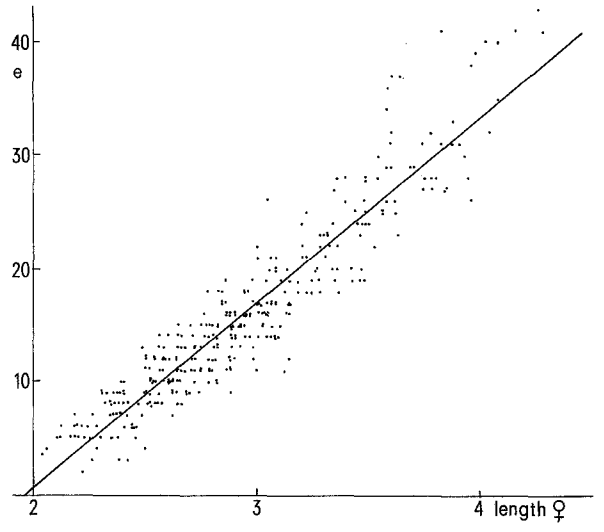

Number of eggs and rounded embryos in the marsupium of female *Jaera ischiosetosa* (e) as a function of body length (L, mm.). Results from the nine samples are pooled.

three sexual cycles in the lifetime of a female (several months). Precopulae are easily recorded: the male, usually smaller than the female, climbs onto her back for a period of some minutes to several hours; copulation does not necessarily occur, and sperm displacement is possible[15].

Results are shown in the table. There was no definite tendency for large females to enter into precopula, as mating females were larger than the mean from November to April and smaller in August. There was a slight but significant correlation in body length between males and females. The mean length of mating males was 25% smaller than the mean length of mating females (male/female mean length = 0.74 + 0.02); these are the best relationships in size for males to hold on to the back of females, and for them to position the genitalia in front of each other[16].

The ratio of females with oostegites was smaller in the pairs than in the control (pooled results: 28.7% + 1.7 in the pairs versus 44.2% + 1.6 in the control). This was partly a bias due to the first mating of females occurring before any cycle of oogenesis. When considering the only females larger than 2.4 mm, which all have reached maturity in every sample, the proportions are still 36.1% + 2.3 versus 65.9% + 1.7 respectively.

The fertility of females of similar size in the two groups may be easily compared, as the number of eggs in the control (fig.) is linearly related to body size (slope: 16.33 eggs/mm; intercept = −31.91 mm; correlation: 0.94; N: 332). This linearity is a possible consequence of the ovaries being two slender rows of maturing oocytes in line, end to end with the axis of the body. When the values of the mating females are plotted on the fertility curve of the control, they appear significantly more fertile than the average, i.e. most of them range above the regression line (100/68 individuals above/below the regression line, vs. 157/175 in the control; P(X2) = 0.01). Comparisons with the fertility curves computed from each seasonal sample yield the same result (table; pooled result: 108/60).

Assuming that non-random pairing in *Jaera ischiosetosa* is an adaptive outcome of sexual selection, the modalities of competition peculiar to each sex need to be considered. Competition between females depends on their rate of survival, on their generation time and on their fertility throughout adult life, while competition between males depends on the broods they contribute to. The reproductive success of a male will therefore depend on (a) the size of its mates, (b) the time elapsing between copulation and the fertilization of the eggs (hence biasing mating records towards non-ovigerous females), and (c) the fertility of its mates as compared to the average fertility (biasing mating records towards the most fertile ovigerous females); the optimal mate size is likely to vary seasonally.

Students of behavioral evolution have paid much attention to competition between members of the sex investing less in its progeny, usually through genetic material only, despite the fact that genetic variation in fitness is assumed to be very low in a natural population[17,18]. On the other hand, a wide variation in fitness is expected in the sex investing more. This variation may be purely environmental; a correlation in fitness exists, however, between individuals sharing a part of their offspring. Behavior could therefore evolve in one sex as a response to a non-genetical variation in the fecundity of the other sex: the sex investing less would evolve discrimination between members of the sex investing more. This would explain the non-random distribution of precopulae in female *Jaera*.

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Defensive alkaloid in blood of Mexican bean beetle *(Epilachna varivestis)*[1]

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Summary. The blood of the Mexican bean beetle *(Epilachna varivestis)* contains a homotropane alkaloid, euphococcinine (**1**). The beetles 'reflex bleed' when disturbed, thereby deploying the alkaloid, which is provenly deterrent to spiders and ants. Newly emerged adults lack the alkaloid, but the compound builds up to deterrent levels in their blood within days. Eggs and larvae of *Epilachna* are devoid of the compound.

Key words. Coleoptera; chemical defense; feeding deterrent; alkaloid; homotropane.

Mexican bean beetles *(Epilachna varivestis;* family Coccinellidae) are well protected against predation. When attacked, they 'reflex-bleed', emitting droplets of blood from the tibio-femoral joints of the legs (fig. 1). The fluid coagulates quickly on exposure to air, and may foil small predators such as ants by gumming up their mouthparts and appendages[3]. We here report that *Epilachna* blood is also chemically deterrent, by virtue of a homotropane alkaloid that it contains. The chemical, which we propose to call euphococcinine, builds up in concentration during the first days following adult emergence of the beetle, eventually achieving levels that are effectively protective against spiders and ants.

Initial tests (fig. 2, top chart) with jumping spiders *(Phidippus regius)* showed *Epilachna* to be fully edible on the first day of adult life. 1-day-old beetles (10 of each sex) that were offered singly to individually caged *Phidippus* were all promptly taken and eaten. Although they reflex-bled when seized, either from one or several legs, the fluid seemed to take no effect, and each beetle was reduced to a thoroughly sucked out, tightly compacted, particulate mass. 7-day-old beetles (10 of each sex; different set of spiders) fared differently. Only two were totally eaten; most were rejected uninjured, either immediately (< 5 sec; 9 beetles) or after short delay (5–120 sec; 4 beetles), while the remainder (5 beetles) were killed but only partly eaten (most of body left recognizably intact). Reflex bleeding was noted to occur in each case.

Extraction of whole *Epilachna* led to the isolation of an alkaloid, absent at the time of adult emergence, but present in older beetles. After several exploratory fractionations, the following isolation scheme was adopted. Several hundred beetles (8–25 days old) were extracted, first with hexane to remove inactive lipids, and then with dichloromethane (8 × 150 µl per beetle). The organic phase was extracted with 2 N hydrochloric acid and the alkaloid was recovered, after neutralization of the acid with 2 N sodium hydroxide, by re-extraction with dichloromethane. Two successive gel filtrations (Sephadex LH-20, eluted with 1:1 dichloromethane/methanol) yielded a pure sample (judged by TLC analysis on silica gel and GLC on OV-1 and OV-17) of a single compound, m.p. 28 °C, in amounts corresponding to circa 15 µg per specimen. The molecular formula $C_9H_{15}NO$ was determined by high resolution mass spectrometry. Other physical and spectroscopic data ($[\alpha]_D$, infrared, ^{1}H-NMR, and mass spectra) were in excellent agreement with the data reported for (+)-9-aza-1-methyl-bicyclo-[3.3.1]nonan-3-one (**1**), previously isolated from the Australian plant *Euphorbia atoto*[4], as well as from the Australian coccinellid *Cryptolaemus montrouzieri*[5]. The term *euphococcinine* that we coin for the compound alludes to its dual botanical and insectan origin.

Gas chromatographic analysis showed the alkaloid to be present in the blood of the beetle. In order to obtain a measure of the rise in concentration of the compound in blood following adult emergence, quantities of blood were 'milked' from beetles of known age and analyzed gas chromatographically. Milking was effected by squeezing the beetles gently in forceps until they bled from one or more legs, and taking up the emitted droplets in microcapillaries for subsequent weighing. As is clear from the results (fig. 2, center chart) the alkaloid titre rises in both sexes, to maxima that are achieved within 1–2 weeks after adult emergence, and are on the average higher in males (1.6 µg/mg blood) than in females (0.9 µg/mg blood). The amount of blood released by reflex bleeding, about 0.5 mg/leg, does not vary with sex or age (fig. 2, bottom chart). Beetles release blood from one

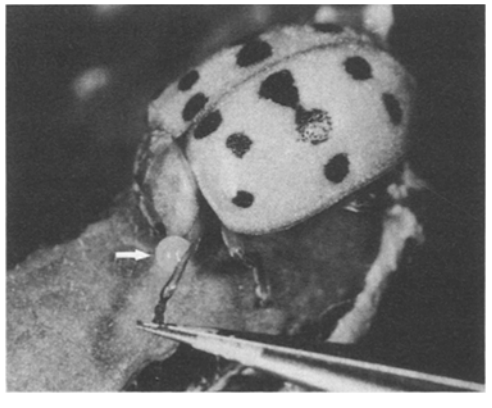


Figure 1. *Epilachna varivestis* on bean leaf, responding to pinching of left foreleg by discharging blood droplet (arrow) from knee joint of that leg. Such reflex bleeding, usually restricted to one leg when stimulus is localized, tends to occur from most or all legs when beetles are handled, squeezed in forceps, or otherwise broadly stimulated.

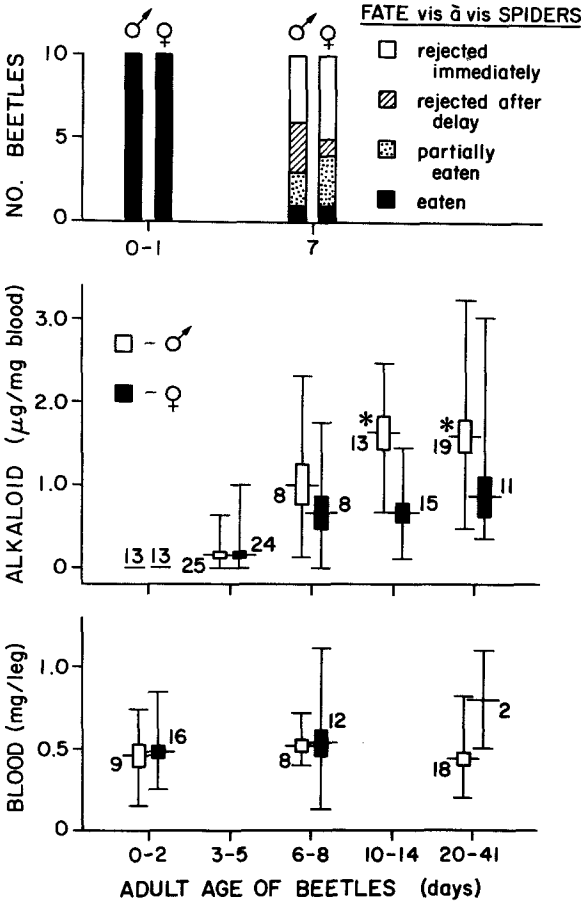


Figure 2. Top: Fate of newly-emerged and 7-day-old adult *Epilachna varivestis* offered to jumping spiders *(Phidippus regius)*. Details in text. The difference in percentage eaten (100% vs 10%) of the two groups is highly significant ($p \ll 0.001$, $\chi^2 = 32.7$, df = 1). Center: Alkaloid content of adult *Epilachna* blood, plotted as a function of beetle age. The increase in blood alkaloid over time is significant ($p < 0.005$) for both sexes ($F = 30.7$, df = 4, 73 for males; $F = 10.8$, df = 4, 66 for females). Asterisks indicate a significantly higher alkaloid content in males relative to females of that age group ($p < 0.001$, $t = 4.3$, df = 26 for 10–14-day group; $p < 0.05$, $t = 2.2$, df = 28 for 20–41-day group). Bottom: Blood emitted ('reflex bleeding') by adult *Epilachna* in response to stimulation, plotted as a function of beetle age. Categories do not differ significantly ($p \gg 0.25$, $F = 0.20$, df = 2, 62 for males and females lumped). Horizontal lines, vertical lines, and bars indicate means, ranges, and standard error of means, respectively.

or more legs, depending on the intensity and degree of localization of the offending stimulus[3]. Under attack conditions, blood emission can therefore be expected to vary on average from 0.5 to 3 mg/beetle. For males and females (> 2 weeks old) this means an externalized defensive deployment of, respectively, 0.8–5 µg and 0.5–3 µg alkaloid/beetle.

Evidence was obtained that the alkaloid itself accounts, in part at least, for the deterrency of *Epilachna* blood to spiders. The testing procedure, described in detail elsewhere[6], involved offering individual *Phidippus regius* standardized food items (freshly killed *Drosophila melanogaster*), treated topically by addition of alkaloid, or left untreated (controls). Fate of item was scored as follows: eaten (reduced to a small mass of solid remains); partially eaten (part of body rejected intact); rejected (dropped within less than 30 sec after seizure). The alkaloid was tested at 3 dosages (0.1, 1.0, 10.0 µg). Application to *Drosophila* was effected in dichloromethane solution (1 µl); controls were treated by application of solvent only. Time was allowed for evaporation of solvent prior to presentation of item to spider. The results (fig. 3) show clearcut deterrency at a dosage of 1 µg, an amount roughly equivalent to what beetles (> 2 weeks old) eject with blood from 1–3 legs.

The alkaloid proved active also as a feeding deterrent to ants. Feeding preference tests were set up in which ants (*Monomorium pharaonis*) were offered a choice between 10^{-1} M aqueous sucrose (a highly acceptable food that served as control) and 10^{-1} M aqueous sucrose with added alkaloid. The tests were carried out near a natural colony of the ant, at a location baited with honey drops to which the ants had laid a network of foraging trails. Testing protocol was as previously described[7]. Alkaloid was tested at 3 concentrations (10^{-2}, 10^{-3}, 10^{-4} M). Each test involved presentation of a given alkaloid solution together with the control. Ant visitation frequency to the two solutions was scored, and provided the basis for calculation of relative acceptability of the two samples. Tests were replicated four times for each alkaloid concentration. It is clear from the results (fig. 4) that the alkaloid was undeterrent at the most dilute level tested, but highly deterrent at a concentration of 10^{-2} M. The latter concentration (≅ 1.5 µg/mg) is roughly equivalent to the alkaloid concentration in the blood of (> 2-week-old) beetles. *Epilachna* blood can be expected to be deterrent to ants even in 3–5-day-old adults. At that age blood alkaloid concentration is in the order of 10^{-3} M, a level that proved significantly deterrent in the ant tests.

Analysis of extracts of larvae and pupae of *Epilachna* showed total absence of alkaloid, as did analysis of extracts of eggs. The alkaloid is therefore restricted to adults, and there is no maternal transmission of the compound to the offspring. Whether eggs and larvae are protected by alternative chemical factors remains unknown.

The gradual buildup of a defensive compound following adult emergence in an insect raises questions. If adults have fixed annual emergence times, one would expect the first individuals that appear in the population, given their relatively unprotected status, to be particularly subject to predation. In *Epilachna* such contingency may not arise. The beetles overwinter as adults[8], and may at the time of spring emergence be expected to be fully alkaloid-laden. Predators, including arthropod predators such as spiders, which are known to be capable of learning[9], may on the basis of early seasonal experience with protected adults discriminate against later emergents. Newcomers, while still potentially vulnerable during their early adult existence, might thus derive benefit by masquerading as Batesian 'automimics'[10] of their protected conspecifics.

Many defensive alkaloids, of which precoccinelline (**2**) is a typical example, have been isolated from coccinellids[11]. The alkaloids do not appear to be present in the coccinellids' diet, but are synthesized de novo from acetate, presumably via a polyketide pathway[12]. The bicyclic homotropane adaline (**3**), characteristic of ladybugs belonging to the genus *Adalia*[13,14], is structurally

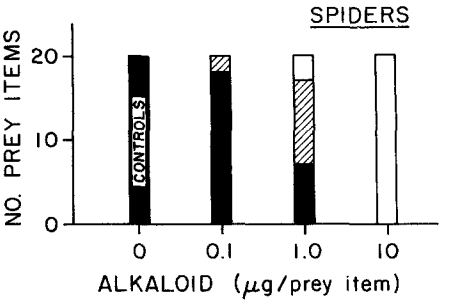


Figure 3. Fate of individual food items (freshly killed *Drosophila melanogaster*), pretreated by topical addition of alkaloid at dosages indicated, offered to jumping spiders (*Phidippus regius*). Black = eaten; cross-hatching = partially eaten; white = rejected. Details in text. Results (eaten vs sum of rejected and partially eaten) for either control or the 0.1-µg group differed significantly from the effect at the 1.0-µg and 10-µg levels ($p < 0.005$ for all comparisons; $\chi^2 \geq 15.8$, df = 1).

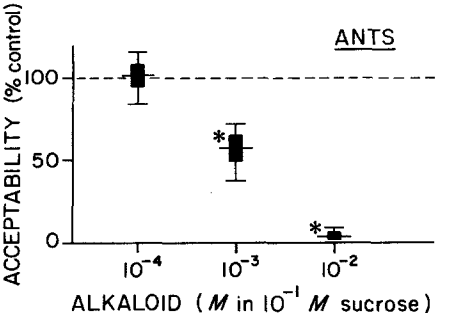
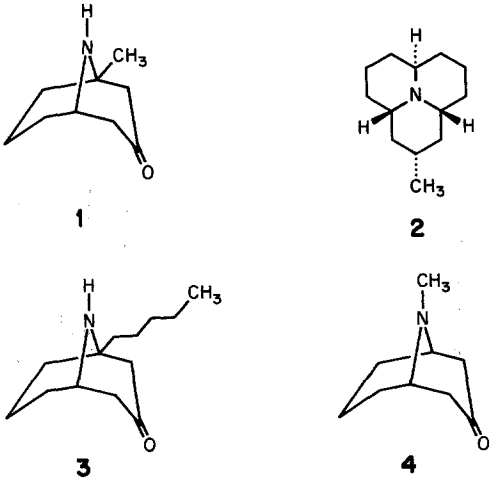


Figure 4. Acceptability of alkaloid solution to ants (*Monomorium pharaonis*), expressed as percent of acceptability of control (10^{-1} M sucrose). Details in text. Horizontal lines, vertical lines, and bars indicate means, ranges, and standard error of means, respectively. N = 4 for each molarity. Asterisks indicate acceptabilities significantly lower than control ($p \ll 0.005$ for both cases, $\chi^2 \geq 92$, df = 1).



very closely related to the lysine-derived pomegranate bark alkaloid, pseudopelletierine (**4**)[15]. Nevertheless, the substitution pattern in adaline has led to the suggestion that this alkaloid is actually a polyketide[12,13]. In any event, it seems likely that adaline and euphococcinine arise via common biosynthetic pathways.

Deterrency to ants has been demonstrated for one other coccinellid alkaloid (coccinelline)[16]. The possibility that these alkaloids generally, including euphococcinine, are deterrent also to non-arthropodan enemies remains open. Potentially, at least, these compounds could fulfill antivertebrate, antihelminthic, and antimicrobial roles. The laboratory finding[16] that quail prefer alkaloid-free over alkaloid-laden coccinellids is intriguing in this regard.

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Biosynthesis of 1-aminocyclopropanecarboxylic acid: Steric course of the reaction at the C-4 position

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Summary. Under the action of the appropriate synthase from ripe tomatoes a 1:1 mixture of (3S,4R)-[3,4-$^2H_2$] and (3R,4S)-[3,4-$^2H_2$]-(2S)-adenosylmethionine is transformed into a 1:1 mixture of the two meso forms of [$^2H_2$]-1-aminocyclopropanecarboxylic acid, a result which proves the operation of an inversion mechanism and which is consistent with direct nucleophilic displacement of the leaving group in the substrate.

Key words. 1-Aminocyclopropanecarboxylic acid; ACC-synthase; PLP-catalyzed reaction; stereochemistry.

The amino acid 1-aminocyclopropanecarboxylic acid (ACC) plays an important role in higher plants as the precursor of the phytohormone ethylene[1,2]. Formation of ACC from S-adenosylmethionine (SAM) is catalyzed by a PLP-dependent synthase[9] and is expected to proceed according to scheme 1. Recently we have demonstrated that the Si-methylene group of ACC stems specifically from the C-4 methylene group of the substrate and thus that the reaction involves an inversion at the α-center[3]. To complete the stereochemical picture we decided to analyze the steric course of the substitution which takes place at the C-4 methylene group of the substrate.

An internal alkylation process involving direct displacement of methylthioadenosine is, of course, expected to proceed with inversion at the center of nucleophilic substitution. Alternatively, it is conceivable that the leaving group of the substrate is displaced by an appropriate nucleophile belonging to the protein component prior to the internal alkylation step, a process that would be expressed in an overall retention at the center undergoing the double substitution.

In principle the two possibilities can be distinguished by carrying out the enzymatic reaction with a [4-$^2H_1$]-SAM of known configuration at C-4 and determining the configuration at C-2 of the

Scheme 1