（ネパール）, [3]アイルランガ大学熱帯病センター（インドネシア）

インドネシア国スンバワ島の3個所の村で，集団駆虫後の消化管寄生蠕虫類の再感染調査を実施した．この村は調査に先立つ6-7ヶ月前にアルベンダゾールによる集団駆虫が実施された村である．全部で199人から採便し，寄生虫検査を行った結果，31％（62人）から何らかの寄生虫が検出された．得られた寄生率に性差および年齢差は認められなかった．陽性率の最も高かったのはUtan村の42％であり，次いでLabanka（39％），Penyaringの（16％）順であった．検出された寄生虫は鞭虫（*Trichuris trichiura*）が最優占種であり，次いで鉤虫（hookworm）であった．

A post-treatment survey of intestinal helminth infection was conducted in three villages on Sumbawa Island, Indonesia in August 1997. A total of 199 subjects, who were given a dose of albendazole in December 1996 and/or January 1997, took part in this study by submitting fecal samples. The overall prevalence of infection was 31%, with no significant differences between males (32 %) and females (30 %), or between children (30 %) and adults (over 15 years) (32 %). The highest prevalence was seen in Utan village (42 %) followed by Labanka village (39 %) and Penyaring village (16 %). *Trichuris trichiura* was the most common parasite detected, followed by hookworm, *Ascaris lumbricoides* and others. The mixed infection rate was very low (5 %), as was the infection intensity.

Key Words: Reinfection, Survey, Intestinal parasite, Soil-transmitted helminth, Indonesia

Introduction

Intestinal parasitic infection is one of the major health problems in developing countries. It has been estimated to affect some 3.5 billion people globally, and 450 million are thought to be ill as a result of such infections, the majority being children (WHO, 2000). Infection rates in some areas in developing countries are close to one hundred percent (Imai *et al.*, 1985; Rai and Gurung, 1986) and have been attributed to poor hygiene and sanitary conditions, a lack of education, and poverty. Polyparasitic infections are also common in some areas (Widjana and Sutisna, 2000; Rai *et al.*, 2001). Of these, soil-transmitted helminths are predominant (Auer, 1990; Widjana and Sutisna, 2000).

Indonesia is a vast country in Southeast Asia comprised of numerous islands. As it is a developing country located in a tropical region, many tropical and infectious diseases are ranked in the "top ten" diseases of this country (Rai *et al.*, 1998). The reported prevalence of intestinal parasitosis varies considerably from one study to another (Stafford *et al.*, 1980; Bang *et al.*, 1996; Uga *et al.*, 2002). Soil contamination is common, even in and around big cities like Surabaya on Java Island (Uga *et al.*, 1995).

Sumbawa is a small rugged island with volcanic ridges and fertile green valleys. The present study was conducted in three villages on Sumbawa Island in August 1997 to evaluate the re-infection with helminth parasites of subjects who were given a dose of albendazole either in December 1996 or January 1997.

Materials and Methods

This follow-up study was conducted in August 1997 in three villages, Penyaring, Utan, and Labanka on Sumbawa Island, east of Bali. A total of 199 individuals took part in this study by submitting their fecal samples in clean, dry screw-capped plastic containers distributed earlier. Informed consent was obtained from all subjects. The age, sex, and area (village name) of each individual were noted. Fecal samples were labeled, fixed in 10 % formal-saline and transported to the laboratory of parasitology in the Tropical Disease Center (TDC) in Airlangga University, Surabaya (East Java). Parasite eggs were detected using the formalin-ether sedimentation technique, and fecal concentrates were examined microscopically. The findings were grouped according to age, sex, and location. Statistical analysis was conducted using the chi-square test.

Results

Of the 199 subjects in this study, 62 (31 %) had helminth parasites (**Table 1**). No differences were found between males (32 %) and females (30 %) ($P > 0.05$) or between children (30 %) and adults (over 15 years old) (32 %) ($P > 0.05$). The highest prevalence was seen in Utan village (42 %) followed by Labanka (39 %) and Penyaring (16 %). The prevalence rates between Utan and Penyaring, and between Labanka and Penyaring, were statistically different ($p > 0.05$), but those between Utan and Labanka were not ($p > 0.05$) (**Table 2**). *Trichuris trichiura* was the most common parasite detected, followed by hookworm, *Ascaris lumbricoides*, and others (**Table 3**). Only 5 % of the study population showed mixed infections. The infection intensity was found to be low (one to five parasite eggs/wet mount; 18 × 18 mm).

Discussion

Recently, we reported a low rate of intestinal parasitic infection (10 % by Kato-Katz, 31 % by AMS & 22 % by Sucrose floatation methods) in the

Table 1 Prevalence of intestinal parasitosis on Sumbawa Island, Indonesia

Category		No. of specimen	No. of positive	Percent positive (%)	P value
Sex	Male	103	33	32	>0.05
	Female	96	29	30	
Age-group	Children	86	26	30	>0.05
	Adults*	113	36	32	
Total		199	62	31	

* Over 15 years of age.

Table 2 Prevalence of intestinal parasitosis in three different villages on Sumbawa Island, Indonesia

Village	No. of sample	No. of positive	Percent positive (%)
Penyaring	76	12	16*
Utan	62	26	42**
Labanka	61	24	39***
Total	199	62	31

*, **: P<0.005, *, ***: P<0.005, **, *** : P>0.05.

Table 3 Parasite species detected on Sumbawa Island, Indonesia

Parasite	No. of samples	No. of positive	Percent positive (%)
Trichuris trichiura	199	62	31
Hookworm	199	22	11
Ascaris lumbricoides	199	20	10
Vampirolepis nana	199	2	1
Enterobius vermicularis	199	2	1
Mixed infection*	199	9	5

* Two or more than two parasites.

capital city of Indonesia (Uga *et al.*, 2002). However, in a rural area on Bali that is close to the present study site, the infection rate was reported as being one hundred percent, with a mixed infection rate of over 90% (Widjana and Sutisna, 2000; Stafford *et al.*, 1980). A similar report was made on another area in Indonesia (Bang *et al.*, 1996), where over 85% of the subjects were reported to be positive for parasites. In the present study, however, the helminth egg positive rate was low, as was the intensity. This appeared to be due to the health study program jointly conducted by TDC, Airlangga University and investigators from counterpart Japanese Universities and the consumption

of anti-parasitic drugs by locals (drugs were made available from local health posts called *Puskesmas*). The infection rates in the villages varied from 16 % (Penyaring) to 42 % (Utan), but we were not able to clarify the reason for this. During the first survey conducted in December 1996 and/or January 1997, villagers were given a single dose of albendazole. A precise survey of helminth prevalence after the treatment was not conducted in the three villages. However, about 10 % of the people in Utan village were positive in the survey performed in February 1997 by the local government staff (personal communication). A single dose of albendazole, a drug belonging to the benzimidazole group, cannot eradicate *T. trichiura* infections completely. In some studies, a single dose of albendazole (400 mg) provided a cure rate of only 44 % (Hanjeet and Mathias, 1991). The need for repeat doses of albendazole, especially with moderate and severe *T. trichiura* infections, has also been reported (Hanjeet and Mathias, 1991). In any case, the present study suggested that about 6 to 32 % of the people were re-infected during the preceding six to seven months.

The most common parasite detected in this study was *T. trichiura,* followed by hookworm and others. This appeared to be due to the consumption of anti-helminthic drugs. These re-infections might also have occurred after consumption of the drug six to seven months earlier, as indicated by the presence of *A. lumbricoides* eggs. Recurrences in the incidence of *A. lumbricoides* and *T. trichiura* infections, nearly reaching pre-treatment levels nine to ten months after mass treatment, have also been reported in Indonesia (Imai *et al.*, 1985) and India (Paul and Gnanamani, 1998). Many epidemiological studies on intestinal parasites in Indonesia that found both species of hookworm have been reported. However, the number of the studies that differentiated one from the other is limited. Higgins *et al.* (1993) and Nurdia *et al.* (2001) carried out surveys in the Flores and Java Islands, respectively, and found only *Necator americanus; Ancylostoma duodenale* was not found. Sumbawa Island, where this survey was conducted, is located between these two islands, therefore, the hookworm we found in this survey might be *N. americanus.*

In neighboring Bali, two-thirds of the study population has reportedly been infected with mixed helminth species (Widjana and Sutisna, 2000; Stafford *et al.*, 1980). The low (5 %) rate of mixed infections seen in this study indicates the effectiveness of the anti-parasitic drug taken six to seven months earlier. In addition to the significantly reduced positive rate, the low intensity of the helminth infection in the present study was an important finding.

Geohelminths are known to cause various kinds of morbidity including physical and mental retardation (Rai *et al.*, 2000, 2001; Sakti *et al.*, 1999) as well as death (WHO, 2000). Although the present study showed a reduced incidence and low intensity of infection, indicating the positive effect of chemotherapy, an integrated control program targeted through schools (Bundy *et al.*, 1990) appears to be essential in controlling enteric parasitic infections on this island.

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各種市販および自家製木酢液・竹酢液の変異原性

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Mutagenecity of various commercial and home-made Pyroligneous Acid products
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Attempts to evaluate the mutagenecity of twelve commercial and seven home-made pyroligneous acid (PA) products employing the *umu* test were unsuccessful because of the presence of anti-bacterial components in the products, which inhibited the growth of *S. typhimurium*, the bacteria used for the test. When the assay was conducted with diluted solutions of three selected products in the range $1\sim10^6$ ppm, positive reactions were obtained in the presence of metabolic activator S-9 Mix, although the A_{630} values obtained were too low to confirm their mutagenecity. However, when several PA products were subjected to the *umu* test after removal of the anti-bacterial components on Sep-pak tC18 column, they all showed a positive reaction for mutagenecity. The simple clean-up method employed in the present study appears useful for evaluations of mutagenecity of samples such as PA products that consist of multiple components.

Key words: Mutagenecity, Pyroligneous acid, *umu* test, S. typhimurium, S-9 Mix

市販木酢液12種と自家製木酢液7種の各々の原液について*umu*試験により変異原性を検定したが，木酢液の検定菌に対する抗菌活性のために評価ができなかった．そこで木酢液3種を選んで，検定菌に対する影響を軽減するために$1\sim10^6$ ppmに希釈し同様に変異原性を検定した．検定菌への影響は減少し，S-9 Mix処理条件下

で陽性反応を示したが，得られた吸光度は陽性対照と比較して低いレベルであり，変異原性は確認できなかった．一方，数種木酢液をSep-pak tC18カラムを用いて部分的に抗菌物質を除去した後で同様の検定を行った場合は，供試した木酢液全てがS-9 Mix処理によって明確な変異原性陽性反応を示した．本研究で用いた部分精製方法は，木酢液のような混合成分からなる資材の変異原性を簡便に検定する方法として有用であると考えられる．

はじめに

木酢液・竹酢液（以下木酢液と称する）は木・竹炭生産時の副産物であり，病害虫防除目的で農薬代替資材として宣伝され販売されている（岸本，1996；農文協，2004）．木酢液にはイエバエ（竹井・林，1968）やヤマビル(千葉県衛生研究所，1997)に対して忌避効果があるという報告や，数種カメムシ類（谷田貝，2001）に対して殺虫活性があるという報告がある．

また木酢液は，有機農業ブームに伴ってすでに広く普及していることから，平成15年の農薬取締法改正に伴って新設された農薬登録を要しない特定農薬（特定防除資材）の候補としても注目を集めている（日本農業新聞，2004）．

一方著者らは木酢液の薬効について，著しい害虫防除活性を示した資材には合成ピレスロイドのシペルメトリンが混入されていたことから（本山・Rahman，2004；Rahman and Motoyama，1998），害虫防除効果があるという農家の体験談には，合成化学農薬が混入されていたいわゆる漢方農薬の場合と同様に（駒形・本山，1998；1999），疑義が存在することを暗示した．さらに，実際に各種市販品および自家製木酢液を供試して，通常推奨されている数100倍希釈液ではなく，10倍希釈液という濃厚液を処理しても植物体上の数種植物病原菌に対して防除効果が認められず，数種害虫に対しても10倍希釈液～原液を処理しても防除効果が認められないこと（駒形・本山，2004a；2004b）を確認した．従って，木酢液の農薬代替資材としての実用効果には疑問がある．

また，木酢液を農薬代替資材として使用する場合の安全性については，科学的な知見がほとんど見当たらない．

木酢液，木タールには200を超える成分が含まれているとされており，それらの成分は，有機酸，フェノール類，カルボニル，アルコール，その他中性成分，塩基等に分類できる（木材工業ハンドブック編集委員会，1982年）．

梅津（2003）はこれらの成分中に，多数の変異原性，刺激性，生殖毒性，発ガン性をもつものがあることを指摘している．

著者らは上述した研究（駒形・本山，2004a）において，各種木・竹酢液市販品および炭焼き窯から直接採取した木酢原液について主要成分の分析を行った結果，梅津（2003）が指摘した有害物質も含まれていることを確認した．ただし，市販の木酢液の品質にはばらつきがあり，成分によっては10倍以上の差が見られた．

各成分の単体での毒性については既に多くの情報があり，例えば発ガン性に関していえばIARC（International Agency for Research on Cancer）のような国際機関で発ガン性の評価がなされている．しかし，木酢液については混合物であるということの他に品質のばらつきもあり，また世界的に見て主要な資材とはいえないため，従来マウスに対する急性毒性（池田ら，1964）以外，毒性評価の対象にはされてこなかった．

本論文では，前報（駒形・本山，2004a；2004b）で用いたのと同じ木酢液市販品，および炭焼き窯から直接採取した木酢原液について，毒性評価の一つとして変異原性試験である*umu*試験を行っ

た結果を報告する．

材料と方法

1．供試木酢液

供試した木酢液を**表1**に示した．これらの木酢液・竹酢液の主要成分と抗菌活性ならびに殺虫活性と水生生物に対する影響については前報(駒形・本山，2004a；2004b）に記載した通りである．

2．試薬類

*umu*試験は株式会社日本抗体研究所の試験キット，ウムラック®を用いた．検定菌（*S. typhimurium*），培養液，S-9 Mix，陽性対照物質（Furylfuramideと2-Aminoanthracene），発色基質，停止液はキットに付属のものを使用した．その他の試薬は市販の特級品を使用した．

3．木酢液の部分精製

一部の木酢液（C, F, G, H, M7）について固相抽出を行った．供試木酢液のpHは2.6～4.7の範囲だったので（駒形・本山，2004a），固相カラムはWaters社のSep-pak tC18（1g）を用いた．固相カラムはアセトン10mlを通液した後，蒸留水10mlを通液しコンディショニングを行った．その後，検体である木酢液の原液若しくは対照である蒸留水を100ml通液した．その後，蒸留水50mlを通液してカラムを洗浄した後，約10分間空気を吸引して乾燥させ，アセトン10mlで吸着物を溶出した．抽出液は40℃以下の湯浴で保温しながら窒素気流下でアセトンを乾固させて1mlのジメチルスルホキシド（DMSO）を加えて濃縮物を溶解させた後に，蒸留水を加えて10mlとした．本液（原液の10倍濃縮相当濃度）および更に蒸留水を用いて10倍（原液相当濃度），100倍希釈した液（原液の10倍希釈相当濃度）を精製検体とした．なお，通液速度は各液とも約1ml/minであった．

4．変異原性試験（*umu*試験）

木酢液の変異原性を*umu*試験で検定した．検

表1　供試木酢液

Group	Product Code	Trade Name	Manufacturer, Dealer, or Temperature at which product was collected
Commercial Product	A	Joryu-Mokusakueki	Keiyo Co. Ltd. (Made in Malaysia)
	B	Joryu Seisei Binchotan-Mokusakueki	Apurotto Co. Ltd.
	C	Joryu Chikusaku Geneki	Kamimura Seitohjo Co, Ltd. (Made in China)
	D	Tokusan Chikusakueki	Apurotto Co. Ltd.
	E	Mokusaku Geneki	Yoki Sangyo Co. Ltd.
	F	Shikoku Konpirasama Fumotosan Chikusakueki	Shikoku Tekuno Inc.
	G	Junsei Mokusakueki	Wako Mokuzai Co. Ltd.
	H	Mokuchikusakueki	Made in Otaki, Chiba Prefecture
Commercial Diluted Product	I	Yomogi Sakueki	Airisu Oyama Co. Ltd.
	J	300-fold Diluted Mokusakueki	Takuto Co. Ltd.
	K	Shokubutsu Mokusaku Ikiiki-spray	Shimada Shoji Co. Ltd.
	L	Shokubutsu Chikusaku Ikiiki-spray	Shimada Shoji Co. Ltd.
Home-made Product	M1		(Temperature) 84 ℃
	M2		82 ℃
	M3		86 ℃
	M4		97 ℃
	M5		122 ℃
	M6		186 ℃
	M7		200 ℃

定はキットに添付されているマニュアルに従って以下の手順で行った．冷凍されている検定菌（*S. typhimurium*）用培養液を室温に戻し，1 mlを菌凍結乾燥品に入れ,静かに攪拌し，室温で10分間静置した後，37℃で3時間静置培養した．代謝活性化試験用のS-9 Mixは，凍結乾燥品に蒸留水1mlを入れ,よく攪拌した．検体である木酢液は，実験1では原液を，実験2では公比10の蒸留水希釈液（1～10^5 ppm）を，実験3では前述した方法で部分精製した木酢液を試料として96穴マイクロタイタープレートの各ウェルにそれぞれ10 μ 1ずつ分注した．またS-9 Mixによる代謝活性化を必要としない陽性対照物質として，キットに同包のFurylfuramideを，代謝活性化を必要とする陽性対照物質として同じくキットに同包されている2-Aminoanthraceneを各々DMSOに溶解させた後，蒸留水で希釈し，木酢液と同様に10 μ 1ずつ分注した．調製した菌液を代謝活性化を必要としないウェルに100 μ 1ずつ分注した．残りの菌液には，その10%に相当する量のS-9 Mixを加え，代謝活性化を必要とするウェルに100 μ 1ずつ分注し，プレートを37℃，遮光の条件下で2時間培養した．37℃に予熱しておいた発色基質を全ウェルに100 μ 1ずつ分注し，37℃で1時間インキュベーションした後，反応停止液を全ウェルに100 μ 1ずつ分注した．3分後，色調が安定した後，マイクロプレートリーダー（Elx808, Bio-Tek Inst.）を用いて，発色度合いを波長630nmの吸光度（A_{630}）で測定した．吸光度は各々に該当する対照ウェルの吸光度を引くことにより補正した．なお，Sep-pak tC18カラムで部分精製した木酢液の対照ウェルには，蒸留水100mlをカラムに通液して同様に処理したサンプルを用いた．

結果と考察

陽性対照の濃度－発色（A_{630}）曲線は**図1**に示した．

実験1：原液に対する試験

各種木酢液の原液を用いた*umu*試験の結果は

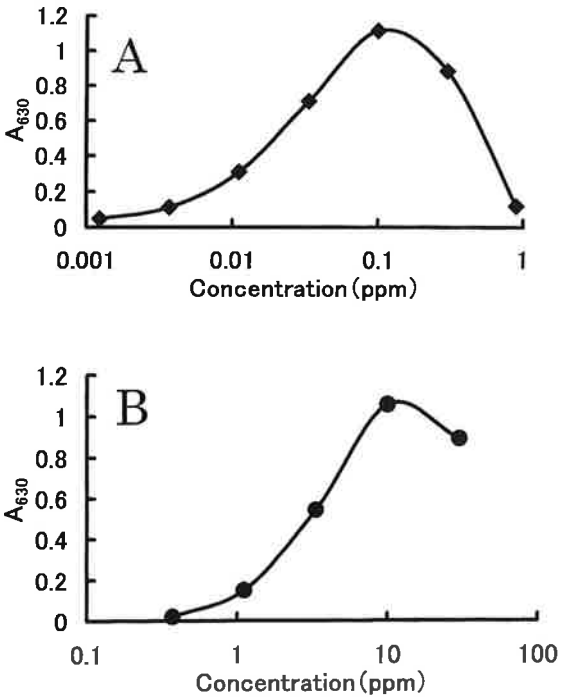

図1　変異原性試験における陽性対照の濃度－発色程度（A_{630}）曲線．A：Furylfuramide（S-9 Mix 非処理），B：2-Aminoanthracene（S-9 Mix 処理）

表2に示した．希釈品（I～L）とS-9 Mix非処理下での木酢液Cを除いた市販木酢液，自家製木酢液はともに吸光度が負の値となった．これは*umu*試験で用いた検定菌*S. typhimurium*の生育が阻害されたことを示している．なお，ここで供試した木酢液の多くは，前報（駒形・本山，2004a）で述べたごとく，原液に近い濃度ではPS培地上の灰色かび病に対して抗菌活性示したということと，また木酢液からは酢酸やホルムアルデヒドなどが検出されていることから，検定菌に対する生育阻害は当然予想されることである．希釈品（I～L）の場合は酢酸をはじめとする成分濃度が著しく低いために生育阻害が起こらなかったのであろう．しかし，これらについても吸光度は低く，陽性対照の最小検出濃度程度もしくはそれ以下（**図1**）であった．なお，S-9 Mix存在下での木

表2 *umu* 試験で検定した各種木酢液のS-9 Mix処理および非処理下における変異原性

Product Code	Concentration Tested	Mutagenecity (A_{630})					
		without S-9 Mix			With S-9 Mix		
		Rep. 1	2	X	Rep. 1	2	X
A	Original Solution	-0.376	-0.364	-0.370	-0.392	-0.410	-0.401
B	ibid.	-0.372	0.326	-0.023	-0.471	-0.503	-0.487
C	ibid.	N.D.	N.D.	N.D.	-0.442	-0.493	-0.468
D	ibid.	-0.381	-0.381	-0.381	-0.441	-0.453	-0.447
E	ibid.	-0.358	-0.373	-0.366	-0.436	-0.478	-0.457
F	ibid.	-0.353	-0.364	-0.359	-0.502	-0.506	-0.504
G	ibid.	-0.385	-0.385	-0.385	-0.476	-0.486	-0.481
H	ibid.	-0.369	-0.379	-0.374	-0.474	-0.499	-0.487
I	ibid.	0.039	0.045	0.042	0.110	0.096	0.103
J	ibid.	0.080	0.085	0.083	0.221	0.098	0.160
K	ibid.	0.030	0.073	0.052	-0.433	-0.478	-0.456
L	ibid.	0.016	0.077	0.047	-0.452	-0.471	-0.462
M1	ibid.	-0.377	-0.372	-0.375	-0.512	-0.514	-0.513
M2	ibid.	-0.437	-0.437	-0.437	-0.493	-0.493	-0.493
M3	ibid.	-0.390	-0.386	-0.388	-0.522	-0.517	-0.520
M4	ibid.	-0.375	-0.379	-0.377	-0.480	-0.485	-0.483
M5	ibid.	-0.387	-0.379	-0.383	-0.496	-0.488	-0.492
M6	ibid.	-0.378	-0.384	-0.381	-0.471	-0.492	-0.482
M7	ibid.	-0.391	-0.395	-0.393	-0.517	-0.512	-0.515

N. D.: No data due to experimental error

酢液Cについては操作上のミスにより信頼できるデータが得られなかったが，後述する実験（実験2，**図2**）において木酢液CはS-9 Mix非処理下では菌の生育阻害を示すことが確認されている．

実験2：希釈液に対する試験

総合的に成分濃度が濃いと思われる（駒形・本山，2004a）木酢液3種類すなわちC, G, Hについて，検定菌に対する抗菌作用の影響を減ずるために希釈液を作成しS-9 Mix処理および非処理下で検定を行った．S-9 Mix非処理下では，いずれの木酢液でも最高濃度である10^5ppm（すなわち原液の10倍希釈液）では，検定菌に対する生育阻害が見られた（**図2**）．しかし，S-9 Mix処理下では生育阻害が見られないことから，生育阻害にはS-9 Mixに含まれる酵素系によって分解される基質が関与するものと推察される．しかし，得られた発色程度（A_{630}値）は陽性対象の検量線の値と比較して著しく低く，変異原性陽性であると判断するのは困難であった．

実験3：部分精製した木酢液に対する試験

前報（駒形・本山，2004a）で報告したごとく，今回供試した木酢液から検出された成分の中には，明らかに変異原性を示すと思われる成分が含まれている．しかし，実験1，実験2において結果が陰性となった理由は2つ推察される．一つは変異原性を示す物質の濃度が低いため，もう一つは木酢液に抗菌活性があるためである．

umu 試験では，変異原性の検定菌に*S. typhimurium*を用いるために，抗菌作用の高い化学物質では陰性になりやすい．通常，変異原性の試験では，水溶解度等の物理化学的条件が許す限り，最高濃度で試験を行うことになっている．しかし，濃度

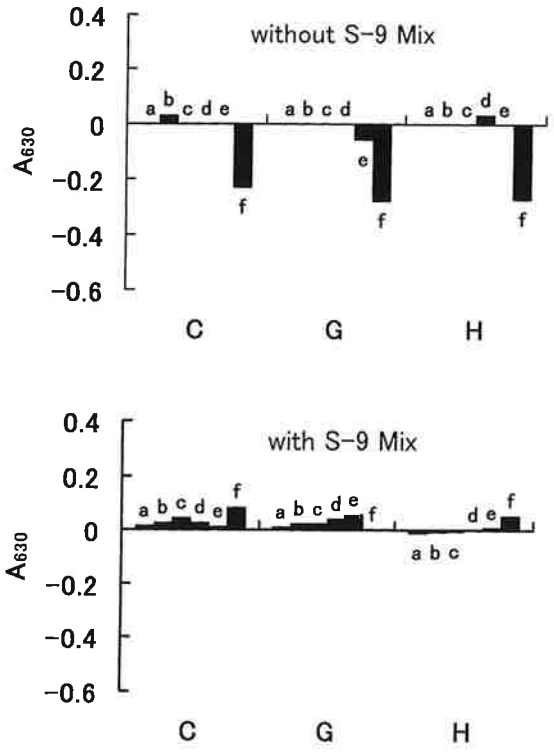

図2　S-9 Mix 処理および非処理下における木酢液C, G, H希釈液の変異原性,（供試濃度）a：1 ppm, b：10 ppm, c：10^2ppm, d：10^3ppm, e：10^4ppm, f：10^5ppm.

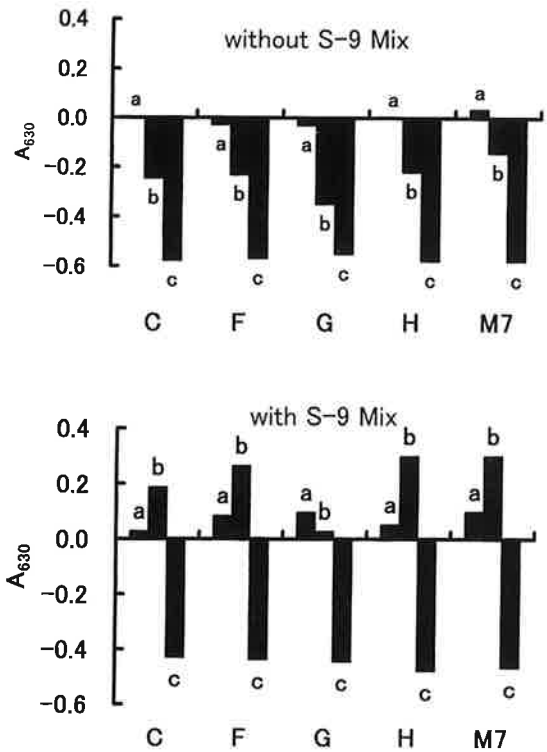

図3　部分精製した木酢液C, F, G, H, M7のS-9 Mix処理および非処理下における変異原性（供試濃度）a：10倍希釈液相当, b：原液相当, c：10倍濃縮液相当

が高くなれば生育阻害の起こる確率も高くなる．特に木酢液の場合はすでに言及したごとく多くの成分が含まれているため(木材工業ハンドブック編集委員会, 1982)，変異原性のある物質が含まれていても，抗菌活性のある物質が共存していれば見かけ上陰性反応を示す可能性もある．

そこで木酢液5種，すなわちC, F, G, H, M 7について固相カラムを用いてカラムに吸着されない抗菌活性物質をある程度除去した試料について同様に変異原性試験を行ったところ，S-9 Mix処理下で全ての供試木酢液は正の吸光度を示し，陽性反応が得られた（**図3**）．

今回の部分精製では，固相カラムを通すことによって木酢液に含まれていた酢酸，アルコール類等の試験系を妨害する抗菌物質は除かれていることが推察されるが，木酢液に含まれる成分は多いため個々の成分の回収率については未検討である．さらに本研究では逆相系のカラムを用いているため，木酢液に含まれているアルコール類等の濃度によっては他の成分の回収率が変化することも推察される．このように本研究では必ずしも変異原性物質を精製する為の条件が最適化されているわけではないにも関わらず，部分精製した木酢液は全てS-9 Mix処理によって吸光度が正となり，検量線と比較して，変異原性を有することを強く示唆した．

一方，木酢液の種類によって陽性反応を示した濃度が異なることは興味深い．例えば，木酢液C, F, H, M 7は原液相当濃度で最も高い陽性反応を示したが，木酢液Gは原液の10倍希釈液相当濃度

でもっとも高い陽性反応を示した．しかし，いずれの木酢液も原液の10倍濃縮液相当濃度では，明確に検定菌に対して生育阻害を示した．従って陽性反応を示した場合も，検定菌に対する生育阻害とのバランスの値に過ぎず，実際の変異原性はもっと強い可能性も考えられる．

試験方法について

一般に，植物抽出液と呼ばれる資材は，構造不明の多くの成分を含む．そのような資材に対しては，農薬等の単成分から成る化学物質の変異原性の試験方法をそのまま適用することは，必ずしも適切ではない．含有成分ごとの変異原性を検定することが望ましいが，木酢液の場合は成分そのものが不明なことに加えて，含まれる成分の種類が著しく多いために，それらの全てについて検定をするのはコスト的に困難を伴う．本研究で採用した固相カラムを用いた部分精製の方法は，抗菌物質を含む混合成分から成る資材の変異原性を検定する上で，簡便かつ有用なひとつの方法と考えられる．

また，本研究で供試した5つの木酢液全てから変異原性陽性の反応が検出されたということは，木酢液を特定農薬の候補資材として考えるにあたっては，長期的摂取に伴う安全性に関して慎重な検討が必要なことを示唆するものである．

木酢液の濃度を変えた至適検定条件下における変異原性の評価，ならびに変異原性物質の単離・同定は今後の検討課題である．なお，木酢液の変異原性については，最近中島ら（2003）が*Ames*試験を用いて一部の木酢液から変異原性陽性の結果が得られたことを報告している．

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Estimating the adult population size of ground beetles (Carabidae) using the removal method

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除去法を用いた地表徘徊性ゴミムシ類成虫の個体数推定　Salah Uddin Siddiquee・中村寛志（信州大学農学部）

長野県にある信州大学農学部構内の森林内とその近くの野菜畑において，除去法を用いた地表徘徊性ゴミムシ類成虫の個体数推定を行った．プラスチック製の境界で区切られた40m²の区画に，乳酸飲料を入れた15個のプラスチック・トラップをセットし，10日間毎日ゴミムシを回収した．調査は森林では2002年の9月末，野菜畑では2003年の10月初めに行われた．森林での優占3種は*Synuchus cycloderus, Pterostichus subovatus, Synuchus nitidus*で，野菜畑では*Harpalus griseus, Harpalus sinicus, Amara simplicidens*であった．全ゴミムシ類と優占3種の個体数およびm²当たりの密度は，いくつかある除去法の中で回帰法と最尤法を用いて行われた．森林内では合計250個体のゴミムシ類が捕獲され，回帰法による推定値は254個体であった．また野菜畑では176個体の採集で，推定値は180個体であった．最尤法による推定値は回帰法とほぼ同じ値で，10回の実際の採集個体数と推定値がほぼ等しかった．またある時点の捕獲個体数とその時点までの累積捕獲個体数の相関係数は－0.9以下であり，推定精度も0.12以下の値であった．ゴミムシ類の個体数推定に応用する上での除去法の前提条件や捕獲回数と推定精度の関係が議論された．

Adult population sizes of ground beetles (Carabidae) in a forest and vegetable field in Nagano Prefecture, Japan were estimated using the removal method. Removal collections using 15 pitfall traps with a lactic acid beverage were conducted at 40-m² survey sites enclosed by a thick plastic sheet for 10 days in September 2002 in the forest site and October 2003 in the field site. Dominant species were *Synuchus cycloderus, Pterostichus subovatus* and *S. nitidus* in the forest, and *Harpalus griseus, H. sinicus* and *Amara simplicidens* in the field. Population sizes within the 40-m² sites and the density (/m²) of total carabid beetles and dominant species were estimated by the regression

and maximum likelihood methods. A total of 250 and 176 carabid beetles were caught in the forest and field sites, and estimates by the regression method were 254 and 180 individuals, respectively. Estimates of dominant species and total carabid beetles by the maximum likelihood method were almost equal to those obtained by the regression method. The observed numbers caught from 10 trappings were almost the same as the estimated values. The correlation coefficients between the number of individuals captured during the *i*th trapping and the total number captured prior to the *i*th trapping were less than −0.9, and the precision level of the estimations was less than 0.12. The prerequisite for the removal method and appropriate number of trappings required for estimating carabid population size were discussed in relation to the precision level of the estimations.

Key words: Ground beetle (Carabidae), population estimation, removal method, regression method, maximum likelihood method, pitfall trap

Introduction

Because of their diversity, ground beetles (Carabidae) have been studied from taxonomical, biogeological and evolutional viewpoints, and recently, their role as potential predators in agroecosystems has been explored. The species composition, seasonal activity and spatial distribution of ground beetles have been studied globally (Yano *et al.*, 1995), and in Japan, important work in paddy fields (Habu and Sadanaga, 1970; Yahiro *et al.*, 1992), and a series of ground beetle studies have been conducted in various agroecosystems (Ishitani and Yano, 1994; Ishitani *et al.*, 1994). It has been established that ground beetles could be used as a biological control in pest management (Holland, 2002), and furthermore, with regards to environmental evaluation, some researches hope to develop ground beetles into a bio-indicator (Ishii *et al.*, 1996; Ishitani, 1996; Villa-Castillo and Wagner, 2002).

To study the ecology of ground beetles and establish them as a predator or bio-indicator, much attention needs to be paid to population estimations in different habitats and seasons. However, population numbers per unit area have yet to be clearly reported, though the spatial distributions and seasonal activity of ground beetles represented by the number of insects collected per trap in various habitats have been previously analyzed (Ishitani *et al.*, 1997; Thomas *et al.*, 2002).

Many methods for estimating the population sizes of animals and insects have been presented. Mark and recapture methods have been mainly used to estimate the population sizes of insects because birth, death and migration occur during their short life spans. The removal method, another population estimation method, has been applied to estimates of the stable population size of rats (Leslie and Davis, 1939) and fish (DeLury, 1951), and involves a series of trapping or collecting without replacement. Inoda and Tsuzuki (2000) tried to estimate population sizes of two *Cybister* species using the removal method.

There are three different approaches to analyzing removal trapping data. In this study we tried to estimate the population density ($/m^2$) of adult ground beetles at two different habitats in Nagano Prefecture, Japan, using the removal method, and

then compared the estimates of three approaches.

Materials and Methods

1. Study sites

Two sites in Minamiminowa Village, Nagano Prefecture were selected to estimate the population size of carabid beetles using the removal method. One site was a small area of experimental forest in the Faculty of Agriculture Campus, Shinshu University (Site 1) dominated by Japanese larch, *Larix leptolepis* Gord., Japanese cypress, *Chamaecyparis obtuse* Endl., and some broadleaf trees. A playing field is located in the northern part of Site 1. The other site (Site 2) was located in a vegetable field on the eastern side of the campus (**Fig. 1**). Tomatoes, eggplants, beans and potatoes were the main crops of this plot.

Field surveys using pitfall traps were conducted in Sites 1 and 2 from September 20 to 29, 2002 and September 30 to October 9, 2003, respectively. Fifteen trap stations spaced 2 m apart lengthwise and 1 m apart widthwise were set in 40-m^2 areas (10 $\times$4 m) in both sites (**Fig. 2**). The survey areas were enclosed by thick plastic sheets 30 cm high above the ground and buried to a depth of 10 cm to protect against invasion of carabid beetles from the outside as well as escape from within.

The prerequisite for this method is that the population must remain stable during the trapping period, that is, there must be no significant natality, mortality or migration (Southwood, 1978). In this study, the adult carabid beetles could not enter or leave the site as a result of the plastic sheet boundary, because these beetles are almost unable to fly. As the surveys were conducted for only 10 days in autumn, the prerequisite mentioned above could be satisfied, even if new emergence and death occurred slightly.

Transparent plastic cups 13.5 cm deep and with an upper and lower diameter of 9 and 6 cm, respectively, were used as traps. Plastic covers were placed 10 cm above the traps to protect them from rainfall

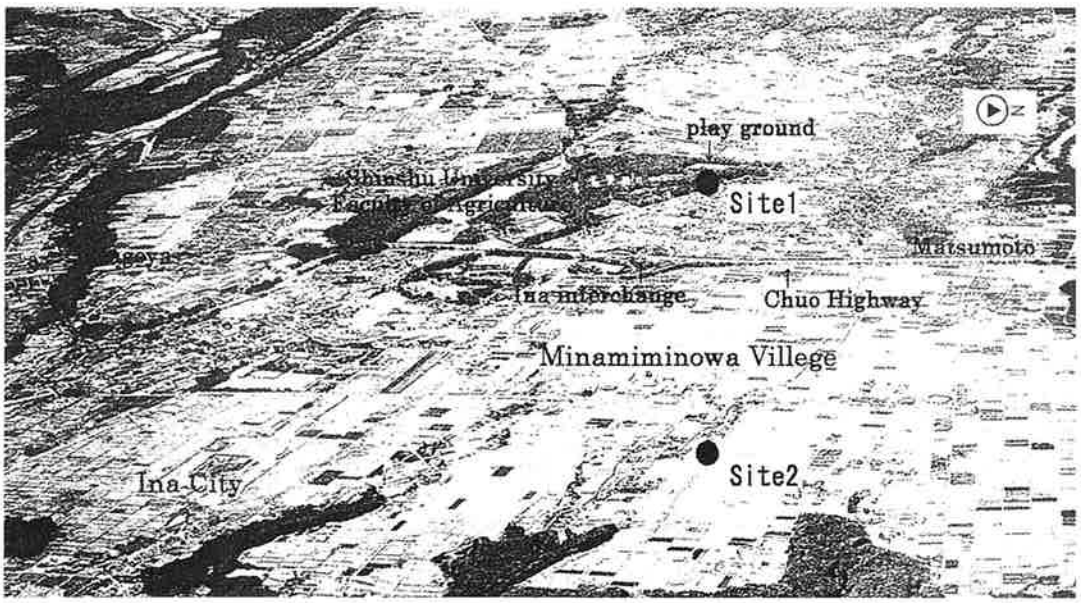

Fig. 1 Map of 2 survey sites in Minamiminowa Village.

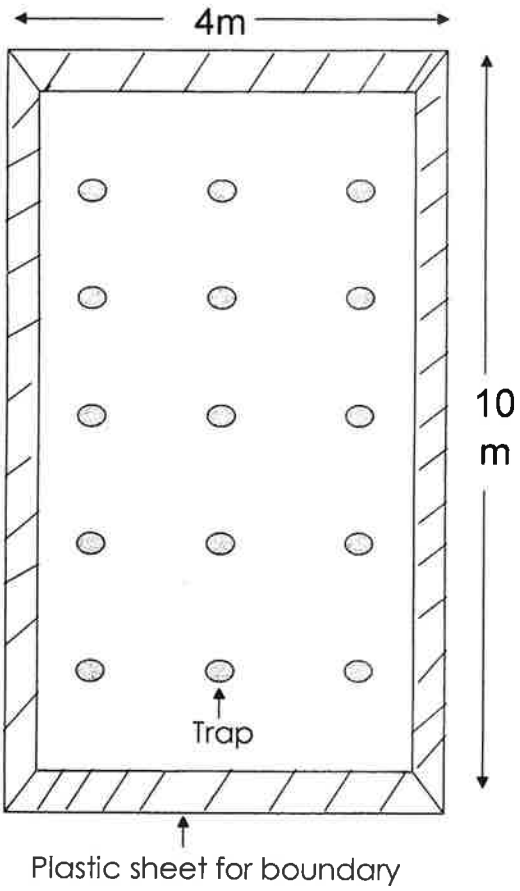

Fig. 2 Arrangement of traps in Site 1 and Site 2

and falling leaves. Inside the traps, lactic acid beverage (Culpis™, Culpis Co., Ltd., Tokyo) was used as bait. Beetle collections were made once a day for ten days incessantly at both sites.

2. Estimation methods using the removal collection data

There are several different approaches for analyzing removal trapping data. In this study we used the regression method (Leslie and Davis, 1939; DeLury, 1947, 1951) and maximum likelihood method (Moran, 1951; Zippin, 1956) to estimate the numbers of the three dominant carabid species and total number of carabid beetles within the 40-m² sites in the two distinguishable habitats. The density per one m² and variance were also estimated.

Regression method: The following liner relation is expected under random trapping:

$$C_i = b\,(N - T_i)$$

where C_i, T_i and N are the number of insects captured during the ith trapping, the total number captured prior to the ith trapping, and the population size, respectively, and b is a constant. As N is equal to T_i at $C_i = 0$, the population size is then estimated as:

$$\hat{N} = \overline{T} + \frac{\overline{C}}{b} \qquad b = -\frac{\sum_{i}^{s} T_i C_i - s\overline{TC}}{\sum_{i}^{s} T_i^2 - s\overline{T}^2}$$

where $\overline{T}$ and $\overline{C}$ are the mean values of C_i and T_i, respectively, and s is the number of trappings. The variance of this estimate $(\hat{N})$ is calculated as:

$$v(\hat{N}) = \frac{\hat{\sigma}^2}{b^2}\left\{\frac{1}{s} + \frac{(\hat{N} - \overline{T})^2}{\sum_{i}^{s} T_i^2 - s\overline{T}^2}\right\} \quad \cdots\cdots (1)$$

Maximum likelihood method: With random trapping, the probability of capturing C_i insects during the ith trapping, given that T_i insects were previously captured is:

$$P(C_i / T_i) = \binom{N - T_i}{C_i} p^{C_i} q^{N - T_i - C_i}$$

where $p = 1 - q$ is the probability of capturing during a single trapping. Based on the maximum likelihood of the joint probability of the catch samples in s trappings, Zippin (1956) showed that population size (N) and variance can be estimated as follows:

$$\hat{N} = \frac{\overline{T}}{(1 - \hat{q}^s)}$$

$$v(\hat{N}) = \frac{\hat{N}(1 - \hat{q}^s)\hat{q}^s}{(1 - \hat{q}^s)^2 - (\hat{p}s)^2 \hat{q}^{s-1}}$$

The estimates of $1 - q^s$ and p are given in Zippin (1956).

As the area of the survey sites in this study is 40 m², the estimates of density (m) per m² and

variance($v(\hat{m})$) *are given as:*

$$\hat{m} = \left(\frac{1}{40}\right)\hat{N}$$

$$v(\hat{m}) = \left(\frac{1}{40}\right)^2 v(\hat{N}).$$

Results

1. Species composition

A total of 250 carabid beetles from 4 subfamilies and representing 14 species were caught in Site 1 (**Table 1**). Three species, *Synuchus cycloderus, Pterostichus subovatus* and *S. nitidus,* were most frequently caught accounting for 187 individuals, which was 74.8 % of the total carabid beetles caught in Site 1. Of these, *S. cycloderus* was most frequently caught, representing 34 % of the total.

A total of 176 carabid beetles from 4 subfamilies and representing 19 species were caught in Site 2 (**Table 1**). Three species, *Harpalus griseus, H.*

Table 1 Species and number of carabid beetles caught in Site 1 and Site 2

Species	No. of individuals	
	Site 1	Site 2
Leptocarabus procerulus (Chaudoir)	16	0
Patrobus flavipes Motschulsky	3	0
Trigonognatha cuprescens Motschulsky	0	11
Pterostichus planicollis (Motschulsky)	0	2
Pterostichus subovatus (Motschulsky)	63	0
Pterostichus microcephalus (Motschulsky)	0	2
Pterostichus nimbatidius Chaudoir	9	2
Dolichus halensis (Schaller)	1	1
Synuchus nitidus (Motschulsky)	39	0
Synuchus cycloderus (Bates)	85	1
Synuchus dulcigradus (Bates)	3	1
Synuchus arcuaticollis (Motschulsky)	17	0
Synuchus sp.	7	0
Amara simplicidens Morawitz	0	22
Amara mocronota ovalipennis Jedlicka	0	9
Anisodactylus signatus (Panzer)	0	6
Harpalus capito Morawitz	1	0
Harpalus jureceki (Jedlicka)	1	0
Harpalus griseus (Panzer)	3	62
Harpalus tridens (Morawitz)	0	14
Harpalus sinicus (Hope)	0	23
Harpalus niigatanus Schauberger	0	2
Harpalus platinotus Bates	0	3
Harpalus corporosus (Motschulsky)	0	5
Harpalus bungii Chaudoir	0	2
Harpalus tinctulus Bates	0	7
Harpalus discrepans Morawitz	2	0
Chlaenius naeviger Morawitz	0	1
Total carabid	250	176

sinicus and *Amara simplicidens*, were most frequently caught accounting for 107 individuals, which was 60.8 % of the total carabid beetles caught in Site 2. Of these, *H. griseus* was most frequently caught, representing 35.2 % of the total.

2. Daily change in the number of trapped individuals

Daily changes in the numbers of the 3 dominant species trapped are shown in **Fig. 3**. The numbers of beetles captured in Site 1 decreased abruptly on the second and third trappings but thereafter showed a gentle reduction (**Fig. 3A**). *S. cycloderus* was not trapped on the tenth trapping, though a total of 10 other carabid beetles were captured. The correlation coefficients between the number of *S. cycloderus*, *P. subovatus* and *S. nitidus* individuals captured during the *i*th trapping (C_i) and the total number captured prior to the *i*th trapping (T_i) were −0.947, −0.925 and −0.968, respectively.

The numbers of beetles captured in Site 2 decreased almost linearly till the fifth trapping and showed a gentle reduction thereafter (**Fig. 3B**). *H. sinicus* was not captured after the sixth trapping, and *H. griseus* and *A. simplicidens* were not trapped on the tenth. The correlation coefficients between the C_i and T_i of *H. griseus*, *H. sinicus* and *A. simplicidens* were −0.977, −0.990 and −0.982, respectively.

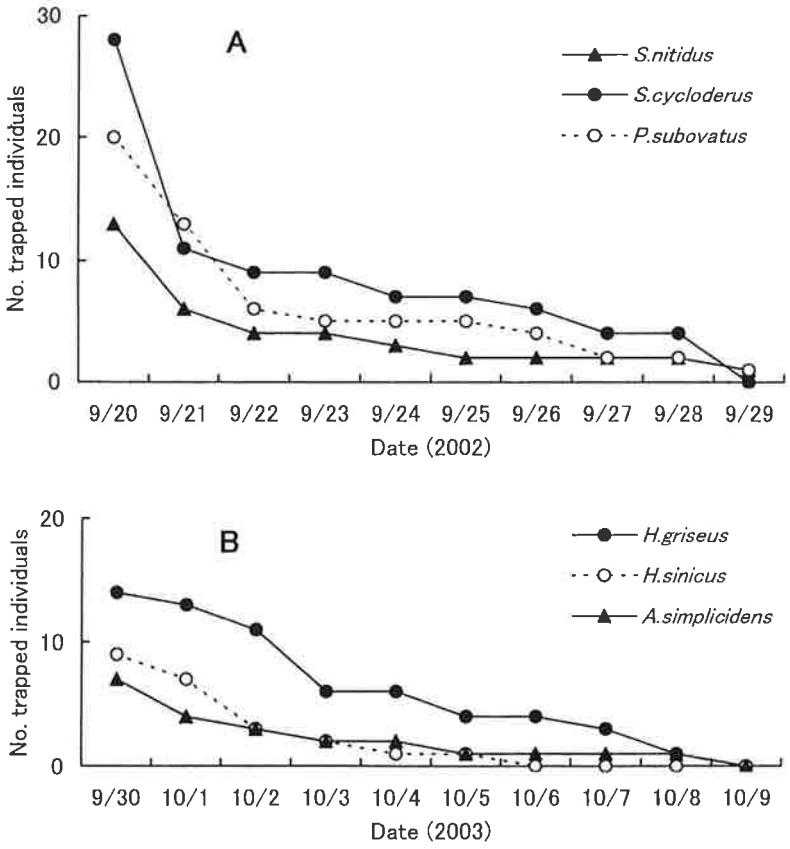

Fig. 3 Daily changes of trapped individuals of 3 dominant species in Site 1 (A) and Site 2 (B).

Daily changes in the total numbers of carabid beetles caught by 15 pitfall traps are shown in **Fig. 4**. A decreasing tendency similar to that of the 3 dominant species in Sites 1 and 2 was observed. No carabid beetles were captured on the tenth trapping in Site 2. The accumulated numbers of individuals captured up till the third and fifth trappings were 56 % and 73.2 % of the total in Site 1, respectively, and 66.5 and 84.7 % in Site 2, respectively.

The correlation coefficients between the C_i and T_i of the total carabid beetles in Sites 1 and 2 were −0.925 and −0.991, respectively.

3. Population estimations

Estimations using the regression and maximum likelihood methods were conducted using the numbers of the 3 dominant species and total carabid beetles trapped at both sites. **Table 2** shows the total number ($\hat{N}$) estimates for the two study sites, the density ($\hat{m}$) per m² and 95 % confidence limits. All data from the 10 trappings were used to calculate the estimates using the regression method. However, with the maximum likelihood method estimates were calculated using only the data sets from the first to the seventh trappings, because the graphs for

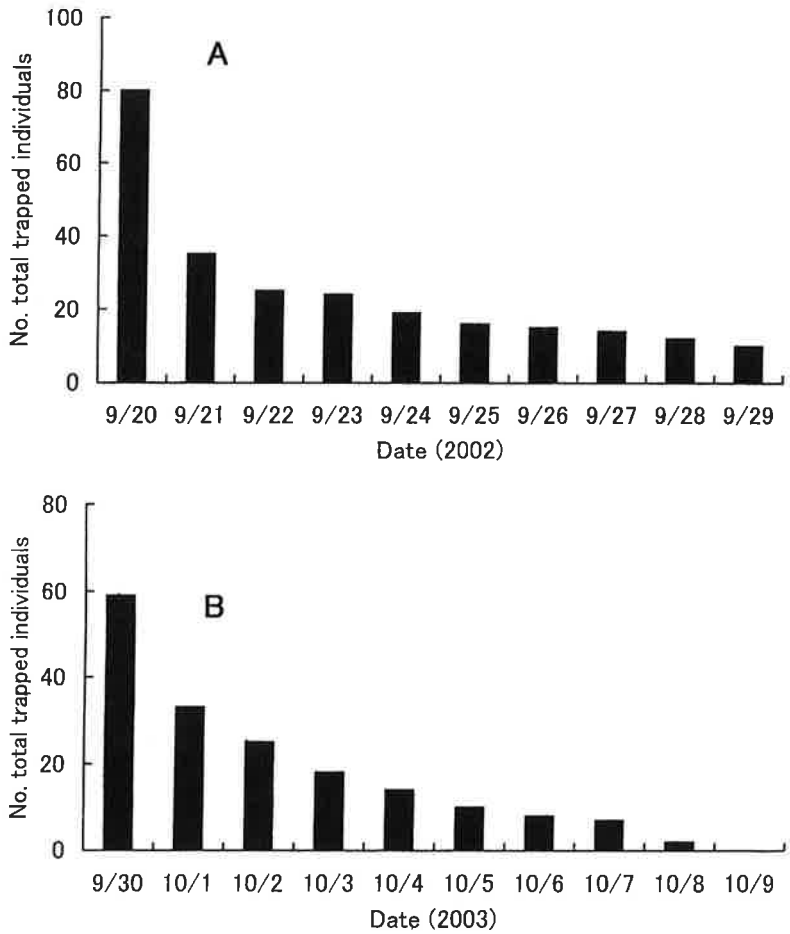

Fig. 4 Daily changes of total trapped individuals of total carabid beetles in Site 1 (A) and Site 2 (B).

Table 2 Estimats of the number of adult carabid beetles within 40 m² and the density per m² by two removal methods using trapping data

	species	Regression method					Maximum likelihood method				
		$\hat{N}$	$v(\hat{N})$	$\hat{m}$ (/m²)	±95% limits	D	$\hat{N}$	$v(\hat{N})$	$\hat{m}$ (/m²)	±95% limits	D
Site 1	*S. cycloderus*	87.7	36.4	2.2	0.30	0.07	91.7	73.8	2.3	0.42	0.09
	P. subovatus	63.9	6.9	1.6	0.13	0.04	66.7	33.4	1.7	0.28	0.09
	S. nitidus	39.1	4.5	1.0	0.10	0.05	37.8	12.3	0.9	0.17	0.09
	Total carabid	253.9	309.1	6.3	0.86	0.07	246.0	123.1	6.1	0.54	0.05
Site 2	*H. griseus*	68.1	6.9	1.7	0.13	0.04	69.0	55.6	1.7	0.37	0.11
	H. sinicus	23.5	0.2	0.6	0.02	0.02	23.7	1.4	0.6	0.06	0.05
	A. simplicidens	22.5	0.5	0.6	0.03	0.03	22.2	7.2	0.6	0.13	0.12
	Total carabid	180.3	14.2	4.5	0.18	0.02	185.6	60.4	4.6	0.38	0.04

estimating $1-q^s$ and p were only given for $s=3$, 4 and 7 trappings (Zippin, 1956).

The population size estimates of the 3 dominant species and total carabid beetles obtained using the regression method were almost equal to those obtained using the maximum likelihood method, and there were no significant differences judging by the 95 % confidence limits.

The standard deviation ($\sqrt{v(\hat{m})}$) to density ($\hat{m}$) ratio ($D=\sqrt{v(\hat{m})}/\hat{m}$) was used to represent the precision level of the estimations (Kuno, 1971). This ratio was less than 0.1 for almost all species. The regression method showed better precision than the maximum likelihood method except for the total number of carabid beetles in Site 1. The observed numbers caught by 10 trappings were almost the same as the estimated values (**Tables 1 & 2**).

Discussion

In this study we used the removal method to estimate the population size of carabid beetles to determine whether or not this method could be applied successfully. There are 3 different approaches to estimating population size with removal trapping data, namely, the regression method (Leslie & Davis, 1939; DeLury, 1947, 1951), the maximum likelihood method (Moran, 1951; Zippin, 1956) and time-unity collecting (Kono, 1953).

The high correlation coefficients of C_i and T_i, and small D values observed here suggest the reliability of the estimates obtained by the regression method. We calculated the 95 % confidence limits using the variance of $\hat{N}$ using Eqn. (1). However, the confidence limits of the regression method can be given more precisely using a solution (x_1, x_2) of the following quadratic equation with cases of less than 10 trappings (Kuno, 1986):

$$\left\{b^2-\frac{\hat{\sigma}^2 t_{s-2}^2(\alpha)}{\sum_i^s (T_i-\bar{T})^2}\right\}x^2-2\bar{C}bx+\bar{C}^2-\frac{\hat{\sigma}^2 t_{s-2}^2(\alpha)}{s}=0$$

Where $t_{s-2}(\alpha)$ is the critical value of t distribution ($df=s-2$) for $(1-\alpha)$ % confidence. The lower and upper limits of $(1-\alpha)$ % confidence are $(\bar{T}+x_1, \bar{T}+x_2)$. According to the above, the 95 % confidence limits of the total carabid beetles trapped in Sites 1 and 2 were (225.8, 307.0) and (173.5, 188.5), respectively. These confidence limits of Site 2 were nearly equal to those obtained by Eqn. (1), but the precision level of the estimates for Site 1 was

lower. This seems to be related to the fact that the correlation coefficient between the C_i and T_i of Site 1 (−0.925) was lower than that of Site 2 (−0.991).

The maximum likelihood method is the most accurate of the 3 approaches (Southwood, 1978), though it strictly requires the chance of being caught and effort of catching to be equal during the trapping period. Our trapping surveys were conducted using identical trapping intervals and identical shapes and numbers of pitfall traps. The fact that the estimates obtained by the maximum likelihood method were not significantly different from those obtained with the regression method, even though only 7 data sets were used, shows that the prerequisites were met and the propriety of this method.

Zippin (1956) showed the relationship between the precision level of the estimates and proportion of individuals removed from a population, and suggested that to obtain a precision level of 0.1, 75 % of a population would have to be removed when the population size was less than 300. Turner (1962) pointed out that for this reason it is impractical to estimate populations of insects caught in pitfall traps when the catching efficiency of these traps is very low. In this study, we used a high trap density (0.375 traps per m^2) and attractive bate so that more than 80 % of the beetles used to estimate population size were removed by the 7th trapping and the precision levels of these estimates with the maximum likelihood method were high (D<0.12) (**Table 2**).

Kono (1953) presented a formula for estimating population size using time-unity collecting data based on the exponential relationship between the number collected and time. Where n_1, n_2 and n_3 are the accumulated numbers of collected individuals at three time points (t_1, t_2 and t_3), such that $(t_1+t_2)/2=t_3$, $\hat{N}$ is estimated as:

$$\hat{N}=\frac{n_3^2-n_1n_2}{2n_3-(n_1+n_2)}.$$

As trappings were carried out daily in this study, n_1, n_2 and n_3 are T_2, T_6 and T_4, respectively, on t_1 = the 2nd trapping day, t_2 = the 6th trapping day and $t_3=(2+6)/2$ = the 4th trapping day. The total carabid beetle population sizes in Sites 1 and 2 according to the above formula were 291.8 and 188.4, respectively. The Site 1 value was overestimated slightly in comparison to the estimate obtained by the regression method, but there was no difference in the Site 2 value, although the variance of the estimate was not given with Kono's method (Kono, 1953).

In this study we estimated population size using the regression and likelihood methods with data from 10 and 7 trappings, respectively. To determine the appropriate numbers of trappings required, the estimates and precision levels were shown in relation to the number of trappings used for the regression method (**Table 3**). The precision level became lower with decreasing trapping times and about half the D values were more than 0.1 when population size was estimated by data from less than 5 trappings. From **Table 3** it can be suggested that at least 5 trappings will give an estimate of carabid beetle population size with a precision level of less than 0.1 using the regression method.

It is still questionable whether the number of carabid beetles caught in pitfall traps (activity-density) accurately reflects the population size (absolute density) in an immediate area (Thomas *et al.*, 2002). Several researchers tried to overcome this problem by additional mark-recapture studies using pitfall traps with barriers (Thomas *et al.*, 1998). In contrast to the capture and recapture method, which is widely used for population estimations, it is not possible to estimate the parameters of population dynamics, such as birth and death rates, from the

Table 3 Estimates of population size and the precision level (D) in relation to the number of trappings

	Sopecies		Number of trappings							
			3	4	5	6	7	8	9	10
Site 1	*S. cycloderus*	$\hat{N}$	53.7	62.6	69.3	76.3	82.2	85.4	88.6	87.7
		D	0.12	0.14	0.12	0.12	0.11	0.10	0.09	0.07
	P. subovatus	$\hat{N}$	48.8	50.4	53.8	57.9	61.1	62.2	63.4	63.9
		D	0.09	0.05	0.06	0.07	0.07	0.06	0.05	0.04
	S. nitidus	$\hat{N}$	26.5	30.1	32.5	33.8	35.2	36.7	38.3	39.1
		D	0.07	0.10	0.09	0.07	0.06	0.06	0.06	0.05
	Total carabid	$\hat{N}$	159.7	181.2	197.5	210.8	223.5	235.5	245.7	253.9
		D	0.09	0.11	0.10	0.09	0.08	0.08	0.07	0.07
Site 2	*H. griseus*	$\hat{N}$	128.2	74.7	74.0	71.2	72.0	72.1	70.1	68.1
		D	0.19	0.22	0.13	0.09	0.07	0.05	0.04	0.04
	H. sinicus	$\hat{N}$	25.5	24.7	24.2	24.3	23.9	26.1	24.3	23.5
		D	0.19	0.09	0.06	0.04	0.03	0.31	0.14	0.02
	A. simplicidens	$\hat{N}$	18.6	19.3	20.8	21.0	21.4	22.0	22.6	22.5
		D	0.09	0.05	0.06	0.04	0.04	0.04	0.04	0.03
	Total carabid	$\hat{N}$	153.5	163.3	170.6	174.7	178.2	181.6	181.4	180.3
		D	0.10	0.06	0.05	0.04	0.03	0.03	0.02	0.02

prerequisite of the removal method. Furthermore, precise estimates need a large part of the population to be removed. This is a critical obstruction for life-table studies. However, it is easier to estimate the population size of ground beetles or other small animals using the removal method because it does not require marking and recapture, which takes time as well as hard labor. The removal method can be used to easily estimate the density of a population per unit area as shown in this study. The removal method using pitfall traps might therefore be useful in quantitatively evaluating whether carabid beetles could be used as a predator or for measuring their biomass.

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Rotenoids in the Yam bean *Pachyrrhizus erosus*: Possible defense principles against herbivores

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対植食者防御の主要因としてヤムビーンに含まれるロテノイド類　アーディーン・ガッサ[1)]・福井昌夫[1)]・佐久間正幸[1)]・西岡孝明[1)]・高橋正三[1)]（[1)]京都大学大学院農学研究科、[2)]現住所：ハサヌディン大学・インドネシア）

ウラナミシジミの雌成虫は，ごく近くに繁殖するフジマメとヤムビーンの花芽に盛んに産卵した．フジマメで見られるウラナミシジミ幼虫による加害は，ヤムビーンでは見られなかった．この幼虫による加害が回避されたのは，ヤムビーンの花芽に含まれる対植食者防御毒が有力と考えられた．ヤムビーンの花芽に加えて葉，種子から，アワヨトウ幼虫の殺虫活性をもとに，殺虫成分としてロテノイドを単離した．単離された*cis*-12a-hydroxymunduserone と *cis*-12a-hydroxyerosone, *cis*-12a-hydroxyrotenone, rotenone の4種のロテノイドの活性と含有量が対食植者防衛の観点から議論された．

Females of the pea blue butterfly *Lampides boeticus* fly to the young flower buds of the yam bean plants *Pachyrrhizus erosus* and the lablab bean plants *Dolichos lablab,* and equally lay eggs on them. No damage to the yam bean induced by the butterfly larvae was observed. But the larvae gave serious damage to the lablab bean growing up in the vicinity of the yam bean. Distinct difference of damages between both plants seemed to be caused by the defense mechanism of the yam bean plants using natural toxins. Possible defense principles were isolated and identified as four rotenoids *cis*-12a-hydroxymunduserone, *cis*-12a-hydroxyerosone, *cis*-12a-hydroxyrotenone and rotenone from flower buds, seeds and leaves of the yam bean on the base of the larvicidal toxicity toward the common armyworm *Mythimna separata.* The activity of each principle was

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quantitatively evaluated. In addition, the relationships between herbivores and the presence of principles were discussed.

Key words: Rotenoids, Yam bean, Common armyworm, Pea blue butterfly

Introduction

Tropical plants are a rich source of bioactive substances. Antifeedants, antihormones, growth inhibitors and other toxicants directed against insects have been investigated to estabish new classes of natural pesticides for commercial application or relationships between these phytochemicals and host plant resistance (Jacobson, 1989). The earliest study on the toxicity of yam bean seed concluded that saponin is toxic (Nag *et al.*, 1935). Further chemical investigations showed the presence of isoflavones, rotenoids and other toxins in the same plant (Norton and Hansberry, 1945; Bickle and Schumid, 1953; Crombie and Whiting, 1963). Although subsequent studies that focused on biogenetic and chemical aspects were performed by Krishnarmurti and Seshadri (1966), and Kalra *et al.* (1977), neither detailed the biological activity toward insects, the distribution nor relative content of these compounds in the plant organs.

Until examination of the yam bean blooms in the autumn, no insect was known to feed on the young leaves, but the pea blue butterfly *Lampides boeticus* flies to the young flower buds and lays eggs on it. However, butterfly larvae feeding on the flower buds were not observed, although the pea blue butterfly grew normally on the flower buds of the natural host plant, the lablab bean *Dolichos lablab.* These observations suggested the presence of toxic materials in young flower buds of the yam bean. In this paper, we describe the isolation and identification of insecticidal compounds from the young flower buds, and quantitatively evaluate their toxicity insecticidal activity against the common armyworm *Mythimna separata.* Also discussed is the ecological role of these compounds toward phytophagous insects. In addition, toxicities of the beans and leaves were quantitatively evaluated for the common armyworm.

Materials and Methods

Extraction

Powdered seeds (240 g) from Bogor, Indonesia, were immersed and then successively extracted three times with hexane, MeOH and ether, respectively. From each solvent, 80 g of oil, 20.1 g of solid materials and 2.2 g of solid materials were gained. Flower buds (12.5 g) and 1.41 kg of leaves were harvested from plants grown on the University campus, and then extracted three times with 400 ml and 6 l of MeOH for one month, respectively.

Instruments and analytical conditions

The HPLC unit consisted of a Hitachi L-6000 Pump equipped with a Waters R401 differential refractometer. The unit was operated using Cosmosil 5Ph packed column [10 mm (ID) × 500 mm, Nacalai Tesque, Kyoto] or Wakosil-II ODS packed column [8 mm (ID) × 250 mm, Wako Pure Chem. Indus. Ltd., Osaka]. EI-MS spectra were measured on Hitachi M-80A Mass Spectrometer at 70 eV using an in-beam direct insertion technique, and ^{1}H-NMR spectrum were measured in $CDCl_3$ on a Brucker AC-300 spectrometer at 300 MHz. Optical rotation was measured using a Jasco Digital Polarometer DIP-370 in $CHCl_3$ with a light path of

1 cm at 30℃.

Insects

Larvae of the common armyworm *Mythimna separata* (Noctuidae, Lepidoptera) were reared on an artificial diet (Insecta LF, Nosan Corporation, Yokohama) under conditions of 25±1℃, 17L-7D, and 50-70% R. H., and the 3rd-instar larvae were subjected to bioassay.

Bioassay method

Crude methanol extracts from flower buds, leaves and seeds of the yam bean and their chromatographic fractions were subjected to bioassay. The samples were dissolved in acetone or $CHCl_3$ to make a solution (1 ml) containing extract from 10 g equivalents of the leaves and seeds, and 300 g equivalents of the flower buds, respectively. The solution (100 μl) was applied to the top and bottom of an artificial diet cube (1 g) with a microsyringe, the applied diet was then air-dried at room temperature for 30 min. The treated diet and 10 3rd-instar instar larvae of the common armyworm were introduced into a plastic petri dish (9 cm ID). For a control, an artificial diet (1 g) was applied with 100 μl of acetone or chloroform, and air-dried in the same manner. The control diet and 10 larvae were introduced into another petri dish. Three replicates were made for each treatment test with each control, and the mortality of the armyworm was checked every 24 h for 10 days.

We focused on effects of time on percentage of kill of the common armyworm larvae. Therefore, larvicidal activity was evaluated using the probit analysis of serial time-mortality data from bioassays (Throne *et al.* 1995) where successive observations are made on the same group of the larvae exposed 1 concentration of a stimulus. By using the analysis, every LT_{50} (50% lethal time) value (day), which is defined as the lethal time value required to kill 50% of the larvae exposed 1 concentration, was calculated from all bioassay experiments. Comparisons of the evaluated larvicidal activities based on LT_{50} value led to the isolation of active principles. All statistics required by the probit analysis were calculated with a program developed by Throne *et al.* (1995).

Results

Isolation of toxic components from the seeds, leaves and flower buds of the yam bean

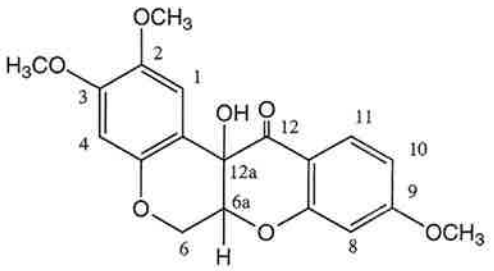
Seeds

Only the methanol extract (20.1 g of solid material) of the seeds indicated insecticidal activity (LT_{50} = 1.9 days) to the common armyworm. The solid suspended in water (1 l) was then extracted three times with 1 l of $CHCl_3$. The toxic components were transferred to the $CHCl_3$ layer (9.1 g). After evaporation the solvent, the $CHCl_3$ layer was applied to a silica gel column [68 mm (ID) × 330 mm, 600 g, Wako C-200, Wako Pure Chem.Indus. Ltd., Osaka] and eluted stepwise with each 5 l of 50% toluene in hexane, 20% and 50% EtOAc in toluene, EtOAc and MeOH. The 20% EtOAc in toluene fraction was active (LT_{50}= 2.2 days). The fraction was applied on a silica gel column [50 mm (ID) × 300 mm, 300 g, Wako-gel] and eluted stepwise with 5 l each of toluene, 2%, 5%, 10%, 20% and 50% EtOAc in toluene. The activity was recovered in 5% and 10% EtOAc in toluene fractions (LT_{50} = 2.5 and 3 days). Both fractions were combined and purified on an ODS column [24 mm (ID) × 230 mm, 70 g, Cosmosil 75 C_{18}-PREP, Nacalai Tesque, Kyoto] with 1 l each of 80% CH_3CN in H_2O and 90% THF in H_2O. The active 80% CH_3CN in H_2O eluate (LT_{50} 1.9 days) was further separated into 7 fractions (250 ml each) on a silicagel column [24 mm (ID) × 320 mm, 90 g, Wakogel C-200] with 30% EtOAc in hexane. The combined active 3rd to 5th fractions (1.65 g; LT_{50}

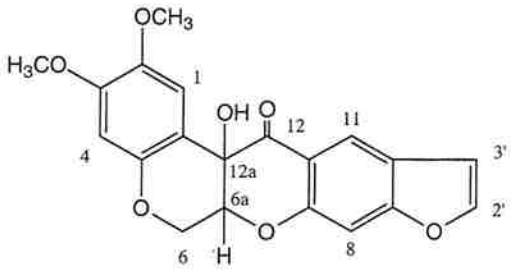
= 2.1 to 2.5 days) were further fractionated by silica gel columns [24 mm (ID) × 370 mm, 90 g, Wako C-200] with 30 % EtOAc in hexane into 54 fractions (25 ml each). The fractions from 24th to 54th contained active compounds (LT_{50} = 2.4 to 4.1 days), and were combined based on HPLC [10 mm (ID) × 250 mm, Cosmosil 5Ph column, 65 % CH_3CN in H_2O as eluent] into three groups: the 24th to 30th fractions, the 31st to 39th fractions and the 40th to 54th fractions. Each combined fraction was separately fractionated with the same Cosmosil 5Ph packed column to give four toxic components **1** (t_R = 28.5 min, LT_{50} = 5.0 days), **2** (30 min, LT_{50} = 4.5 days), **3** (37 min, LT_{50} = 8.2 days) and **4** (47 min, LT_{50} = 2.0 days) (**Table 1**). The quantities of **1, 2, 3** and **4** that were contained in one gram equivalent of the seeds were 1, 1.83, 2.43 and 0.46 mg, respectively.

Flower buds

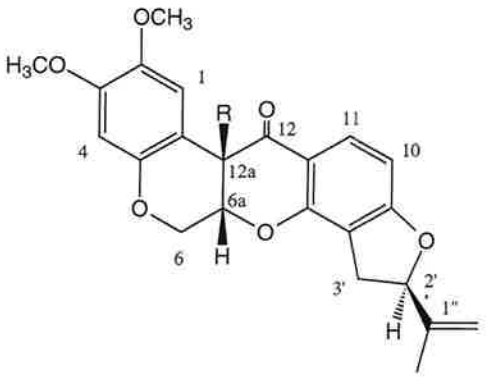
The methanol extract (LT_{50} = 4.6 days) of the flower buds was partitioned three times with 500 ml each of water and $CHCl_3$. The $CHCl_3$ layer that



1: cis-12a-hydroxymunduserone



2: cis-12a-hydroxyerosone



3: cis-12a-hydroyrotenone

R=OH

4: Rotenone

R=H

Fig. 1 Isolation rotenoids from the yam bean.

Table 1 ^{1}H NMR shift data for rotenoids (**1**, **2**, **3** and **4**)

Position	1	2	3	4
Rotenoid skeleton				
H-1	6.54 (s)	6.55 (s)	6.55 (s)	6.78 (s)
H-4	6.49 (s)	6.48 (s)	6.48 (s)	6.45 (s)
H-6	4.50 (dd)	4.60 (dd)	4.46 (d)	4.18 (d)
	4.62 (dd)	4.66 (dd)	4.49 (dd)	4.61 (dd)
H-6a	4.60 (br t)	4.49 (br t)	4.58 (br t)	4.92 (br t)
H-8	6.38 (d)	7.02 (br s)	—	—
H-10	6.58 (dd)	—	6.53 (d)	6.50 (d)
H-11	7.85 (d)	8.20 (s)	7.82 (d)	7.84 (d)
H-12a	—	—	—	3.82 (s)
12a-OH	4.46 (s)	4.43 (s)	4.58 (s)	—
Dihydrofuranoid skeleton				
H-2′	—	—	5.23 (t)	5.24 (t)
H-3′ a	—	—	2.95 (dd)	2.95 (dd)
H-3′ b	—	—	3.29 (dd)	3.32 (dd)
1″ —Me	—	—	1.73 (s)	1.77 (s)
1″ $=CH_2$	—	—	4.90 (s)	4.90 (s)
	—	—	5.10 (s)	5.10 (s)
Furanoid skeleton				
H-2′	—	7.55 (d)	—	—
H-3′	—	6.74 (d)	—	—
Substituents				
OMe	3.72 (s)	3.71 (s)	3.72 (s)	3.77 (s)
	3.79 (s)	3.79 (s)	3.82 (s)	3.81 (s)
	3.81 (s)	—	—	—

Notes: J values (i) for rotenoid skeleton, 6−6 = 12.2 Hz, 6−6a = 2.8 and ca 1 Hz, 6a−12a = 3.6 Hz, 8−10 = 2.4 Hz, 10−11 = 8.5 Hz; (ii) for dihydrofuran skeleton, 2′−3′a = 9.8 Hz, 2′−3′b = 8.3 Hz, 3′a −3′b = 15.9 Hz, 1″ = CH_2 two braod singles; (iii) for furanoid system, 2′−3′ = 2.2 Hz.

showed larvicidal activity (LT_{50} = 4.8 days) yielded 0.97 g of residue which was applied to a silica gel column [26 mm (ID) × 240 mm, 55 g, Wako C-200] and eluted with 1.5 l each of 50 % toluene in hexane, 20 % and 50 % EtOAc in toluene, EtOAc and MeOH. Only the 20 % EtOAc in toluene fraction (200 mg) possessed the activity (LT_{50} = 4.7 days). The fraction was applied to a silica gel column [20 mm (ID) × 240 mm, 15 g, Wako C-200] and then eluted with 750 ml each of toluene 2 %, 5 %, 10 %, 20 % and 50 % EtOAc in toluene. The active 5 % and 10 % EtOAc in toluene fractions (LT_{50} = 5.5 and 6.1 days) were combined (56.7 mg) and purified by an ODS column [24 mm (ID) × 170 mm, 5 g, Cosmosil 75 C_{18}-PREP] with 750 ml each of 80 % CH_3CN in H_2O and 90 % THF in H_2O. The active 80 % CH_3CN in H_2O fraction (LT_{50} = 4.9 days) was subjected to HPLC using a Cosmosil 5Ph packed column (65 % CH_3CN in H_2O as eluent), and two toxic com-

ponents (**1** and **3**, LT_{50} = 5.2 and 7.9 days) were isolated with retention times at 28.5 and 37.0 min. The quantities of **1** and **3** that were contained in one gram equivalent of the flower buds were 0.03 and 0.07 mg, respectively.

Leaves

The methanol extract (226 g, LT_{50} = 6.5 days) from the leaves was dissolved into water (1 l), extracted three times with $CHCl_3$. The $CHCl_3$ (56 g) extract showed larvicidal activity (LT_{50} = 6.1 days). The extract was applied to a silica gel column [80 mm (ID) × 450 mm, 1 Kg, Wako C-200] and eluted stepwise with each 8 l of 50 % toluene in hexane, 20 % and 50 % EtOAc in toluene, EtOAc and MeOH. The active 20 % EtOAc in toluene fraction (LT_{50} = 6.0 days) was applied on a silica gel column [68 mm (ID) × 460 mm, 700 g, Wako C-200] and eluted stepwise with each 5 l of toluene, 2%, 5 %, 10 %, 20 % and 50 % EtOAc in toluene. The active 10 % EtOAc in toluene fraction (LT_{50} = 6.6 days) was subsequently purified by an ODS column [24 mm (ID) × 230 mm, 90 g, Cosmosil 75 C_{18}-PREP] with 1 l each of 80 % CH_3CN in H_2O and 90 % THF in H_2O. The active 80 % CH_3CN in H_2O eluate (LT_{50} = 6.3 days) was separated on a silica gel column [40 mm (ID) × 280 mm, 180 g Wako C-200] with 30 % EtOAc in hexane into 7 fractions (500 ml each). The active 3rd and 4th fractions were combined and then subjected to HPLC using a Wakosil-II ODS packed column (60 % CH_3CN in H_2O as eluent). The eluate from the ODS packed column was subjected HPLC using a Cosmosil 5Ph packed column (65 % CH_3CN in H_2O as eluent), and two fractions of 2 (t_R = 30 min, LT_{50} = 6.8 days) and 3 (37 min, LT_{50} = 8.7 days) showed activity toward the armyworm respectively. The quantities of **2** and **3** that were contained in one gram equivalent of the flower buds were 0.23 and 0.45 mg, respectively.

Structure elucidation of isolated components from the seeds, leaves and flower buds of the yam bean

Since isolated compounds were rotenoids (**Fig. 1**), their structural characterization was carried out by comparing the physical and spectral data with those previously reported. ^{1}H-NMR data for the isolated compounds are shown in **Table 1**. The *cis* stereochemistry of the B/C ring junction was established from the ^{1}H-NMR spectrum as seen in **Table 1**, where H-1 at 6.54-6.78 ppm is strongly deshielded than that in *trans* substituted compounds at 7.82 ppm (Oberholzer *et al.*, 1974).

EI-MS spectrum of compound **1** gave M^+ ion at *m/z* 358 (98 %) and the dehydrated ion (M^+−18) at *m/z* 340 (13 %) with the base ion at *m/z* 208, suggesting the molecular formula of $C_{19}H_{18}O_7$ (Kalra *et al.*, 1977; Dagne *et al.*, 1989). Data of the optical rotation $[\alpha]_D$ +40.4°, melting point (m. p.) 91-92 ℃, and the ^{1}H-NMR spectrum were in agreement with those of cis 12a-hydroxymunduserone (Kalra *et al.*, 1977; Dagne *et al.*, 1989).

EI-MS spectrum of compound 2 gave M^+ ion at m/z 368 (90 %) and the dehydrated ion (M^+−18) at m/z 350 (8 %) with the base ion at m/z 208, suggesting the molecular formula of $C_{20}H_{18}O_7$ (Kalra *et al.*, 1977). Data of the optical rotation $[\alpha]_D$ + 186°, m. p. 282-283 ℃, and the ^{1}H-NMR spectrum were in agreement with those of *cis*-12a-hydroxyerosone although the $[\alpha]_D$ value measured by Kalra *et al.* (1977) is + 170.4°.

The third compound (**3**) was isolated as a colorless solid. Its EI-MS spectrum gave M^+ ion at *m/z* 410 (99 %) and M^+−18 ion at *m/z* 392 (14 %) with the base ion at *m/z* 208, suggesting the chemical formula $C_{23}H_{22}O_7$ (Kalra *et al.*, 1977; Dagne *et al.*, 1989). Data of the optical rotation $[\alpha]_D$ − 164°, m. p. 91-92 ℃, and ^{1}H-NMR spectrum were in agreement with those of *cis*-12a-hydroxyrotenone (3) (Kalra *et al.*, 1977; Dagne *et al.*, 1989).

Table 2 Active compounds isolated from the seeds, flower buds and leaves of the yam bean *Pachyrrhizus erosus* and their toxicities against the common armyworm

Compound	Amount[1]		
	Seed	Flower bud	leaf
cis-12a-hydroxymunduserone (**1**)	1.00 (5.0)[2]	0.03 (5.2)[3]	–
12a-hydroxyerosone (**2**)	1.83 (4.5)	–	0.23 (6.8)
cis-12a-hydroxyrotenone (**3**)	2.43 (8.2)	0.07 (7.9)	0.45 (8.7)
Rotenone (**4**)	0.46 (2.0)	–	–

1) One milligram per gram equivalent of plant organ.
2) Toxicity was estimated using a Probit program for analyzing serial time-mortality data (Throne *et at.*, 1995), and the figures in parenthsis indicate LT_{50} (50 % lethal time) value (days) for 30 armyworms.
3) Test solution of 300 g equivalents of the flower buds was used in the bioassays for bud (see text in details).

Isolated compound **4** was crystallized as a plate from MeOH. Its EI-MS spectrum gave M^+ ion at *m/z* 394 (9 %) and the base ion at *m/z* 208, suggesting $C_{23}H_{22}O_6$ as its molecular formula (Kalra *et al.*, 1977; Dagne *et al.*, 1989). Data of the optical rotation $[\alpha]_D$ -194.2°, m.p. 159-161 ℃, and ^{1}H-NMR spectrum were agreement with those of rotenone (Kalra *et al.*, 1977; Dagne *et al.*, 1989).

Discussion

Comparisons of the LT_{50} values revealed the distribution and relative content of four rotenoids (**1, 2, 3** and **4**) as larvicidal components in the beans, flower buds and leaves of the yam bean (**Table 2**). The seeds contain all four components, whereas the leaves and flower buds lack rotenone and contain two of the medium or low toxic components. The crude methanol extracts of the seeds and leaves showed the high (LT_{50} = 1.9 days) and medium (LT_{50} = 6.5 days) larvicidal activity. In the flower buds, their content was found to be small; several percent of that found in the seeds. During the first step of extraction of the toxic components from the flower buds, the crude methanol extract from 10 g equivalents was bioassayed for lavicidal activity using 3rd-instar larvae. Consequently, the extract did not show any activity. However, against 1st-instar larvae in a separate experiment it exhibited effective larvicidal activity (LT_{50} = 0.85 day) and all larvae died within the first three days. In the isolation process of the active principles, the concentration of test solution (1 ml) from the flower bud extract was adjusted to 300 g equivalents of the buds. These results suggested that the tiny flower buds contain so reasonable amount of rotenoids to kill younger 1st-instar larvae, so that they can protect themselves with less efforts of defense as compared with defense aginst the 3rd-instar larvae (**Table 2**).

Until examination of the blooms of yam bean in the autumn, no insect was known to feed on the young leaves, but the pea blue butterfly *Lampides boeticus* flies to the young flower buds and actually lays eggs on it (**Fig. 2**). However, butterfly larvae feeding on the flower buds were not observed. On the other hand the butterfly grew normally on the flower buds of the natural host plant the lablab bean *Dolichos lablab* in the proximity of the yam bean. Above observations suggest that the young butterfly larvae probably have the same sensitivity to these toxins as the 1st-instar larvae of the armyworm do, because the 1st-instar larvae of the butterfly that

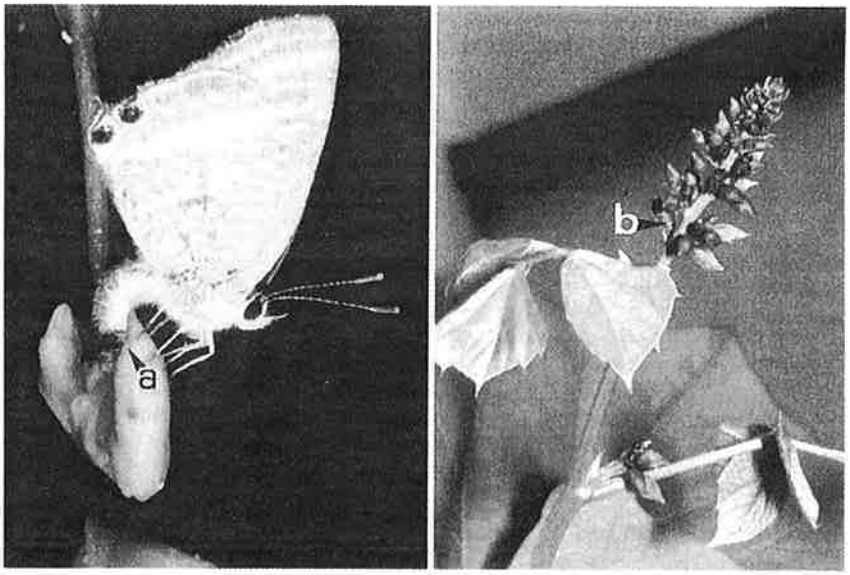


Fig. 2 L: The pea blue butterfly *Lampides boeticus* laying eggs on the young flower buds of the lablab bean *Dolichos lablab* (host plant). a: the ovipositor of the butterfly. R: The pea blue butterfly eggs (b) laid on the young flower buds of the yam bean (non host plant).

hatched from the eggs were not observed on the flower buds (per. observation). Thus, the flower buds have succeeded in arming themselves with natural toxins against damage caused by herbivorous insects.

The fragrance of flower buds of the lablab bean and the yam bean were collected using a headspace method on absorbent Tenax and eluted with a solvent. Coupled GC-EAD responses of the female butterfly antennae toward the eluates were recorded. The antennae responded positively toward species-specific and also commonly overlapped GC peaks of both fragrances desorbed from the absorbent (Gassa and Fukui, unpublished data). This suggests that the butterfly antennae are sensitive to fragrance chemistry, and can recognize host odors accurately due to fragrant chemicals in both flower buds, because behavioral mediating compounds show high electrophysiological activity (Andersson, 2003). We also observed that non-herbivore bees and butterflies foraging as pollinators often visit the flowers of both bean plants during the same season in the field. This shows that fragrances play a role as attractants of herbivores as well as non-herbivore, such as bees and butterflies.

Armbruster (1997) notes that the two commonest interactions between plants and animals are herbivory and pollination: Most plants experience, simultaneously or sequentially in the course of their lifetimes, interaction with both herbivores and pollinators. Our present study supports this. During such interactions, the yam bean plant appears to defend itself using rotenoids from herbivore attack. To make the above explanation more accurate, further studies are needed. The results should reveal the biological role of rotenoids in flower buds with respect to the interactions between the yam bean plant and the pea blue butterfly (**Fig. 2**).

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Survey on the infestation of houses by *Incisitermes minor* (Hagen) in Kansai and Hokuriku areas

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関西地域および北陸地域におけるアメリカカンザイシロアリの被害調査　Yuliati Indrayani[1)]・吉村　剛[1)]・藤井義久[2)]・簗瀬佳之[2)]・岡久陽子[1)]・今村祐嗣[1)]（[1)]京都大学生存圏研究所，[2)]京都大学大学院農学研究科）

アメリカカンザイシロアリによる住宅の被害について，関西地域および北陸地域（9県）のシロアリ防除会社に郵送によるアンケート調査を行った．回答があった96社のうち兵庫県，大阪府，和歌山県，富山県，福井県の31社からアメリカカンザイシロアリによる被害例が報告された．被害にあった家屋の約83%が木造で，最も多く発見された階級は職蟻（ニンフ）であった．被害は主に部材より落下した糞により発見された．垂木，軒，野地板，梁，柱のような屋根部材や，窓枠，戸枠，柱，障子のような内装部材が被害を受けやすいことが明らかになった．最も一般的な防除対策は殺虫剤の噴霧であった．

The infestation of houses by *Incisitermes minor* (Hagen) was surveyed in Kansai and Hokuriku areas (9 prefectures) by means of a postal questionnaire sent to termite control companies. Of the 96 companies that responded, 31 of them, located among Hyogo, Osaka, Wakayama, Toyama, and Fukui Prefectures, reported finding *I. minor* attacks in houses they had inspected. Approximately 83% of the infested houses were wooden post and beams constructions. Worker (nymph) castes were the most commonly found caste in infested houses. The infestation was usually detected by fecal pellets under the infested timbers. Roofing materials such as rafters, trim, boards, beams, pillars, and lathing boards, and interior materials such as window frames, door frames, pillars, poles, and paper doors were the most susceptible parts. Spraying with insecticide was the most common countermeasure taken to combat infestation.

Key words : Postal survey, Termite infestation, *Incisitermes minor*, Kansai and Hokuriku areas

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Introduction

Incisitermes minor (Hagen), a dry-wood termite native to the western region of the United States (Weesner, 1970), is called the western dry-wood termite. It is the most common structure-infesting dry-wood termite in the southwestern part of the United States (Cabrera and Scheffrahn, 2004). Su and Scheffrahn (1990) stated that *I. minor* was one of the five most economically important and destructive termites in the United States.

A colony of *I. minor* may contain 2,000 termites, and is found in both natural and man-made environments. Although colony development is slow, severe structural damages may be caused by the presence of multiple-colony infestation (Su and Scheffrahn, 1990). Colonies of *I. minor* live entirely within sound dry wood (Cabrera and Rust, 2000). Due to these ecological characteristics, *I. minor* is easily transported in infested wood products by various human activities. In a mobile society, the introduction of such exotic species is difficult to prevent, as remarked by Grace *et al.* (1991).

Japan is one of the major countries importing wood and wood-based materials that can serve as a natural habitat of *I. minor.* The first infestation of *I. minor* in Japan appeared in Tokyo, followed by appearances in the west Chiba Prefecture (Mori, 1976), and by the time of this report infestation had been sporadically reported in Hyogo, Wakayama, Kanagawa, Hiroshima, Osaka, Yamaguchi, Kagoshima, and Okinawa Prefectures, (Yamano, 1998 ; Yasuda *et al.*, 2003). However, no national or even area survey has yet been conducted to assess the distribution of infestation of *I. minor.*

In this study, the infestation of *I. minor* in Kansai and Hokuriku areas (9 prefectures) was surveyed by means of a postal questionnaire sent to termite control companies.

Methods

This study was conducted by means of a postal survey. The questionnaire was sent to 154 termite control companies listed as members of the Japan Termite Control Association, Kansai-Branch, consisting of Shiga, Kyoto, Nara, Hyogo, Osaka, Wakayama Prefectures, and Hokuriku-Branch, consisting of Toyama, Ishikawa, and Fukui Prefectures. The questions were :

1. Have you ever seen an *I. minor* infestation?
   a. Yes b. No c. No, but heard of that
2. When did you find the first *I. minor* infestation?
   (years after construction)
3. Structure of the house:
   a. What kind of building?
      1. House 2. Office 3. Warehouse
      4. Other
   b. What major constructional type?
      1. Wooden post and beams
      2. Rein forced concrete (RC)
      3. Combination of wooden post and beams and RC
      4. Unknown
   c. Did you treat the house before construction?:
      1. Treated 2. Not-treated 3. Unknown
   d. In what City/Town is the infested house located?
4. How did you find the damage?
   a. I found the fecal pellets but did not find attacked timbers
   b. I did not find the fecal pellets but found attacked timbers
   c. I found both the fecal pellets and attacked timbers
   d. Other
5. a. Did you find termites? 1. Yes 2. No
   b. What caste of termite?
      1. King or Queen 2. Alate

3. Nymph/worker 4. Soldier

c. Did you identify the termite species?

1. Yes 2. No

6. What parts of the house had been attacked by *I. minor*?

7. What timber species had been attacked by *I. minor*?

8. Countermeasures taken against the infestation:

a. Did you perform extermination of termites?

1. Yes 2. No

b. What kind of countermeasures was taken against the infestation?

1. Replacement of damaged parts

2. Spraying of insecticides

3. Fumigation 4. Other

Results

Number of responses

Out of 154 companies receiving the survey, 96 (62.3%) completed and returned the questionnaire. The numbers of collected postal questionnaires in this survey are shown in **Table 1**. As a result, 31 companies (32.3% of total recovery) reported finding *I. minor* attacks in houses they had inspected, 61 companies (63.5% of total recovery) had found no infestation, and 4 companies (4.2% of total recovery) reported that they had heard of attacks by *I. minor*. Locations of the *I. minor*-infested houses reported in this survey are shown in **Table 2**.

Termite infestation in houses

The survey showed that 58 houses infested by *I.*

Table 1 Number of collected postal questionnaires

Total number of questionnaires	Response			Total Recovered questionnaires
	No infestation found	Infestation found	Heard of infestation	
154	61	31	4	96

Table 2 Location of *I. minor*-infested houses in Kansai and Hokuriku areas

City / Town	Prefecture	Numbers of infested houses
Kishigawa	Wakayama	1
Kokawa	Wakayama	20
Kozagawa	Wakayama	2
Naka	Wakayama	2
Susami	Wakayama	4
Uchita	Wakayama	6
Wakayama	Wakayama	3
Amagasaki	Hyogo	1
Kobe	Hyogo	5
Kakogawa	Hyogo	1
Nishinomiya	Hyogo	6
Takarazuka	Hyogo	2
Osaka	Osaka	3
Fukui	Fukui	1
Himi	Toyama	1

minor were found in Kansai and Hokuriku areas. These houses were classified by construction type, years after construction, and whether there was preventive treatment before construction (**Table 3**). Regarding construction type, 48 houses (82.8%) were wood frame, 1 house (1.7%) was RC, and 6 houses (10.3%) were combination (wood and RC). When the infestation was discovered, five houses (8.6%) were less than 5 years old and 46 houses (79.3%) were over 5 years old. Preventive treatment before construction was conducted only in 6 (10.3%) houses; 41 (70.7%) houses were not treated before construction.

Table 4 shows termite castes and the manner of damage found in infested houses. The majority of infestations (55 : 94.8%) were detected by both fecal pellets and attacked timbers, and 3.5% and 1.7% of infestations were detected only by the presence of attacked timbers and the wings of alate, respectively. Concerning the termite caste, worker (nymph) was the most commonly found caste in infested houses, followed by soldier and alate. Termites found in 41 (70.7%) infested houses were identified as *I. minor*, while those in 10 (17.2%) were not identified. For seven (12.1%) infested houses, the companies did not answer whether the species of termite had been identified.

The summarized data of attacked parts of houses

Table 3 *I. minor*-infested houses classified by construction type, years after construction, and preventive treatment before construction

Construction type[1)]	No. of infested house (%)	Years after construction			Preventive treatment before construction		
		<5 yr	>5 yr	Unknown	Yes	No	Unknown
W	48 (82.8)	2	40	6	5	32	11
RC	1 (1.7)	-	1	-	-	1	-
W + RC	6 (10.3)	3	2	1	1	5	-
Unknown	3 (5.2)	-	3	-	-	3	-
Total	58 (100)	5	46	7	6	41	11

1) W : Wood post and beams RC : Reinforced concrete, W + RC : Wood + Reinforced concrete

Table 4 Termite caste and the manner of damage found in infested houses[1)]

Construction type[2)]	Termite caste[3)]			Manner found damage[4)]			Termite identification		
	A	Wo	S	Wi	AT	P+AT	Yes	No	Unknown
W	14	37	28	1	1	47	34	9	6
RC	-	1	-	-	-	1	-	1	-
W + RC	-	4	4	-	-	5	4	-	1
Unknown	1	3	3	-	1	2	3	-	-
Total	15	45	35	1	2	55	41	10	7

1) Data collected from 58 infested houses.
2) For legends see Table 3.
3) A : Alate, Wo: Worker, S : Soldier.
4) Wi : Wings, AT : Attacked Timber, P + AT : Pellet + Attacked Timber.

are shown in **Table 5.** Data were collected from 58 infested houses without categorization by construction type. As shown in the table, roofing materials such as rafters, trim, beams, boards, pillars, and lathing boards, and interior materials such as window frames, door frames, pillars, poles, and paper doors were the most frequently attacked in the houses infested by *I. minor*. Timbers in the wall such as pillars and poles, and other materials such as sills, door jambs, shutters, gates, and imported furniture were the second-most frequently attacked parts. Materials under the floor such as floor lumber, girders, bases, joists were hardly attacked.

Table 6 shows timber species in houses attacked by *I. minor*. Out of 58 infested houses, the companies responded to attacks of the species in timber in 38 houses. Pine (*Pinus* spp. and Douglas fir) timbers were the most susceptible to *I. minor*, followed by hemlock (*Tsuga* spp.), hinoki (*Chamaecyparis obtusa* Endl.), sugi (*Cryptomeria japonica* D. Don), and lauan (*Shorea* spp. and other tropical hardwood species).

Countermeasures against infestation

In some of the infested houses in this survey, multiple control measures were taken against the infestation. Spraying with insecticide was the most common practice, followed by replacement of damaged parts, fumigation, and other treatments such as painting (**Table 7**).

Discussion

Figure 1 shows the distribution map of *I. minor* based on the data gathered by the present survey. The results clearly show infestation by this termite in coastal areas of Hyogo, Osaka, and Wakayama Prefectures. This may be closely related to the fact that *I. minor* was introduced to Japan in infested timber imported from overseas. In addition, we

Table 5 Wooden parts in houses frequently attacked by *I. minor*[1)]

Attacked parts	Number of infested houses
1. Roofing materials: rafters, trim, beam, boards, pillars, lathing boards	47
2. Interior materials: window frames, door frames, pillars, poles, paper doors	40
3. Timber in the wall: pillars, poles	29
4. Other: sills, door jambs, shutters, gates, imported furniture	25
5. Materials under the floor: floor lumber, girder, base, joist	9

1) Data collected from multiple answers in 58 infested houses.

Table 6 Timber species in houses attacked by *I. minor*[1)]

Timber species attacked	Number of positive answers
1. Pine (*Pinus* spp. and Douglas fir)	32
2. Hemlock (*Tsuga* spp.)	22
3. Hinoki (*Camaecyparis obtuse* Endl.)	6
4. Sugi (*Cryptomeria japonica* D. Don)	5
5. Lauan (*Shorea spp.* and other tropical hardwood species)	1

1) Data collected from multiple answers in 38 infested houses.

Table 7 Countermeasures against the infestation that were used in Kansai and Hokuriku areas[1)]

Control treatment	Number of infested houses
1. Spraying of insecticide	49
2. Replacement of damaged parts	4
3. Fumigation	2
4. Other (Painting)	1
5. No treatment	8

1) Data collected from multiple answers in 58 infested houses.

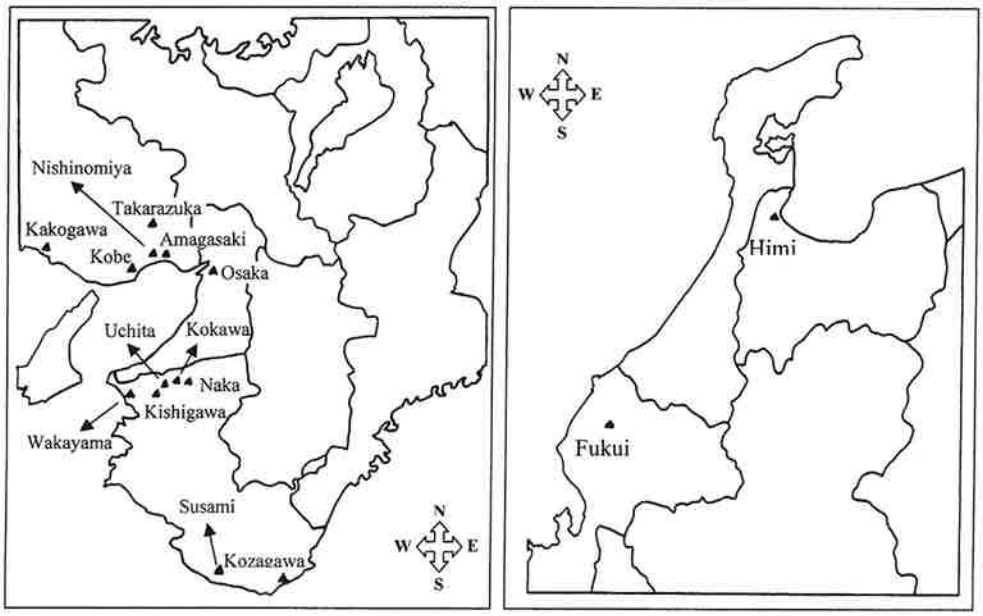

Fig. 1 Distribution map of *I. minor* in Kansai (left) and Hokuriku (right) areas.

obtained information on the infestation of *I. minor* in Fukui and Toyama Prefectures by this survey. From these results, although no infestation was found in Shiga, Kyoto, Nara, or Ishikawa Prefectures, we may conclude that the possibility of infestation by *I. minor* is much higher than previously estimated.

The highest infestation rate occurred in Kokawa, Wakayama Prefecture (20 houses), followed by those in Nishinomiya, Hyogo Prefecture (6 houses), Uchita, Wakayama Prefecture (6 houses), Kobe, Hyogo Prefecture (5 houses), Susami, Wakayama Prefecture (4 houses), and Osaka, Osaka Prefecture (3 houses), respectively. The main source of multiple infestations in a specific area seems to be natural spread via alate flight. As Morimoto (2004) stated, some alates of *I. minor* could fly distances 800m-1600m. On the other hand, the introduction of infestation to other areas appears to be caused by transportation of infested wood products by human

activity.

The present survey clearly indicated that the highest rate (83.3%) of infestation was found in wooden houses over 5 years after construction. This may reflect the facts that older houses have a lower moisture content than that of new houses, and that early detection of *I. minor* infestation is difficult. Roofing materials such as rafters, trim, and boards, and interior materials such as window frames, door frames, and pillars, were the most frequently attacked parts of the houses infested by *I. minor*. These results were very consistent with *I. minor* infestations in California reported by Harvey (1934 a), who stated that the original colonizing pairs of termites entered from the roof between the shingles and the sheathing. Another infestation in Louisiana found particularly in window frames was reported by Messenger and Scheffrahn (2002). These results strongly indicate that those parts of a house should be carefully inspected and provided adequate treatment. This type of infestation is unlike those resulting from subterranean termite attacks, which mostly occur in floors, joists, beams, and sills (Sornnuwat, Y. *et al.*, 1996), structural lumbers (Scheffrahn *et al.*, 1988), and sub-floors (Mankowski and Morrell, 2000). The habitat of *I. minor*, infesting wood with moisture content of less than 12% (Western exterminator company, 2004), may have a close relationship to this attacking manner.

Worker (nymph) castes were the caste most commonly found in infested houses, followed by soldier and alate castes. This is understandable, given that the nymph population represents the majority of a colony. Five-year colonies of *I. minor* typically consist of a primary king and queen, 20 soldiers, and 500 nymphs. In 10-year colonies, one or more supplementary reproductives are present in addition to the primaries plus 70 soldiers and 1600 nymphs. In 15-year colonies, there is one or more supplementary reproductives plus 120 soldiers and 2600 nymphs (Harvey, 1934 b).

The majority of infestations (55 houses: 94.8%) were identified by detection of both fecal pellets and attacked timbers. Based on the results of this survey, we can say that the presence of an infestation is mainly detected by accumulations of fecal pellets under the infested part. Currently, non-visual methods of detection of dry-wood termites such as acoustic emission monitoring, sound amplifier, metabolic gas detection, and canine olfaction (Su and Scheffrahn, 2000), and microwave detection (Mizutani, 2003), can be employed.

Among woods, pine (*Pinus* spp.) and Hemlock (*Tsuga* spp.) were the most susceptible to *I. minor*. Because some companies did not know the scientific names of those timbers, "pine" timbers may include Douglas fir and *Pinus* spp. In addition, "hemlock" timbers include western hemlock from North America.

Regarding countermeasures taken against infestation, spraying with insecticide was the most common practice. This might be because this method is inexpensive compared to that of fumigation and replacement of damaged parts. The following other non-chemical control measures could be also employed, as Su and Scheffrahn (2000) stated:

a. Whole-structure treatments
   Non-chemical: heat
b. Compartmental treatments
   Non-chemical: heat, cold and liquid N_2
c. Local or spot treatments
   Non-chemical: -wood surface (electrocution and microwave)
   -wood injection (nematodes and fungi)

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Short Communication

Larvae of the coffee bean weevil, *Araecerus fasciculatus* (DeGeer) (Coleoptera: Anthribidae), feeding on pith of the giant ragweed in Osaka, Central Japan

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オオブタクサ茎の髄も摂食するワタミヒゲナガゾウムシ *Araecerus fasciculatus* (DeGeer) (Coleoptera: Anthribidae) の幼虫

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綿の種子，コーヒー豆，柑橘類などの汎世界的な害虫であるワタミヒゲナガゾウムシの幼虫が，オオブタクサの枯茎に穿孔しているのを，大阪市大和川河川敷において冬季に発見した．本種の幼虫はオオブタクサ茎の髄を摂食し，その中で蛹化，羽化し，脱出した．発見された幼虫のサイズと発育期間から推測すると，産卵はオオブタクサが生育を終えた秋から初冬に行われたと考えられた．オオブタクサの枯茎が本種の自然下での増殖場所として機能し，その結果として本種が柑橘類や貯蔵農作物に被害を与える可能性がある．

We observed that the larvae of coffee bean weevil *Araecerus fasciculatus* DeGeer (Coleoptera: Anthribidae), a cosmopolitan pest infesting various stored crops such as cottonseed, coffee bean and orange fruits, bored into dead stems of the giant ragweed *Ambrosia trifida* (Asteraceae) at the riverbank of the Yamato River, Osaka City, central Japan. The larvae fed on the pith of the giant ragweed, pupated and eclosed in the pith and exit from it. Judging from the size of the larvae that were found in the field and developmental period which has been reported so far, oviposition may have occurred from autumn to early winter when the giant ragweed ceased its growth. The giant ragweed may function as a breeding site for *A. fasciculatus* in natural setting, resulting in infestation to orange fruits and stored crops.

Key words: Coffee bean weevil, *Araecerus fasciculatus*, Field, Host plant

The coffee bean weevil, *Araecerus fasciculatus* DeGeer [=*A. coffeae* (Fabricius)] (Coleoptera: Anthribidae), is a well-known cosmopolitan pest infesting various stored crops such as cottonseed and coffee bean (e. g. El Sayed, 1940; Childers and Woodruff, 1980). Recently, orange fruits are frequently infested by *A. fasciculatus* (Childers, 1982a, b; Etoh *et al.*, 1996; Grout *et al.*, 2001). *Araecerus fasciculatus* originates from South and Southeast Asia (El Sayed, 1940). Although diverse plant materials have been recorded as hosts for *A. fasciculatus* in stored or agricultural conditions of the world (El Sayed, 1940; Childers, 1982a), hosts of *A. fasciculatus* have infrequently been recorded in natural setting. To regulate the populations of pest insects, hosts around farmlands and in natural setting should be known and managed properly. We found *A. fasciculatus* larvae feeding on stems of the giant ragweed, *Ambrosia trifida* L. (Asteraceae) at a riverbank of central Japan, so their feeding habit is reported in this short paper.

We found coleopteran larvae in dead stems of the giant ragweed (**Fig. 1a, b**) at the riverbank of the Yamato River (34° 35′ N, 135° 30′ E, 10 m a. s. l.),

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Osaka City, Osaka Prefecture, central Japan on 30 December 2003. The riverbank was located at an urban area. The stems (in total 2.5 m in length) infested by the larvae were cut off, placed in plastic bags and brought to the laboratory. The collected stems were split and the larvae were observed in the laboratory. Then, the larvae were found boring into pith of the stems (**Fig. 1c**). Thus, we split all the stems, the pith was enclosed in two 200-ml plastic cups, and the larvae were reared under laboratory conditions (ca. 15-20℃). As a consequence, the larvae pupated in the pith and thirteen adults of *A. fasciculatus* eclosed from early February to late March 2004.

The giant ragweed, originating from North America, is an invasive alien plant and negatively affects native vegetation in disturbed or early successional habitats of Japan (Washitani, 2002). The giant ragweed is an annual herb, grows up to 3 m until late autumn and overwinters as seeds (Shimizu *et al.*, 2001). Since *A. fasciculatus* larvae found in the stems were medium-sized (<3 mm in body length) and the developmental period for *A. fasciculatus* from egg to adult (ca. one month under 26.7℃, Vitelli *et al.*, 1976) seemed to prolong because of low winter temperature, oviposition by *A. fasciculatus* adults appeared to occur from autumn to early winter.

The main stem of the giant ragweed grows up to 40 mm in diameter and bears soft thick pith (Shimizu *et al.*, 2001; Yamazaki and Sugiura, *pers. obs.*). In order to inspect whether *A. fasciculatus* larvae feed on stems of other weeds, we split several stems of the tall goldenrod *Solidago altissima,* the hairy beggarticks *Bidens pilosa* and the Japanese mugwort *Artemisia indica* var. *maximowiczii* (Asteraceae). But, the stems of other weeds were not infested by *A. fasciculatus* larvae. Thick soft pith of the giant ragweed may be suitable for oviposition and subsequent larval feeding. Dead stems of the giant ragweed may serve as cradles for development

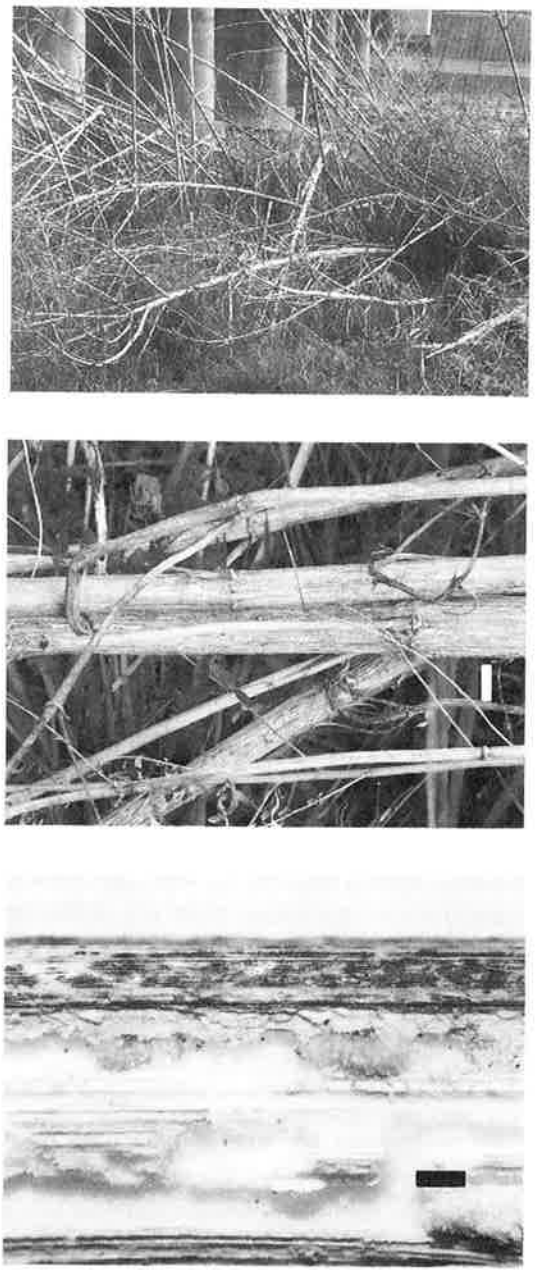

Fig. 1 (a) A senescent patch of the giant ragweed in which *A. fasciculatus* larvae were found, (b) Dead stems of the giant ragweed. White scale line : 10 mm, (c) Two *A. fasciculatus* larvae in a pith of the giant ragweed. Black scale line: 1.0 mm.

of *A. fasciculatus* larvae. Taking into account of recent injury by *A. fasciculatus* to orange fruits, it is recommended that the giant ragweed is removed in and around orange orchards.

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資　料

大阪府北部の住宅団地における チョウ類相

青柳　正人

㈱地域環境計画

Butterflies in urban housing complexes in Northern Osaka Prefecture. Masato Aoyagi (Chiiki-Kankyo-Keikaku Co., Takatsuki, Osaka 569-1123, Japan). *Jpn. J. Environ. Entomol. Zool.* **15**: 273－284 (2004)

Butterfly communities were studied in four urban housing complexes: Satsukigaoka-danchi (Satsukigaoka), Higashitoyonaka-daiichi-danchi (Higashitoyonaka), Asahigaoka-danchi (Asahigaoka), and Kori-danchi (Kori), in northern Osaka in 1998. The numbers of species and densities (km^{-1}) ranged from 16-25 and 14.1-24.6, respectively. The most abundant species was *Pseudozizeeria maha* in 3 sites, and *Pieris rapae* in Kori. Rebuilding of a housing complex led to the reduction of species and density. For example, densities in new building areas were 0.3-0.4 times those in old ones. This reduction is considered to be due to the loss of the grass around buildings.

Key words: Butterfly community, Urban environment, Rebuilding, Osaka

はじめに

住宅地におけるチョウ類群集を明らかにした例はきわめて少なく，吉田（1997, 1998）と青柳・吉尾（2002）の報告があるにすぎない．吉田（2002）は都市環境の指標にチョウ類群集を用いることに関して，生息密度が低い種の割合が相対的に高いため，単年度の調査結果に不安定さを与えていると問題点を指摘している．本研究ではそういった問題点を含みつつも，都市空間に生息するチョウ類相を明らかにし，都市化に強いチョウ類や都市化の進行にともなうチョウ類の分布や生息状況の変化などの貴重な記録を提供すると考える．

本研究は都市基盤整備公団による旭ヶ丘団地および東豊中第一団地建替えに関する調査設計の基礎調査として行ったものである．また本調査は団地におけるチョウ類相を明らかにするのが主たる目的であったため，特定のセンサスルートを設けずに行った．そのため，他の研究報告と厳密な比較を行うことはできないが，前述したようにデータを公表することに意義があると考える．

調査地および方法

1．調査地

調査は大阪府北部の池田市にある五月ヶ丘団地（以下，五月ヶ丘），豊中市にある東豊中第一団地（東豊中）および旭ヶ丘団地（旭ヶ丘），枚方市にある香里団地（香里）で行った（**図1**）．いずれの

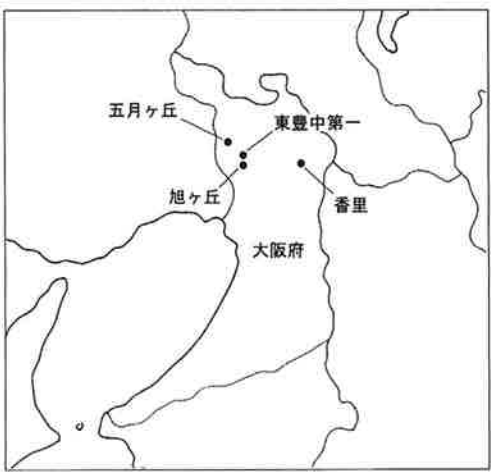

図1　調査地として設定した大阪府池田市五月ヶ丘団地，豊中市東豊中第一団地，同旭ヶ丘団地，枚方市香里団地の概略地点．

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団地も都市基盤整備公団（当時は日本住宅公団）により建設された団地である．東豊中以外の団地は一部で建替えが行われていた．

五月ヶ丘は1959年（昭和34年）に入居が始まった面積10.0haの団地である．調査時には老朽化した建物の建替えが行われており，約5.1haが建替え後の区画，あるいは建替え工事中であった．建替え前の区画における主な植栽樹木は住棟の南側でケヤキ，サツキ，ヒラドツツジ，住棟の北側でネズミモチ，サンゴジュ，ウバメガシで，主な草本植物はススキ，ヒメジョオン，セイタカワダチソウ，チガヤ，クズ，イタドリであった．建替え後の区画における主な植栽樹木はクロマツ，ソメイヨシノ，サザンカ，コクチナシ，シャリンバイ，シモツケ，ユキヤナギで，主な草本植物はカバープランツとして植栽されていたリュウノヒゲ，シバであった．

東豊中は1960年（昭和35年）に入居が始まった面積14.0haの団地で，2階建ての低層住宅と4～5階建ての中層住宅からなっていた．調査時には建替え工事は行われていなかった．主な植栽樹木はメタセコイア，コナラ，クロマツ，ハリエンジュ，アラカシ，ピラカンサ，ツツジ類，カイヅカイブキ，ユキヤナギで，主な草本植物はヨモギ，エノコログサ，スズメノテッポウ，アレチヌスビトハギ，セイタカアワダチソウであった．

旭ヶ丘は1959年（昭和34年）に建設された15.2haの広さの団地である．調査時には老朽化した建物の建替えが行われており，約8.1haが建替え後の区画，あるいは建替え工事中であった．建替え前の区画では5階建ての中層住宅と2階建ての低層住宅からなり，建替え後の区画は主として8階建ての高層住宅となっていた．建替え前の区画では主な植栽樹木がケヤキ，ネズミモチ，ハリエンジュ，クロマツ，ヒラドツツジ，トベラで，主な草本植物はヒメジョオン，クズ，シバ類，チガヤ，ニワゼキショウ，シマスズメノヒエ，ススキ，アレチヌスビトハギなどであった．また住棟付近では住民による草花の植栽も盛んであった．建替え後の区画では主な植栽樹木がケヤキ，クスノキ，ムクゲ，サルスベリ，カナメモチ，シラカシ，ユキヤナギ，ツツジ類，アベリアなどであった．一方，住棟間は駐車場で占められていることが多く，草地の面積が建替え前の区画に比べて狭かった．そのためか草本植物はシバ類やカタバミの他は少なく，また住民による草花の植栽はほとんどみられなかった．

香里は1958年（昭和33年）に入居が始まった面積155haの大規模団地である．調査時には老朽化した建物の建替えが行われており，約9.3haが建替え後の区画，あるいは建替え工事中であった．建替え前の区画の主な植栽樹木はトウカエデ，クロマツ，ケヤキ，トウネズミモチ，ヒラドツツジで，主な草本植物はエノコログサ，メリケンカルカヤ，ススキ，クズ，セイタカアワダチソウ，ヒメジョオン，ヘクソカズラであった．建替え後の区画の主な植栽樹木はアラカシ，イロハモミジ，コナラ，アメリカハナミズキ，クロマツ，アベリア，ヤマブキであり，主な草本植物はススキ，ヨモギ，カバープランツとして植栽されていたシバやコグマザサであった．

2．方法

調査は1999年の5月から10月にかけて，特定のルートを決めず，しかし踏査したルートが重複しないように調査地全域にわたって歩いた．基本的に時速約2kmの速度で歩き，範囲を決めないで確認されたチョウ類は全て記録した．調査ルートが一定していないために，正確な比較を行うことはできないが，なるべく各団地間で出現チョウ類相を比較できるように，1km当たりの出現個体数に補正を行い，議論できるように努めた．以下，1kmあたりの出現個体数を生息密度とする．各団地における調査日毎の調査時間，気象条件，踏査距離を**附表1-1～1-4**に示した．

結果と考察

1．各調査地のチョウ類相

各団地においてルートセンサスで確認されたチョ

ウ類を**表1**に示した．

チョウ類の確認種数は五月ヶ丘で25種，東豊中で21種，旭ヶ丘で20種，香里で16種となった．生息密度は東豊中が24.6，五月ヶ丘が20.9，旭ヶ丘が18.6，香里が14.8であった．香里は種数および生息密度ともに調査地の中で最も低い値を示した．各調査地での調査日ごとの確認個体数を**附表2-1～2-4**に示した．

表1　大阪府北部の4つの調査地（池田市五月ヶ丘，豊中市東豊中，同旭ヶ丘，枚方市香里）で確認されたチョウ類の1kmあたりの目撃個体数（実数）

種　名		五月ヶ丘	東豊中	旭ヶ丘		香　里
				1999年	1998年[1)]	
アオスジアゲハ	*Graphium sarpedon* L.	0.61 (12)	0.87 (16)	1.34 (29)	0.87 (26)	0.47 (6)
ナミアゲハ	*Papilio xuthus* L.	2.76 (55)	3.20 (55)	1.83 (41)	1.03 (31)	1.27 (16)
キアゲハ	*P. machaon* L.		0.05 (1)			
クロアゲハ	*P. protenor* Cramer	0.38 (8)	0.05 (1)	0.14 (3)	0.03 (1)	0.09 (1)
ナガサキアゲハ	*P. memnon* L.	0.11 (2)				
カラスアゲハ	*P. bianor* Cramer	0.09 (2)	0.05 (1)			
モンキチョウ	*Colias erate* Esper	0.29 (6)	0.12 (2)	0.13 (3)	0.10 (3)	2.22 (25)
キチョウ	*Eurema hecabe* L.	1.86 (35)	3.35 (40)	1.56 (33)	1.70 (51)	3.19 (39)
モンシロチョウ	*Pieris rapae* L.	1.50 (29)	4.03 (66)	2.12 (44)	1.80 (54)	1.00 (12)
ウラギンシジミ	*Curetis acuta* Moore	0.22 (4)	0.06 (1)	0.32 (7)	0.30 (9)	0.08 (1)
ムラサキシジミ	*Narathura japonica* Murray	0.06 (1)	0.13 (2)		0.03 (1)	
トラフシジミ	*Rapala arata* Bremer				0.03 (1)	
ベニシジミ	*Lycaena phlaeas* L.	1.05 (21)	1.40 (25)	0.81 (18)	0.23 (7)	1.74 (20)
ウラナミシジミ	*Lampides boeticus* L.	0.51 (9)		0.71 (14)	0.63 (19)	
ヤマトシジミ	*Pseudozizeeria maha* Kollar	6.33 (117)	5.41 (88)	3.72 (76)	1.57 (47)	1.43 (18)
ツバメシジミ	*Everes argiades* Pallas	0.66 (12)	0.90 (15)	1.75 (35)	1.07 (32)	0.25 (3)
ルリシジミ	*Celastrina argiolus* L.	0.16 (3)		0.22 (5)	0.43 (13)	
テングチョウ	*Libithea celtis* Fuessly	0.05 (1)			0.03 (1)	
ツマグロヒョウモン	*Argyreus hyperbius* L.	1.98 (38)	2.34 (39)	1.49 (30)	0.47 (14)	1.02 (12)
コミスジ	*Neptis sappho* Pallas	0.06 (1)			0.27 (8)	
ホシミスジ	*N. pryeri* Butler	0.93 (19)	1.50 (24)	1.10 (25)	0.43 (13)	0.42 (5)
キタテハ	*Polygonia caureum* L.				0.03 (1)	
ヒメアカタテハ	*Cynthia cardui* L.	0.22 (4)	0.12 (2)	0.32 (6)	0.13 (4)	0.16 (2)
アカタテハ	*Vanessa indica* Herbst				0.10 (3)	
コムラサキ	*Apatura metis* Freyer	0.11 (2)		0.09 (2)		
ゴマダラチョウ	*Hestina japonica* C. et R. Felder	0.05 (1)		0.04 (1)		
ヒメウラナミジャノメ	*Ypthima argus* Butler			0.41 (9)	0.37 (11)	0.32 (4)
ヒメジャノメ	*Mycalesis gotama* Moore		0.26 (4)	0.13 (3)	0.03 (1)	
コノマチョウ属の一種	*Melanitis* sp.		0.06 (1)			
キマダラセセリ	*Potanthus flavum* Murray	0.06 (1)	0.06 (1)			
チャバネセセリ	*Pelopidas mathias* Fabricius	0.79 (14)	0.50 (8)	0.33 (7)	0.33 (10)	0.81 (10)
イチモンジセセリ	*Parnara guttata* Bremaer et Grey	0.12 (2)	0.12 (2)		0.27 (8)	0.31 (4)
	確認種数	25	21	20	26	16
	合　計	20.9 (399)	24.6 (394)	18.6 (391)	12.3 (370)	14.8 (179)

1）青柳・吉尾（2002）より．

各団地におけるチョウ類の季節消長を**図2**に示した．各団地に共通した傾向として5月から7月にかけて個体数が増加していき，8月にいったん個体数が落ち込み，9，10月に個体数を増やすという季節消長が確認された．

団地間で比較すると，香里では6月を除いて概ね個体数が少なかった．特に10月は草刈り直後であったこと，天候が曇りであったことなどが起因して，他の団地に比べて極端に少ない確認個体数になったことが考えられた．

東豊中では8月以降，生息密度が他の団地に比べて約10個体程度多かった．特に8月は他の団地でおしなべて10個体未満の低い生息密度に留まったのに対して，18.3個体と比較的高い生息密度が確認された．

旭ヶ丘では1998年に調査を行っているので，簡単な比較を行った（**表1**）．1999年は5種の減少が確認されたが，これは調査回数の少なさに起因していると考えられる．上位5種を構成するチョウ類はヤマトシジミ，モンシロチョウ，ナミアゲハ，ツバメシジミ，キチョウと共通していた．両年の共通種は18種で，1998年のみ確認された種は8種，1999年のみ確認された種は2種であった．

2．団地の建替えとチョウ類相

団地の建替えが行われている五月ヶ丘，旭ヶ丘，香里で建替え前の区画と建替え後の区画におけるチョウ類の種数と生息密度を比較した（**表2**）．ただし団地境界より外（公園・小学校など）で確認された個体はこの解析から除いた．

建替え前の区画と建替え後の区画でのそれぞれの延べ調査距離（km）は五月ヶ丘で12.18と6.55，旭ヶ丘で12.09と5.64であり，建替え前の区画における調査距離が長かったが，香里では5.95と6.42で，建替え後の区画の調査距離が長かった．

種数を比較すると，五月ヶ丘では建替え前の区画で23種のチョウ類が確認された．これは全確認種の92%にあたる．同様に旭ヶ丘では全体の86%にあたる18種，香里では全16種が建替え前の区画で確認された．一方，建替え後の区画の確認種数は，五月ヶ丘で全体の52%にあたる13種，旭ヶ丘で48%にあたる10種，香里で63%にあたる10種にとどまった．

生息密度に関しては，建替え前の区画は21.58～27.18であったのに対して，建替え後の区画では7.61～8.41で，建替え前の区画の方が多く，建替え後の区画のおよそ2.5～3倍に達した．

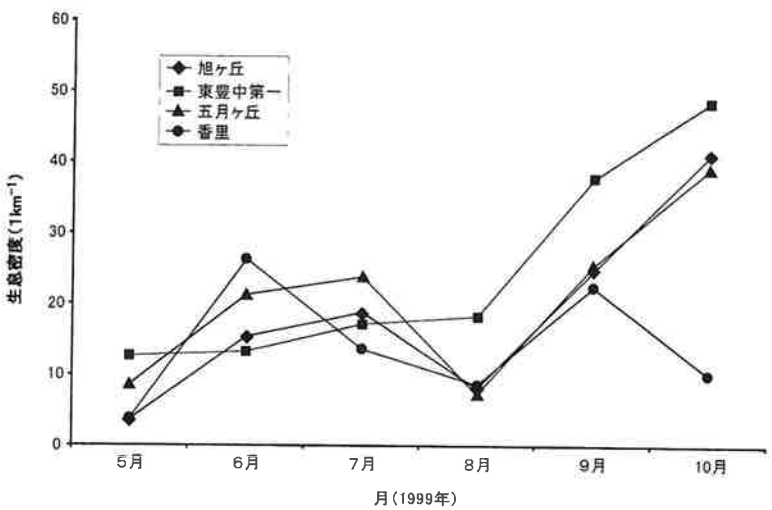

図2　1999年5～10月に各調査地において目撃されたチョウ類の生息密度の季節的変化．

表2　大阪府北部の3つの調査地（池田市五月ヶ丘，豊中市旭ヶ丘，枚方市香里）において建替え前，建替え後のそれぞれの区画におけるチョウ類の1kmあたりの目撃個体数（実数）

種名	五月ヶ丘		旭ヶ丘		香　里	
	建替前	建替後	建替前	建替後	建替前	建替後
アオスジアゲハ	0.73 (9)	0 (0)	1.17 (13)	0.60 (4)	0.82 (5)	0.13 (1)
ナミアゲハ	3.52 (44)	0.95 (6)	2.24 (27)	0.61 (4)	1.34 (8)	1.07 (7)
クロアゲハ	0.35 (5)	0.36 (2)	0.31 (3)	0 (0)	0.19 (1)	0 (0)
ナガサキアゲハ	0.18 (2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
カラスアゲハ	0 (0)	0.12 (1)	0 (0)	0 (0)	0 (0)	0 (0)
キチョウ	2.57 (30)	0.44 (3)	2.15 (24)	1.21 (7)	4.76 (28)	1.25 (8)
モンキチョウ	0.48 (6)	0 (0)	0.15 (2)	0 (0)	3.59 (19)	1.11 (7)
モンシロチョウ	2.10 (25)	0.27 (2)	2.14 (24)	0.52 (3)	1.07 (6)	0.97 (6)
ウラギンシジミ	0.27 (3)	0.17 (1)	0.41 (5)	0 (0)	0.16 (1)	0 (0)
ムラサキシジミ	0 (0)	0.17 (1)	0 (0)	0 (0)	0 (0)	0 (0)
ベニシジミ	1.51 (19)	0.35 (2)	0.42 (5)	0.50 (3)	2.77 (15)	0.82 (5)
ウラナミシジミ	0.81 (9)	0 (0)	1.00 (11)	0 (0)	0 (0)	0 (0)
ヤマトシジミ	7.82 (91)	4.12 (25)	4.47 (51)	2.41 (14)	2.14 (13)	0.81 (5)
ルリシジミ	0.18 (2)	0 (0)	0.37 (5)	0 (0)	0 (0)	0 (0)
ツバメシジミ	1.06 (12)	0 (0)	2.37 (27)	0.56 (2)	0.55 (3)	0 (0)
テングチョウ	0.08 (1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
ツマグロヒョウモン	2.94 (35)	0.15 (1)	1.46 (16)	0.49 (3)	2.14 (12)	0 (0)
コミスジ	0.09 (1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
ホシミスジ	0.73 (9)	0.13 (1)	1.27 (14)	1.36 (8)	0.55 (3)	0 (0)
ヒメアカタテハ	0.26 (3)	0.35 (2)	0.37 (4)	0 (0)	0.17 (1)	0.17 (1)
コムラサキ	0.09 (1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
ゴマダラチョウ	0.08 (1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
ヒメウラナミジャノメ	0 (0)	0 (0)	0.77 (9)	0 (0)	0.55 (4)	0 (0)
ヒメジャノメ	0 (0)	0 (0)	0.07 (1)	0 (0)	0 (0)	0 (0)
キマダラセセリ	0.09 (1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
チャバネセセリ	1.08 (12)	0.35 (2)	0.42 (5)	0.16 (1)	0.65 (4)	0.98 (6)
イチモンジセセリ	0.18 (2)	0 (0)	0 (0)	0 (0)	0.33 (2)	0.30 (2)
確認種数	23	13	18	10	16	10
生息密度（実数）	27.18 (323)	7.94 (49)	21.58 (246)	8.41 (49)	21.78 (125)	7.61 (48)
延べ調査距離（km）	12.18	6.55	12.09	5.64	5.95	6.42

＊団地境界より外で確認された個体は除外した．

このようにチョウ類は種数，生息密度ともに建替え前の区画に多く出現する傾向が認められ，すべてが建替えられると，チョウ類が減少することが予想された．既に建替えが進んでいる旭ヶ丘，五月ヶ丘，香里の生息密度が建替えを行っていない東豊中と比べて少なかったのは，建替えによる環境の変化のためと考えられた．

建替え前の区画に出現する傾向の強い種はウラナミシジミ，ツバメシジミ，ヒメウラナミジャノメであった．ウラナミシジミは移動性が強いものの，明るく開けた空間を好むことから（福田ら，1984b），高層化により日陰が多くなった建替え後の区画には出現しにくいと予想される．建替え前の区画の住棟周囲には，ツバメシジミが食餌植物とするコマツナギやシロツメクサが生育するような草地があったが，建替え後の区画では草地が駐

車場に置き換わり，コマツナギやシロツメクサがほとんどなくなったため，建替え後の区画では極めて少ないと考えられた．またヒメウラナミジャノメは草地を好み，イネ科およびカヤツリグサ科草本植物を食餌植物とする（福田ら，1984c）．前2種と同様に建替え後の区画における草地環境の少なさが本種の生息空間に適さない環境となったと考えられた．これら3種は建替えの影響を最も受けたチョウ類といえよう．

一方，建替え後の区画に各団地で共通して出現した種はヤマトシジミであった．ヤマトシジミは「人為的環境に最もよく適応した種の一つ」（福田ら，1984b）と言われ，また食餌植物とするカタバミは比較的乾燥した場所やコンクリートの割れ目にも生育できる．このような性質から建替え後の区画に優占する種となったと考えられる．

ヤマトシジミ以外は団地ごとに異なっていた．五月ヶ丘では生息密度が0.95であるナミアゲハがヤマトシジミについで建替え後の区画に出現した．旭ヶ丘では生息密度が1.00以上であったキチョウとホシミスジ，香里では生息密度が1.00以上であったキチョウ，モンキチョウ，ナミアゲハ，同じく0.98であったチャバネセセリ，0.97であったモンシロチョウが建替え後の区画に多く出現していた．

建替えという環境変化は，チョウ類の種数および生息個体数の減少をもたらすことが明らかになった．これは建替えにより建物が高層化し日陰が増えたこと，住棟の間の草地が駐車場に置き換わったことなどが影響していると考えられた．

3．都市化に強いチョウ類

各団地における優占種上位10種を**表3**に示した．4団地のうち，香里を除いた3団地でヤマトシジミが最優占種であった．またヤマトシジミ，ナミアゲハ，キチョウの3種はすべての団地において優占上位5種に入っており，これら3種は住宅団地で最も普通なチョウ類と言える．さらにすべての団地で優占上位10種に入っているチョウ類がモンシロチョウ，ツマグロヒョウモン，ベニシジミ，アオスジアゲハ，ホシミスジである．これら5種に先の3種を加えた8種が大阪府北部の団地で普通に見られるチョウ類であると考えられる．

前述の団地で普通に見られるチョウは都市適応種，すなわち都市化に強いチョウ類と考えられる．これら8種のうち，カタバミを食草とするヤマトシジミとギシギシなどのスイバ属を食草とするベニシジミ以外は，すべて人為的に植栽される植物を利用することのできる種である．またヤマトシジミの利用するカタバミは，前述のようにコンクリートの割れ目にも生育できる都市環境にも強い草本植物である．このように都市環境に適応できたチョウ類の特徴は人為的な植栽，あるいは都市化に強い植物を利用することができた点にあると考えられる．

香里で上位に入っていたモンキチョウは，シロツメクサなどのマメ科草本を食草としており（福田ら，1984a），一般的に極めて普通の種であるが，香里以外の調査地では目撃数が少なかった．モンキチョウが多いことが香里のチョウ類相を特徴づけているが，特にマメ科草本植物が多いと言うこともないので，モンキチョウが他の団地より多い理由は明らかではない．モンキチョウは特定の団地でのみ多かったことから，都市化に強いチョウ類に含めることはできない．

吉田（1999）は関西における都市適応種として，モンシロチョウ，ヤマトシジミ，ナミアゲハ，ツマグロヒョウモン，キチョウ，アオスジアゲハ，イチモンジセセリの7種をあげている．先の8種の大阪北部の団地で普通に見られるチョウ類と比べると，ベニシジミとホシミスジが含まれずに，イチモンジセセリが加わっている点が異なっている．ベニシジミはおそらく団地内の草地に生育するギシギシなどの寄主植物を利用できるために生息していると考えられる．都市化が進むと，ギシギシなどが生育するような草地が失われていくので，ベニシジミの場合は条件付きの「都市適応種」と見るべきであろう．ホシミスジはおそらくユキヤナギやシモツケなどの寄主植物が団地に多く植

表3　大阪府北部の4つの調査地（池田市五月ヶ丘，豊中市東豊中，同旭ヶ丘，枚方市香里）における優占チョウ類上位10種の1kmあたりの出現個体数（実数）

順位	五月ヶ丘	東豊中	旭ヶ丘	香　里
1	ヤマトシジミ 6.33 (117)	ヤマトシジミ 5.41 (88)	ヤマトシジミ 3.72 (76)	キチョウ 3.21 (39)
2	ナミアゲハ 2.76 (55)	モンシロチョウ 4.03 (66)	モンシロチョウ 2.17 (45)	モンキチョウ 2.13 (25)
3	ツマグロヒョウモン 1.98 (38)	キチョウ 3.35 (40)	ナミアゲハ 1.83 (41)	ベニシジミ 1.48 (17)
4	キチョウ 1.86 (35)	ナミアゲハ 3.20 (55)	ツバメシジミ 1.81 (36)	ヤマトシジミ 1.27 (16)
5	モンシロチョウ 1.50 (35)	ツマグロヒョウモン 2.34 (39)	キチョウ 1.56 (33)	ナミアゲハ 1.11 (14)
6	ベニシジミ 1.05 (21)	ホシミスジ 1.50 (24)	ツマグロヒョウモン 1.49 (30)	ツマグロヒョウモン 1.02 (12)
7	ホシミスジ 0.93 (19)	ベニシジミ 1.40 (25)	アオスジアゲハ 1.34 (29)	モンシロチョウ 1.00 (12)
8	チャバネセセリ 0.79 (14)	ツバメシジミ 0.90 (15)	ホシミスジ 1.10 (25)	チャバネセセリ 0.81 (10)
9	ツバメシジミ 0.66 (12)	アオスジアゲハ 0.87 (16)	ベニシジミ 0.81 (18)	アオスジアゲハ 0.47 (6)
10	アオスジアゲハ 0.61 (12)	チャバネセセリ 0.50 (8)	ウラナミシジミ 0.71 (14)	ホシミスジ 0.42 (5)

栽されていることと深い関係があると考えられる．イチモンジセセリが団地で上位種に入らない理由は明らかではない．イチモンジセセリは移動性が強いとは言え，主たる生息場所は水田であるから（福田ら，1984c），都市における個体数の多少は供給源である水田の有無や水田面積の大小などが左右すると考えられる．

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附表 1-1　1999年 5 ～10月に大阪府池田市五月ヶ丘団地においてチョウ類ラインセンサスを行った調査日，調査時間，気象条件，および調査距離

調査日	調査時間	天　候	風	気温（℃）	調査距離（km）
5月 14 日	10：05-12：00	晴	弱	26.0-28.2	3.84
6月　8日	9：48-12：10	晴	微-中	24.5-28.3	3.48
7月　8日	10：02-12：13	晴	弱-中	27.5-29.6	3.36
8月 20 日	13：29-14：25	晴	弱-中	32.4-31.2	2.86
9月 14 日	9：39-11：34	晴	微-中	28.3-31.3	2.95
10月 12 日	9：32-11：49	晴／曇	無-弱	26.8-27.8	2.97

附表 1-2　1999年 5 ～10月に大阪府豊中市東豊中第一団地においてチョウ類ラインセンサスを行った調査日，調査時間，気象条件，および調査距離

調査日	調査時間	天　候	風	気温（℃）	調査距離（km）
5月 26 日	10：15-11：20	曇	弱	22.9-22.6	2.45
6月 14 日	9：52-11：33	晴	弱-中	-	3.17
7月　9日	10：00-11：44	晴	無-弱	26.5-29.5	3.21
8月 17 日	9：58-11：40	晴／曇	微-弱	30.5-32.2	3.01
9月 21 日	10：22-12：12	曇	無-弱	29.0-29.0	2.63
10月 13 日	9：45-12：44	曇	微-弱	25.3-28.7	2.61

附表 1-3　1999年 5 ～10月に大阪府豊中市旭ヶ丘団地においてチョウ類ラインセンサスを行った調査日，調査時間，気象条件，および調査距離

調査日	調査時間	天　候	風	気温（℃）	調査距離（km）
5月 25 日	10：35-12：01	晴／曇	弱-中	23.6-25.0	4.11
6月　9日	10：08-12：21	晴	無-弱	25.6-28.1	3.91
7月　7日	9：59-12：06	晴	微-弱	28.3-30.5	3.46
8月 13 日	9：54-11：44	晴／曇	微-弱	31.1-31.5	3.69
9月 13 日	9：37-12：17	曇／晴	無-弱	28.2-29.6	3.85
10月 14 日	9：57-12：36	曇	無-中	24.4-26.0	3.10

附表 1-4　1999年 5 ～10月に大阪府枚方市香里団地においてチョウ類ラインセンサスを行った調査日，調査時間，気象条件，および調査距離

調査日	調査時間	天　候	風	気温（℃）	調査距離（km）
5月 21 日	13：00-14：23	晴	無-弱	30.7-28.7	2.39
6月 10 日	10：00-11：56	晴／曇	微-中	26.5-27.7	1.94
7月　6日	10：00-11：46	晴	微-弱	25.3-27.0	1.84
8月 12 日	10：28-12：32	曇／晴	微-弱	31.1-31.6	2.08
9月 10 日	9：38-11：16	晴	無-微	29.2-30.0	2.14
10月 15 日	11：32-12：47	曇	微-弱	24.1-22.9	1.99

附表 2-1　1999年に大阪府池田市五月ヶ丘団地において目撃されたチョウ類の個体数

種　名	5月14日	6月8日	7月7日	8月20日	9月14日	10月12日	合計
アオスジアゲハ	2	2	4	2	1	1	12
ナミアゲハ	12	16	10	8	7	2	55
クロアゲハ	5		1	1	1		8
ナガサキアゲハ					1	1	2
カラスアゲハ	2						2
キチョウ		13	1	1	11	9	35
モンキチョウ		6					6
モンシロチョウ	1	12	3		3	10	29
ウラギンシジミ						4	4
ムラサキシジミ						1	1
ベニシジミ	4	3	8	1	1	4	21
ウラナミシジミ					1	8	9
ヤマトシジミ	5		31	4	37	40	117
ルリシジミ			1		2		3
ツバメシジミ		2		1		9	12
テングチョウ		1					1
ツマグロヒョウモン	2	5	14	1	5	11	38
コミスジ					1		1
ホシミスジ		14	4		1		19
ヒメアカタテハ			1		1	2	4
コムラサキ			1		1		2
ゴマダラチョウ			1				1
キマダラセセリ						1	1
チャバネセセリ					1	13	14
イチモンジセセリ				2			2
種　数	8	10	10	8	13	12	25
個体数	33	74	80	21	75	116	399

附表 2-2　1999年に大阪府豊中市東豊中第一団地において目撃されたチョウ類の個体数

種　名	5月26日	6月14日	7月9日	8月17日	9月21日	10月13日	合計
アオスジアゲハ			9	5	2		16
ナミアゲハ	6	14	9	7	17	2	55
キアゲハ		1					1
クロアゲハ			1				1
カラスアゲハ			1				1
キチョウ	3	5	2	7	14	9	40
モンキチョウ		1			1		2
モンシロチョウ	7	5	11	3	16	24	66
ウラギンシジミ					1		1
ムラサキシジミ						2	2
ベニシジミ		9	5	4	3	4	25
ヤマトシジミ		1	7	13	24	43	88
ツバメシジミ		1		5	3	6	15
ツマグロヒョウモン		1	7	6	12	13	39
ホシミスジ	13	3	3	2	2	1	24
ヒメアカタテハ		1				1	2
ヒメジャノメ	2				1	1	4
コノマチョウ属の一種						1	1
キマダラセセリ				1			1
チャバネセセリ				1	2	5	8
イチモンジセセリ				1	1		2
種　数	5	11	10	12	14	13	21
個体数	31	42	55	55	99	112	394

附表 2-3　1999年に大阪府豊中市旭ヶ丘団地において目撃されたチョウ類の個体数

種　名	5月25日	6月9日	7月7日	8月13日	9月13日	10月14日	合計
アオスジアゲハ	3	1	16	6	3		29
ナミアゲハ		11	10	5	15		41
クロアゲハ	1		2				3
キチョウ	3	2	8	1	10	9	33
モンキチョウ		2			1		3
モンシロチョウ	2	14	6		3	19	44
ウラギンシジミ					5	2	7
ベニシジミ		9	3	4	1	1	18
ウラナミシジミ				1	3	10	14
ヤマトシジミ		3	8	9	20	36	76
ルリシジミ					5		5
ツバメシジミ		3		3	7	22	35
ツマグロヒョウモン	1	1	5	1	6	16	30
ホシミスジ	5	13	5		1	1	25
ヒメアカタテハ			1			5	6
コムラサキ		1			1		2
ゴマダラチョウ					1		1
ヒメウラナミジャノメ			2		6	1	9
ヒメジャノメ					3		3
チャバネセセリ					4	3	7
種　数	7	11	11	8	18	12	20
個体数	15	60	66	30	95	125	391

附表 2-4　1999年に大阪府枚方市香里団地において目撃されたチョウ類の個体数

種　名	5月21日	6月10日	7月6日	8月12日	9月10日	10月15日	合計
アオスジアゲハ	2		1	3			6
ナミアゲハ	4	2	3	2	5		16
クロアゲハ			1				1
キチョウ	1	12	3	2	17	4	39
モンキチョウ		21	2	1	2		26
モンシロチョウ	1	4	1	2		4	12
ウラギンシジミ				1			1
ベニシジミ		7	9	2	1	1	20
ヤマトシジミ			1	2	13	2	18
ツバメシジミツ		2		1			3
マグロヒョウモン		1	5	1	3	2	12
ホシミスジ		2	1		1	1	5
ヒメアカタテハ					1	1	2
ヒメウラナミジャノメ	1	1	1		1		4
チャバネセセリ				1	4	5	10
イチモンジセセリ				1	3		4
種　数	5	9	11	12	11	8	16
個体数	9	52	28	19	51	20	179