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Termite Trail-Following Effects of *Houttuynia cordata* Extracts

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ドクダミ抽出物のシロアリ道しるべ活性　大村和香子[1)]・三枝道生[2)]・山本幸一[1)]・大平辰朗[1)]・加藤　厚[1)]（[1)]独立行政法人森林総合研究所，[2)]岡山県木材加工技術センター）

ミゾガシラシロアリ科（Rhinotermitidae）に属するシロアリではそれらの道しるべフェロモン（*Z, Z, E*）-3, 6, 8-dodecatrien-1-olと部分構造が一致している物質のあとをたどって歩くことが知られている．我々は，イエシロアリ（*Coptotermes formosanus*）がドクダミ（*Houttuynia cordata*）のメタノールおよび*n*-ヘキサン抽出物のあとをたどって歩くことを発見した．そこで化学分析と生物検定とを組み合わせることにより，ドクダミ抽出物の道しるべ活性物質を検索した．*n*-ヘキサン抽出物をシリカゲルカラムクロマトグラフィーにて酢酸エチル/*n*-ヘキサン系で順次極性を上げて分画した．得られた画分のうち5, 10, 15%酢酸エチル/*n*-ヘキサン画分が活性を示した．この各画分をGC-MS分析した結果，5％と10%酢酸エチル/*n*-ヘキサン画分に含まれる2-undecanoneと1-nonanolが活性を示すことが明らかとなった．

Rhinoterimitid termites trail-follow chemicals with structures the same in part as their trail pheromone, (*Z, Z, E*)-3, 6, 8-dodecatrien-1-ol. We found that the termites were attracted to and trail-followed the methanol and *n*-hexane extracts of a plant, *Houttuynia cordata* Thunb. Active compounds causing trail-following from *H. cordata* were investigated by chemical analysis and a bioassay with *Coptotermes formosanus* as the test termite. The *n*-hexane extracts were fractionated by silica-gel column chromatography with a mixture of *n*-hexane and ethyl acetate with polarity increasing in steps. Fractions that were 5％, 10%, and 15% ethyl acetate were active. GC-MS analysis of the 5％ and 10% fractions gave peaks corresponding to 2-undecanone and 1-nonanol, respectively, which compounds were the active ones.

Key words: Trail-following activity, Termite, *Houttuynia cordata, Coptotermes formosanus*

Introduction

Termites are likely to trail-follow chemicals, which is not endogenous. Becker (1966) reported that *Reticulitermes flavipes* workers follow traces drawn in a certain ballpoint pen ink, and identified the active compounds as diethylene glycol monomethyl and monobutyl ethers. Chen *et al.* (1997) found that 2-phenoxyethanol in ballpoint pen ink causes trail-following by *Coptotermes formosanus* Shiraki.

A perennial, *Houttuynia cordata* Thunb. (common name, chameleon plant) is widely grown in East Asia including Japan. The herb in the flower season is called "juuyaku" in Japanese, and is a folk medicine used as a diuretic and laxative, to treat gonorrhea, women's diseases, and so on. The diuretic components in the herb are potassium salt (Ohta, 1942) and flavone glycosides, quercitrin and isoquercitrin (Nakamura *et al.*, 1936; Kimura and Nishikawa, 1953). Components responsible for the characteristic odor of the herb are 2-undecanone, 3-ketodecanal, and methyl lauryl sulfide (Kameoka *et al.*, 1972).

We have found that Japanese cedarwood stakes treated with methanol extracts of *H. cordata* were selected for attack by subterranean termites, *R. speratus* in preference to untreated stakes. Both methanol extracts and *n*-hexane extracts caused trail-following by *R. speratus* and *C. formosanus*. We are interested in the relation between termite behaviors and the extracts, and try to elucidate trail-following activity of the *H. cordata* extracts in this study.

Materials and Methods

Plant material

All portions above the ground of *H. cordata* were collected in the precincts of the Forestry and Forest Products Research Institute, Tsukuba, Japan in June 1996 and 2000.

The plants were air-dried in the shade, and then extracted with methanol or *n*-hexane for 5-7 days. The extracts were concentrated under reduced pressure before being bioassayed or fractionated. The *n*-hexane extracts (1.36 g) were fractionated by silica-gel column chromatography (silica-gel, 230-400 mesh, Merck; column, i.d. 32 mm and length 450 mm) with a mixture of *n*-hexane and ethyl acetate (EtOAc) with successive increases in polarity (150 mL of the mixture in each run). The active fractions were analyzed by gas chromatography (GC) and GC-mass spectrometry (MS) for identification of the components.

General methods

GC was done on a Shimadzu GC-14B apparatus equipped with a flame ionization detector under the following conditions: column, CBP-1 (50 m × 0.25 mm i.d., Shimadzu); injection port temperature, 250 ℃; detector temperature, 350 ℃; and carrier gas, helium (velocity, 60 cm/s). The column temperature was programmed to stay at 60 ℃ for 1 min, rise from 60 ℃ to 315 ℃ at 20 ℃/min, and then to stay at 315 ℃ for 10 min.

Capillary GC-MS was done on a Hewlett Packard HP-6890 GC system connected with an HP-5973 MS spectrometer equipped with an ATD 400 (Perkin Elmer). Analytical conditions were as follows: column, HP-INNOWax (30 m × 0.25mm i.d.); injection port temperature, 250 ℃; detector temperature, 350 ℃; and carrier gas, helium. The column temperature was kept at 60 ℃ for 8 min, from 60 ℃ to 200 ℃ at 3 ℃/min and 200 ℃ to 220 ℃ at 4 ℃/min, and then kept at 220 ℃ for 5 min.

Termites

Externally undifferentiated mature larvae (workers) of *C. formosanus* were obtained from a laboratory colony that was originally collected in

Miyazaki Prefecture and maintained in the dark at 26 ± 2 ℃ and approximately 65% relative humidity for at least 15 years at the Forestry and Forest Products Research Institute.

Stake test

Sapwood of Japanese cedar (*Cryptomeria japonica* D. Don) was sawn to measure 20 (T) × 20 (R) × 200 (L) mm, and treated with a methanol extract of *H. cordata* to give 6.30% (w/w) dry weight of extracts per a stake. Five pairs of treated and five pairs of untreated stakes were buried in a soil tank at the institute, and kept for at least a month to test their biological resistance.

No-choice bioassay

An acryl pipe with a hard plaster bottom (diameter, 9 cm; height, 5 cm) was used as the test container. The bottom was covered with 10 g of sea sand (15-20 mesh; Junsei Chemical Co., Ltd. (Tokyo, Japan)), and moistened with 2 ml of deionized water. Paper discs (diameter, 25 mm; Whatman International Ltd., Maidstone, England) were impregnated with 40 μl of a methanol extract. The amount of extract retained was 6.30, 0.63 and 0.063% (w/w) (dry weight of the test solution per disc). The control discs were untreated. The discs were dried at 60 ℃ for 12 h and then in a desiccator under reduced pressure for 24 h. Two plastic saucers containing either a test disc or a control disc were placed symmetrically 12 mm away from the center of the test container, and 100 termites were introduced into the test container. After 3 days, the discs were taken out and dried as above and the weight loss by each disc was calculated. Three replications were done for each test compound. All bioassay procedures were at 26 ± 2 ℃ and approximately 65 % relative humidity.

Trail-following bioassay

The trail-following bioassay (**Fig. 1**) was done by the method of Ohmura *et al.* (1995). A 15-cm-long circle was drawn in pencil on clay-coated paper, and 2 μl of a sample solution made from an extract or fraction prepared as described above, was applied along the circle with a 5-μl micropipette. After evaporation of the solvent, a plastic ring (1.5 cm i.d. and 1 cm high) with two openings was placed on the penciled line so that the openings on the circle. A Petri-dish lid wrapped in red cellophane was placed over the test circle to cut off draughts and decrease the amount of light, after a worker termite was put inside the plastic ring. Ten workers were used in each of the different sample solutions, and five termites were tested in each circle. Two circles were drawn for a sample. The distance it walked along the circle was measured. When the mean walking distance of 10 termites was above 5 cm (1/3 of the circumference), the sample was regarded as having the activity. All bioassays were at 26 ± 2 ℃ and approximately 65 % relative humidity.

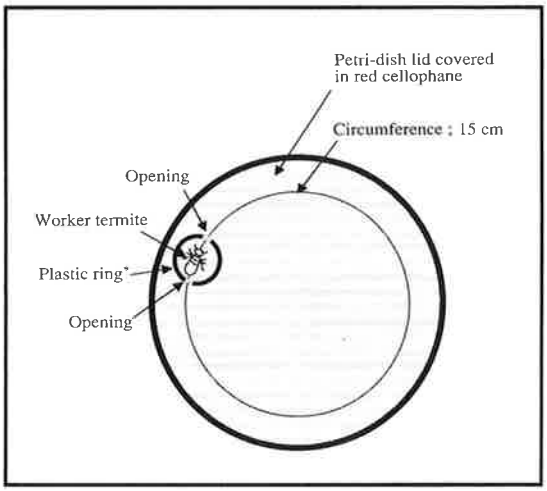


Fig. 1 Trail-following bioassay. * 1.5 cm i.d. and 1 cm high.

Results and Discussion

Feeding-preference

About one month after the wood stakes were

buried, *R. speratus* termites had invaded the soil tank through the ground, and attacked the stakes treated with methanol extract (**Fig. 2**). However, the weight losses of treated and untreated discs differed little (**Fig. 3**), so feeding-stimulant effects were unclear under no-choice conditions. Perhaps the attractants in the extracts evaporated or decomposed during heating of the disc at 60℃.

Trail-following effects

Both methanol and *n*-hexane extracts caused trail-following. Results of *n*-hexane extracts, three active fractions, 5%, 10% and 15% EtOAc, were active (**Fig. 4**); the 10% EtOAc fraction was most active (termites walked a mean of 11.0cm). The both 5% and 10% EtOAc fractions, when further examined, gave two major peaks in 5% EtOAc fraction by GC, and the retention time (t_R) of one of the peaks was same as that of 2-undecanone, t_R 13.3 min. Results of GC-MS showed a ratio of ion intensities of a peak with t_R of 21.9 min (**Fig. 5a**) due to the molecular ion (*m*/*z*; 170). The diagnostic fragment ions were nearly equal to those of 2-undecanone, previously found in *H. cordata* by Kameoka *et al* (1972). One of the active components of the 5% EtOAc fraction was therefore 2-undecanone (see **Fig. 6**).

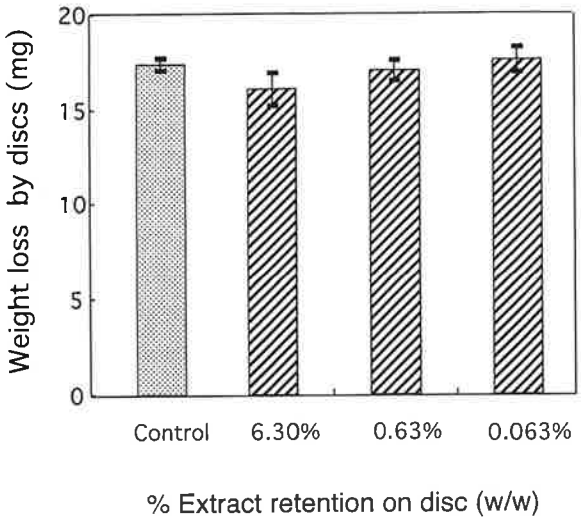


Fig. 3 Results of no-choice feeding bioassay.

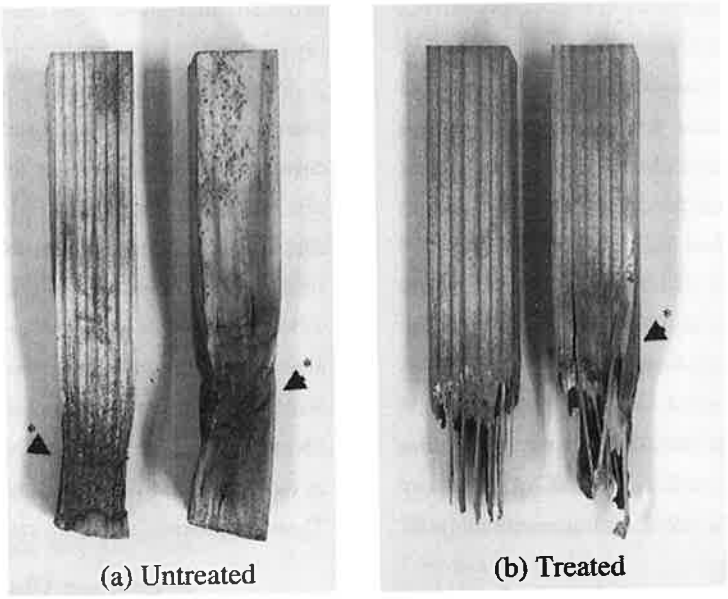


Fig. 2 Untreated wood stakes (a) and stakes treated with methanol extracts (b) of *Houttuynia cordata*. *Wood stakes shrank after being dried because of soft-rot.

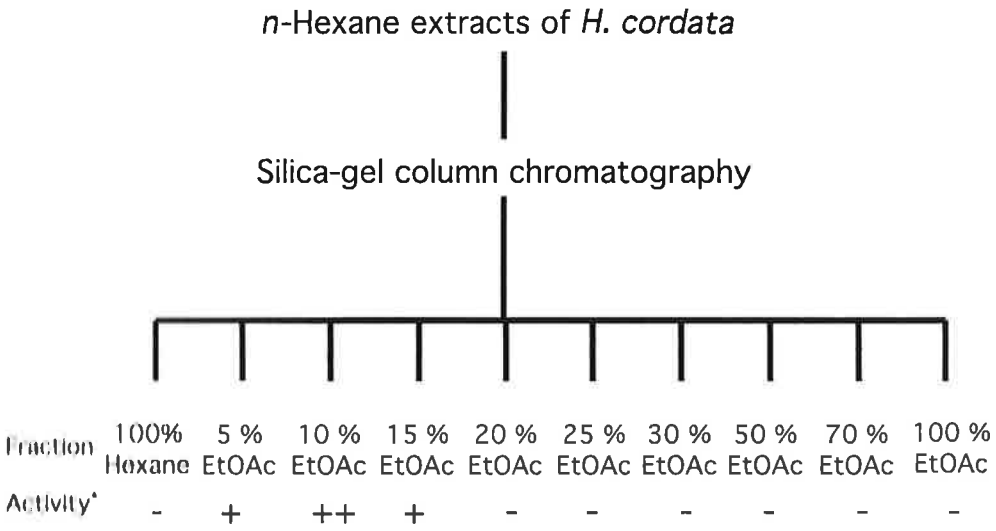


Fig. 4 Fractionation of *Houttuynia cordata* extracts. * ++ : mean walking distance ≥10cm, + : mean walking distance ≥5 cm, − : mean walking distance <5 cm.

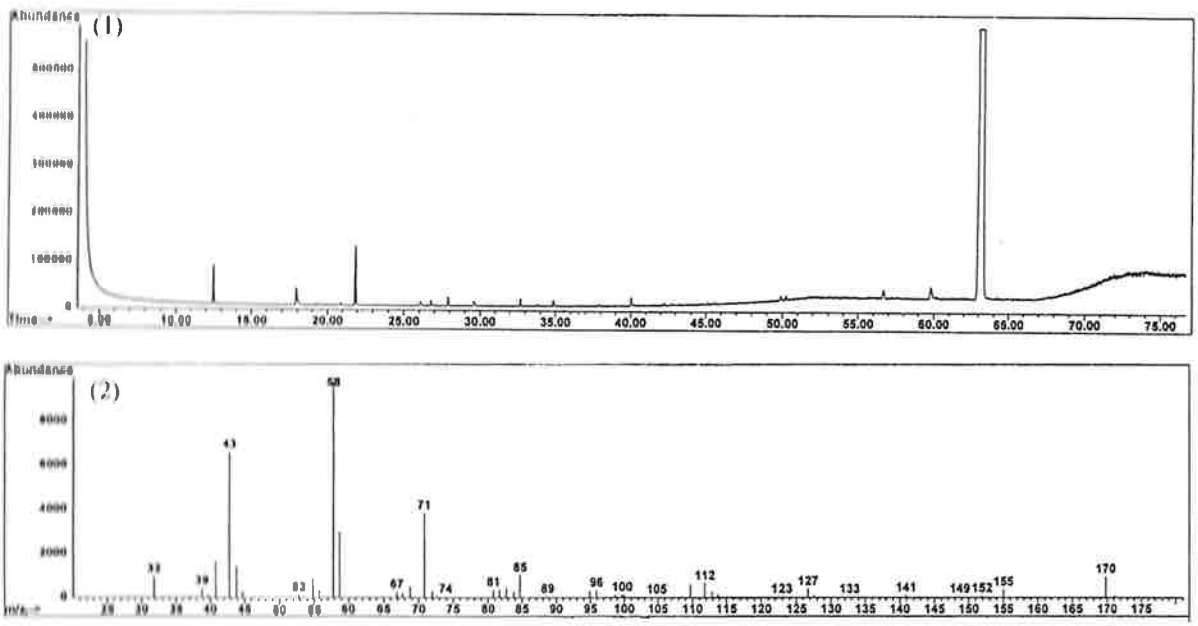


Fig. 5 a GC-MS of 5 % ethyl acetate fraction in *n*-hexane. (1) Total ion chromatogram. (2) MS spectrum at a retention time of 21.9 min (2-undecanone).

The bioassay with 2-undecanone at the concentration of 0.1 % showed trail-following effects. (*Z, Z, E*)-3, 6, 8-dodecatrien-1-ol (**Fig. 5 b**), which is a trail pheromone of *C. fomosanus*, had such effects even at the concentration of 10^{-6} % (Tai *et al.*, 1971). The effects of (*Z, Z, E*)-3, 6, 8-dodecatrien-1-ol were therefore as 10^5 times those of 2-undecanone.

GC-MS of the 10 % EtOAc fraction gave many

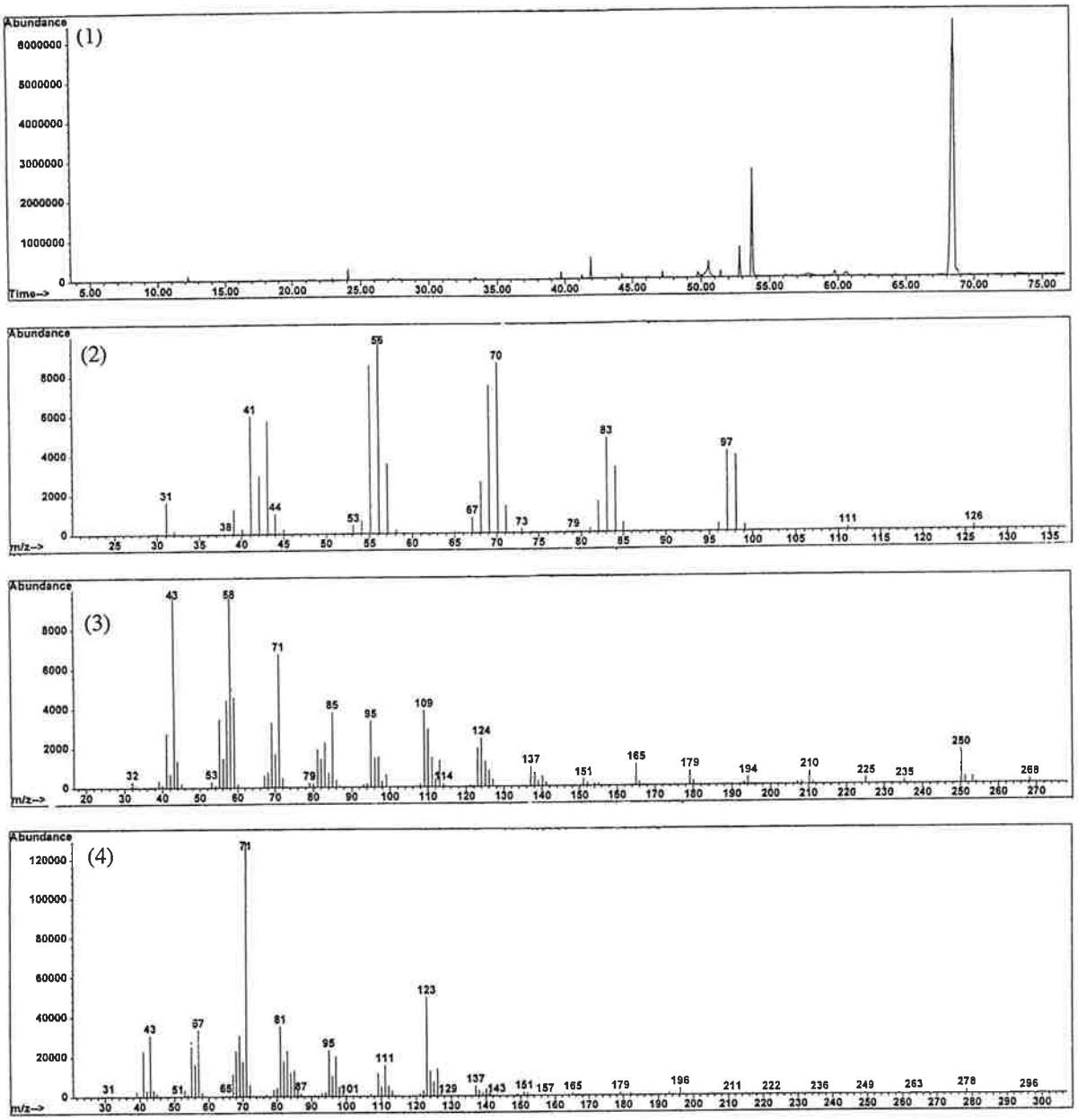
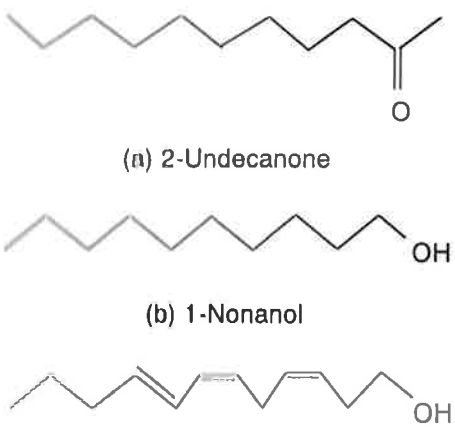


Fig. 5b GC-MS data of 10% ethyl acetate fraction in *n*-hexane. (1) Total ion chromatogram. (2) MS spectrum at a retention time of 24.1 min. (1-nonanol). (3) MS spectrum at a retention time of 39.7 min. (6, 10, 14-trimethylpentadecan-2-one). (4) MS spectrum at a retention time of 53.7 min. (Phytol).

peaks, and three were identified as 1-nonanol (t_R, 24.1 min), 6, 10, 14-trimethylpentadecan-2-one (t_R, 39.7 min), and phytol (t_R, 53.7 min), respectively (**Fig. 6**). 1-Nonanol had as much activity as high 2-undecanone, but the two other compounds were inactive in the concentration range of 0.01-10%.

Most of the peaks found in GC and GC-MS are still unidentified. The trail-following effects of *H. cordata* extracts were not high, but other plants may be found that are better semiochemical sources for termite control.



(a) 2-Undecanone

(b) 1-Nonanol

(c) (*Z.Z.E*)-3.6,8-Dodecatrien-1-ol

Fig. 6 Chemical structures of trail-following substances in *Houttuynia cordata* extract (a, b) and trail pheromone of *Coptotermes formosanus* (c).

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(1*Z*)-and (1*E*)-2-Nitroethenylbenzenes, and 2-Nitroethylbenzene as Natural Products in Defense Secretions of a Millipede *Thelodesmus armatus* Miyosi (Polydesmida : Pyrgodesmidae)

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ヒメヨロイヤスデ（オビヤスデ目，ハガヤスデ科）の防御分泌物から，(1*Z*)-2-及び(1*E*)-2-ニトロエテニルベンゼンと2-ニトロエチルベンゼンの同定　桑原保正[1)]・森直樹[1)]・佐久間正幸[1)]・田辺　力[2)]([1)]京都大学大学院農学研究科，[2)]徳島県立博物館)

ヒメヨロイヤスデ*Thelodesmus armatus* Miyoshiからヘキサンで抽出した防御分泌物の組成を，GC/MS分析で調べた．主成分として(1*E*)-2-ニトロエテニルベンゼンを，微量成分として(1*Z*)-2-ニトロエテニルベンゼンと2-ニトロエチルベンゼンを検出し，合成により同定した．本種は青酸ガスを発生させないオビヤスデ目として3番目の確認であり，天然物としての2-ニトロエテニルベンゼンの発見は，ウチカケヤスデ*Eucondylodesmus elegans Miyosi*についで2番目である．

The defense secretions of *Thelodesmus armatus* Miyosi, a mixture consisting of three components, (1*Z*)-2- and (1*E*)-2-nitroethenylbenzenes, and 2-nitroethylbenzene, were detected by GC/MS. (1*E*)-2-Nitroethenylbenzenes was the major component, while the other two were minor. Their chemical structures were identified by synthesis. It was the third example of the species that possessed non-cyanogenetic defense secretions among Polydesmida, and the second that emitted 2-nitroethenylbenzenes as natural products.

Key words : Millipede, *Thelodesmus armatus* Miyoshi, (1*Z*)-2-Nitroethenylbenzene, (1*E*)-2-Nitroethenylbenzene, 2-Nitroethylbenzene, Defense secretion

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Introduction

It is known that Polydesmida is as an order of cyanogenetic millipedes who produce benzaldehyde as a major component of the defense secretion together with hydrogen cyanide, that is, mandelonitrile (IV) emitter (Eisner *et al.*, 1978), and that IV is formed from L-phenylalanine (Duffey et al., 1974). Whereas two exceptional species have recently been found as summarized in **Fig. 1**. A mixture of (1*E*)-2- and (1*Z*)-2-nitroethenylbenzenes (I and II) has been identified as the defense secretion from *Eucondylodesmus elegans* Miyosi (Polydesmida; Doratodesmidae) (uchikake-yasude in Japanese), and these compounds are also evidenced to come from the same precursor *L*-phenylalanine as stated above (Kuwahara *et al.*, 2002). It is the first exceptional species among the order, who possesses no trace of cyanogens. Then, the Japanese endemic millipede *Niponia nodulosa* (Polydesmida: Cryptodesmidae) (makuragi-yasude in Japanese) has been realized to be the second one. The species is known as an emitter of earthy smells composed of a mixture of oct-1-en-3-ol and geosmin, that function as the alarm pheromone of the species (Ômura *et al.*, 2002). Origins of these volatile are unknown at present.

Based on these facts, it may be not always true to assume that Polydesmida is an order of cyanogenetic species, and several large species such as *Parafontaria laminata* (kisha-yasude in Japanese) group, Nedyopus venustus (tateobi-aka-yasude in Japanese), *Oxidus graculis* (yake-yasude) and *Chamberlinius hualienensis* (yannbaru-tosaka-yasude), migrate seasonally or periodically by outbreak to households causing severe public nuisances and to railroads stopping train services (Niijima and Arimura, 2002). To obtain more evidence on non-cyanogenetic species among Polydesmida, we have collected *Thelodesmus armatus* Miyosi (Polydesmida: Pyrgodesmidae) (himeyoroi-yasude in Japanese) in the organic soil at the backyard of Kyoto laboratory, and studied its defence secretion. The species is small and slim (body length about 5 mm) than *E. elegans* and its body is creamy white. It was collected together with *E. elegans* in the summer season. Its population around Kyoto laboratory seemed to be very scarce. We obtained only three individuals for analysis and also for species identification.

As the defensive secretion of *T. armatus*, a mixture of the nitro compounds as mentioned above was identified by GC/MS, together with a trace amount of the related compound.

Materials and Methods

Used millipedes

Three adults of *Thelodesmus armatus* Miyosi (Polydesmida: Pyrgodesmidae) were collected from organic soil in Kyoto University; intact one, freshly killed one with unknown reasons and damaged one during the collection. The intact one was dipped in n-hexane (20 μl) for 3 min after rearing for three days in the laboratory, and the hexane extract (2 μl) was subsequently analyzed by a gas chromatograph coupled with a mass spectrometer (hereafter abbreviated as GC/MS) as mentioned later. The other two ones were each extracted immediately after collection, and subjected for GC/MS analysis. The remaining body after removal of *n*-hexane layer was stored in 70% EtOH for species identification.

GC/MS analysis

GC/MS spectra were obtained by an HP-5989B Mass Spectrometer, operated at 70 eV, using an HP-5 capillary column (0.25 mm x 30 m, 0.25 μm in film thickness) as a split-less mode with He as carrier gas (1.23 ml/min) at a temperature programmed condition from 60 ℃ to 290 ℃ at a

rising rate of 10 ℃/min with an initial 2 min hold.

Preparation of (1*E*)-2- and (1*Z*)-2-nitroethenylbenzenes (I and II)

The compound (I and II as a mixture, purity more than 98%) was prepared from benzaldehyde and nitromethane with sodium hydroxide in aqueous methanol as described earlier (Worrall, 1948). The product was consisted of the mixture of I and II (*E*/*Z* ratio: 56/1) by GLC analysis, and gave the identical results to those of GC/MS and NMR as reported previously (Kuwahara *et al.*, 2002).

Catalytic hydrogenation of a mixture of (1*Z*)-2- and (1*E*)-2-nitroethenylbenzenes (I and II) to nitroethylbenzene (III)

Synthetic mixtures of I and II (10 mg) dissolved in EtOH (10 ml) was hydrogenated with a catalytic amount of PtO_2 under atmospheric pressure at room temperature. After one hour of the reaction, I and II was consumed and the reaction product gave a complex mixture by GC/MS analysis. The target compound III was detected in the mixture at t_R 10.347 min with the following mass spectrum; M^+ ion at m/z 151 (2%), 104 (100%), 91 (6%), 79 (24%), 77 (28%), 65 (5%) and 51 (14%).

Results and Discussion

Gas liquid chromatograms (obtained by GC/MS) of the hexane extract from three individuals were all composed of three peaks (A, B and C), though their contents and ratios were varied among specimens. The hexane extract from an intact specimen, supposedly at the most natural condition among three individuals, was composed of the following abundances; the peak C (t_R: 11.73 min, 91.82%) was the major component, and the other two peaks; A (t_R: 10.31 min, 0.06%) and B (t_R: 10.675 min, 8.12%), were minor, as shown in **Fig. 2**.

Mass spectrum of the major component (peak C) gave the molecular (M^+) and the base ions at m/z 149 (100%), together with the following diagnostic fragments; m/z 132 (21%), 119 (6%), 102 (64%), 91 (43%), 77 (81%), 65 (14%) and 51 (28%) (**Fig. 3**). The fact that M^+ was obtained as an odd number with high intensity, and diagnostic

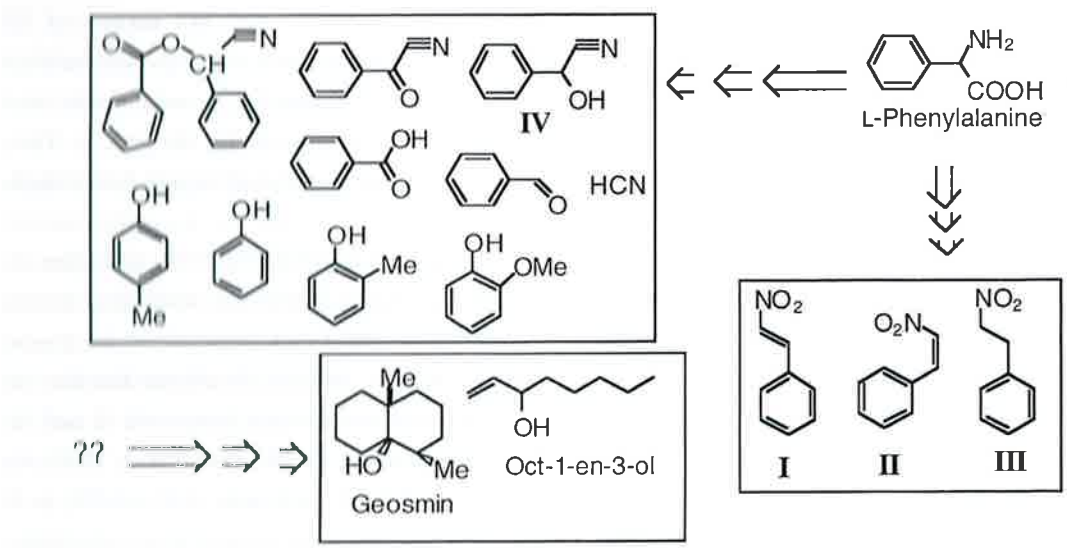

Fig. 1 Secretory compounds from polydesmid millipedes and biosynthesizing precursors of their major compounds.

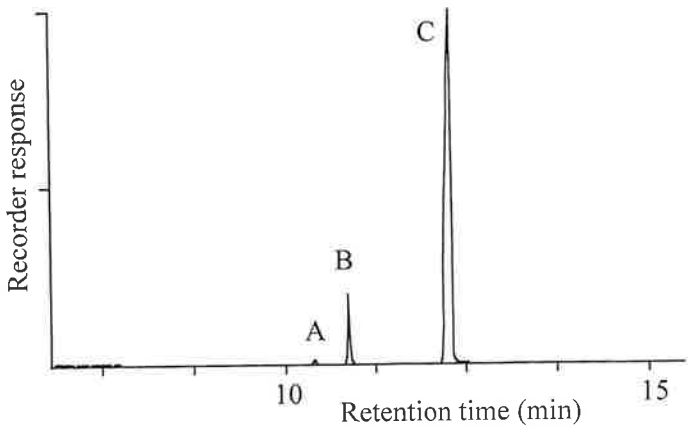

Fig. 2 A typical gas liquid chromatogram (monitored by GC/MS) of the hexane extract from *T. armatus*.

ions at m/z 91 and m/z 77, supported the structure to be a mono-substituted benzene containing odd number of nitrogen atoms. The GC t_R at 11.74 min (peak C) was identical to that (11.73 min) of the other white millipede *E. elegans* reported and also of synthesized standard of (1*E*)-2-nitroethenylbenzene (I, 11.74 min) (Kuwahara *et al.*, 2002). All of three mass spectra were also identical each other, and then, the compound in the peak C was identified as I.

The component of the peak B gave GC t_R at 10.68 min, and the mass spectrum indicated the M^+ ion at m/z 149 (36%), and the base ion at m/z 77 (100%), together with the following diagnostic ions; 132 (47%), 121 (14%), 102 (51%), 91 (59%), 65 (24%), and 51 (44%) (**Fig. 3**). All these results were identical to those of the minor components with GC t_R 10.73 min as reported (Kuwahara *et al.*, 2002), and also of the minor component of synthesized 2-nitroethenylbenzene (a mixture of I and II). Therefore, the structure of the peak B component was concluded to be II. Total content of compounds (I and II) was estimated to be less than 10 μg in individual millipede, based on their peak responses by GC/MS analysis.

The component of peak A indicated a M^+ ion at m/z 151 (2%) and the base ion at 104 (100%), together with following fragment ions; 117 (4%), 91 (7%), 79 (20%), 77 (26%), 65 (4%), 63 (4%), and 51 (9%) (**Fig. 3**). Both the molecular and the base ions appeared at each m/z 2 higher mass number than those of peaks B and C, as well as, the synthetic I and II mentioned above. These facts suggested the structure of the compound to be 2-nitroethylbenzene (III). GC/MS analyses of III prepared by hydrogenation of the synthetic mixture of I and II gave the same GC t_R and the same mass spectrum as those observed for the peak A. Then, the structure of the compound in peak A was identified as III.

No traces of mandelonitrile (IV) and other related compounds, commonly distributed among Polydesmida (**Fig. 1**), are detectable in the present species (**Fig. 2**). Because the defense function has been reported for the major compounds (I and II) against a species of ant (Kuwahara *et al.*, 2002), the present mixture of compounds (I, II and III) in *T. armatus* may also serve as the defense compounds.

A mixture of I and II (*Z*/*E* ratio: 1/157 for males and 1/154 for females) have been known from *E.*

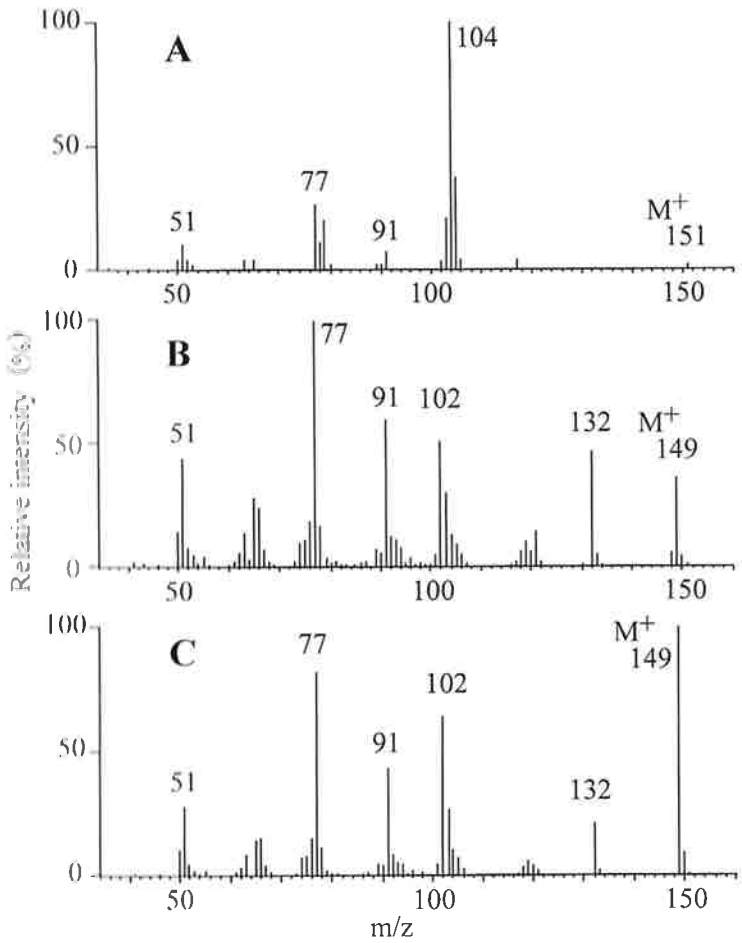

Fig. 3 Mass spectra of natural 2-nitroethylbenzene (A, III), (1*Z*)-2-nitroethenylbenzene (B, II) and (1*E*)-2-nitroethenylbenzene (C, I).

elegans, not only as the first identification of the defense compound but also as natural products (Kuwahara *et al.*, 2002). Accordingly, the present identification of I and II (*Z*/*E* ratio: 1/11) from *T. armatus* was the second example. The proportion of II in the mixture (I and II), based on a single analysis from an intact millipede, was larger than that of *E. elegans*, though further data accumulation should be required for discussing these discrepancy of the ratios between the two species.

The compound (III) has been described as a fragrant principle of *Dennettia tripetala* fruits (Okogun and Ekong, 1969), and as a components of essential oil, not only from fruits (up to 68.38%) (Ekundayo *et al.*, 1992), but also from leaves of the plant (up to 53.7%) (Adeoti *et al.*, 2000). The compound (III) is also known as a principle of cinnamon odor in leaves and bark of two South American Lauraceae *Aniba canelilla* and *Ocpotea pretiosa* (Naranjo *et al.*, 1981), and known as antifungal agents (Ajaiyeoba *et al.*, 1999). A high toxicity of III has been demonstrated to yeast especially *Candida albicans* (Oger *et al.*, 1994). Although III has been known in plants as stated above, it is the first identification not only

among millipedes but also among species in the animal kingdom.

The compounds (I, II and III) are each closely related derivatives as indicated in **Fig.** 1. It is no wonder to assume in the present species that all these compounds might be biosynthesized from *L*-phenylalanine, based on the following fact of *E. elegans* that α, β,2,3,4,5,6-*d*$_7$-(1*E*)-2-nitroethenylbenzene is detectable in the secretion after feeding with α, β, β,2,3,4,5,6-*d*8-*L*-phenylalanine by GC/MS analysis (Kuwahara *et al.*, 2002).

As a result, the third species *T. armatus* belonging to Polydesmida was found to possess three none cyanogenetic compounds (I, II and III) as its defence secretion. Two compounds (I and II) have been shared with another species *E. elegans* belonging to Doratodesmidae, therefore, these compounds may be distributed other than the present two families. The other cryptodesmid species *N. nodulosa* possesses two other compounds as mentioned. Then, we could assume that species belonging to these three families are non-cyanogenetic, corresponding to a total of 19 Japanese species (Pyrgodesmidae; 9 species, Doratodesmidae; 4 species, and Cryptodesmidae; 6 species). In Japan, about 300 millipede species belonging to 10 orders are distributed, among which 181 species belong to Polydesmida, the most diversified order of millipedes (Tanabe, 2001). About 10% of Polydesmida are, then, calculated as non-cyanogenetic and might be an interesting target to study, together with other orders such as Gromerida and Polyzoniida (Kuwahara, 1999). On the other hand, these non-cyanogenetic Polydesmida at present seems to be practically harmless to human, because of their small bodys sizes (less than 1 cm), and no records available of their outbreaks.

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Wandering Behavior as a Response to Crowding is Coupled with the Mechanism Controlling Development Rate in *Plodia interpunctella* (Lepidoptera: Phycitidae)

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ノシメマダラメイガの密度への反応としての這い出し行動は幼虫発育を制御する機構とカプリングする Muhammad Naeemullah, 竹田真木生（神戸大学自然科学研究科）

ノシメマダラメイガを発育の早いものと遅いものに選抜をかけ，2つの系統（FDLとSDL）をつくった．それらを100, 400, 1000個体/30g（米ぬか）の3つの密度区で飼育し，発育日数，幼虫サイズ，成虫サイズ，死亡率，這い出し率等を比較した．両系統とも，高密度区では羽化が遅れたが，その他の形質では2つの系統間で異なった効果が現れた．這い出し率は，FDLでは顕著に下ったが，SDLでは選抜は顕著な効果をもたらさなかった．生存率はFDLでは各密度区であまり変わらなかったが，SDLでは高密度区で下がった．幼虫サイズはFDLでは高密度区へ向かって小さくなったが，SDLではその減少はFDLほどではなかった．一方，成虫サイズについては，FDLは低密度区で最大となり，高密度で著しく減少したが，SDLでは高密度で小さくなるものの，その効果はFDLに比べ比較的少なかった．発育速度への選抜が，他の形質に影響を及ぼしたが，貯穀害虫である本種は広範な遺伝的多型を含んでいて，餌の枯渇，共食いなど個体群的な淘汰圧に対して，体サイズ（＝蔵卵数）の増加，発育速度の短縮，餌からの離脱による共食いからの逃避，共食いによる密度調節，休眠などさまざまな適応形質を調和的に調節しながら生息環境に適応し，繁殖率を高めるのであろう．

Behavioral and developmental responses to crowding were investigated in the two lines of *Plodia interpunctella* selected for fast (FDL) and slow (SDL) development rates. The two lines were reared at three density conditions, i. e., 100, 400 and 1000 eggs

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/30 g rice bran. The development time increased in both the lines as density level increased. However, the FDL developed significantly faster than the SDL under all the conditions examined. The SDL produced heavier larvae upon wandering than the FDL, especially at 400 eggs/30 g rice bran. The FDL produced heaviest adults under the least crowded condition of the three rearing densities examined while in the SDL reduction of weight at high densities was less apparent than in the FDL. Survival was higher in the SDL than in the FDL under less crowded conditions. The selection also affected the behavior of the larvae. Some larvae left rice bran when they reached maturity (wandering larvae) while the others stayed in the bran to spin cocoon (non-wandering larvae). Wandering was less prominent in the FDL than in the SDL but the selection for fast development further lowered the rate of wandering, although no selection was imposed on this particular trait. The selection for slow development, on the other hand, did not increase the rate of wandering. The present result showed that the mechanisms controlling developmental rate are somehow coupled with the mechanisms that control other developmental and behavioral traits, notably wandering behavior. Some pleiotropic or epigenetic interplay seems to help avoid a mass extinction upon starvation and cannibalism while maximize the reproductive rate under favorable conditions.

Key words: *Plodia interpunctella,* Artificial selection, Development rate, Crowding

Introduction

Density-dependent regulation of life cycles is common in insects (Uvarov, 1921; Utida, 1972). Population density influences insect life cycles in various ways, altering the wing morph, body size, developmental fate and rate, fecundity, behaviors, pigmentation and other life cycle parameters (Peters and Barbosa, 1977). In the housefly, *Musca domestica* L., an increase in larval density decreased the development rate and adult body weight and different strains differed in the duration of larval development under crowded conditions (Sullivan and Sokal, 1963). In *Ephestia kuehniella,* crowding decelerated the larval development and decreased the adult body weight (Smith, 1969). In the black carpet beetle, *Attagenus elongatus,* increased levels of larval crowding resulted in slow larval development and delayed pupation (Barak and Burkholder, 1977). In this species, selection for early emergence reduced the sensitivity to larval crowding in only six generations (Barak and Burkholder, 1977). Crowding delayed pupation also in *Tribolium freemani* (Nakakita, 1983; Kotaki and Fujii, 1995). Tsuji (1963) investigated the effect of crowding on diapause determination in *Plodia interpunctella,* and demonstrated that high larval density induced diapause in the last larval instar, though this diapause is averted at high temperatures. He has reported three different types of diapause induced by three different cues: short photoperiod, temperature fall and crowding. Each type has a distinctive sensitive stage to a specific cue. He also showed that inbreeding and continuous rearing of larvae under constant conditions in the laboratory resulted in modification of develop-

mental characteristics including a reduction in the rate of density-dependent diapause.

By selecting fast and slow developing individuals from a population of *P. interpunctella,* two selection lines were established in our laboratory at 25℃ under LD 16 : 8. Subsequent experiments using these selection lines revealed that artificial selection not only altered development rate and metabolic rate but also responses to photoperiod and temperature (Naeemullah and Takeda, 1998). The selection, however, had no or very little effect on the cold-hardiness (Naeemullah *et al.*, 1999).

This paper investigates the effect of artificial selection for the response to crowding. The regulation of development rate is a metabolic regulation, while behaviors are regulated by the nervous system. Ultimate goal for this study is to identify a pleiotropic mechanism and the consequence of selection to alter the steady-state point of canalized condition, if such exists (Schlichting and Pigliucci, 1998).

Materials and Methods

Procedure of selection

A stock culture of *P. interpunctella* was established on rice bran from approx. 200 adults of mixed age at 25℃ under LD 16 : 8 with 50% R. H. from a population that had naturally colonized on wheat bran. From this culture two selection lines were established; the FDL for fast development and SDL for slow development. The detailed procedure of selection has been published elsewhere (Naeemullah and Takeda, 1998). Briefly, adults that had emerged on the first day (day 34) were selected for the FDL and those which emerged at the tail of emergence period (day 62) were selected as the SDL. From these adults, 100 eggs were placed under controlled conditions on 30 g of diet; rice bran and glycerol mixed at 10 : 3 by weight. To ensure high survival, eggs laid for the second 24 hrs of adult life were used for experiments, since the majority of the first laid eggs were infertile (Sardesai, 1968). The same procedure was repeated in the following generations.

Effects of selection for fast and late development on developmental responses to crowding

Groups of 100, 400, and 1000 eggs from both the selection lines were established on 30 g diet in glass jars measuring 9 x 10 cm and reared at 25.6±0.5 ℃, under LD 16 : 8 and R. H. 62±2.8%. Under optimum rearing conditions, most larvae, when full grown, come out (wander) of the food and spin cocoons or remain as larvae under sheltered places or on the walls of container. Corrugated cardboard was introduced into the jars for pupation / hibernation sites. The jars were checked daily to count the larvae which entered the corrugated sheets or were wandering. These larvae were transferred to new jars. The number of adults both from the individuals that pupated in the corrugated sheets and those which remained in the food were counted and weighed within 24 hours after emergence, separately for males and females. The experiment was replicated thrice.

Effects of the selection on the weight of wandering larvae

Another set of glass jars containing 100, 400 and 1000 eggs in 30 g of diet was set up for both the selection lines. These jars were checked daily from the time the first larva started wandering until when no larvae were found on the food. Larvae were weighed in groups without checking the sex on the assumptions that the sex ratio should not be too biased and transferred to new jars.

Effects of the selection on the proportion of larvae wandering out of food

Eggs of generations 18 (FDL) and 19 (SDL), were incubated at densities of 100, 400 and 1000 as described above. At the wandering stage, larvae