Hemorrhage in a Coccinellid Beetle and Its Repellent Effect on Ants

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Science, New Series, Vol. 134, No. 3475. (Aug. 4, 1961), pp. 329-331.

Table 1. P as a function of trials.

Trials	Mean of P	.05 Fiducial limits of mean
1–30	.593	.562–.624
31–60	.660	.625–.694
61–90	.672	.636–.708

may develop. For both of these reasons, reinforcement of corrections should not change P as much as reinforcement of initial responses. By the rules of the correction procedure, P of all reinforcements for M will follow initial responses; the corresponding proportion for L is $(1-P)$, since L is the initial response on $(1-P)$ of the trials. It is known (*2*) that after a few trials P becomes larger than .5, so that most of the M reinforcements follow initial responses, while most of the L reinforcements follow corrections.

For the above reasons, a new procedure, called the *nonreinforced trial procedure,* was devised. Only one response is allowed to occur on any one trial. Nonreinforced trials are interspersed with reinforced trials, so that π is controlled.

In the present experiment, 19 rats, deprived of food for approximately 22.5 hr, were subjected to one trial per day for 90 days in a slightly modified version of a T-maze described by Nunis (*4*). The nonreinforced trial procedure was used to reinforce a position preference with $\pi = .67$. Reinforcements were administered in blocks of three, consisting of two M reinforcements and one L reinforcement. At the beginning of a block, whichever response was made was reinforced. When all of the assigned reinforcements had been received for one of the two responses, that response was not reinforced until the assigned number of reinforcements had been obtained for the other response. It so happened that .608 of all trials were reinforced. (Reinforcement consisted of allowing the rat to enter a white goal box from a black alley to eat ground chow for 20 sec. When nonreinforcements were scheduled, a swinging door leading to the nonreinforced goal box was locked. Five seconds after the rat interrupted a photobeam 1.5 in. in front of this door, it was removed from the apparatus.)

Table 1 shows the mean of P during each 30-trial portion of the experiment and .05 fiducial limits for this mean (t-test). Toward the end of the experiment, P is approximately equal to π. Estes (*2*) has shown that asymptotic equality of P and π is implied by certain assumptions about the effect of reinforcement on response probability provided that all trials are reinforced. If our result is to confirm Estes' views, it must be shown that the nonreinforced trials do not affect P. Under the present use of the nonreinforced trial procedure, nonreinforcements of M can occur only during a block of reinforcements in which the two reinforcements of M have already occurred, but L has not yet occurred. The value of P on the trial following the second reinforcement of M during such a block is called *Prr*. If the trial following these two successive reinforcements of M also results in M, this last trial is not reinforced; the value of P after such a nonreinforcement is called *Prrn*. Similarly, the value of P following two such nonreinforcements is called *Prrnn*. These statistics were obtained for each rat and their means were: $Prr = .839$; $Prrn = .788$; and $Prrnn = .664$. This decrease in P as a function of nonreinforcement of M is significant at the .05 level (Kendall $W = .161$, 18 degrees of freedom), indicating that without special assumptions about the interaction of reinforcement and nonreinforcement, the present experiment, like previous probability learning experiments with animals, cannot decisively confirm or reject theories about the effect of reinforcement on response probability.

Important information about the role of the nonreinforcements in the nonreinforced trial procedure is supplied by two proportions, $F(M)$ and $F(L)$. $F(M)$ is the proportion of M occurrences which are reinforced:

$$F(M) = x_\pi / P$$

where x is the proportion of all trials (both M and L) which are reinforced (so that x_π is the proportion of all trials containing reinforced occurrences of M). Similarly, $F(L)$ is the proportion of L occurrences which are reinforced:

$$F(L) = x(1-\pi)/(1-P)$$

It may be verified that when $P < \pi$, $F(M) > F(L)$; that when $P = \pi$, $F(M) = F(L)$; and that when $P > \pi$, $F(M) < F(L)$. I have not been able to relate these mathematically derived facts to the above-mentioned theories (*1, 2*) in any rigorous way. However, if it is assumed that when $F(M) > F(L)$, P increases, and that when $F(M) < F(L)$, P decreases, these facts imply that once P reaches π, it will oscillate around π. (The possibility that P will remain constant at π is ignored because it was empirically shown that $Prr > \pi$.) Presumably, then, the mean of P will approximate π (*5*).

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20 March 1961

Hemorrhage in a Coccinellid Beetle and Its Repellent Effect on Ants

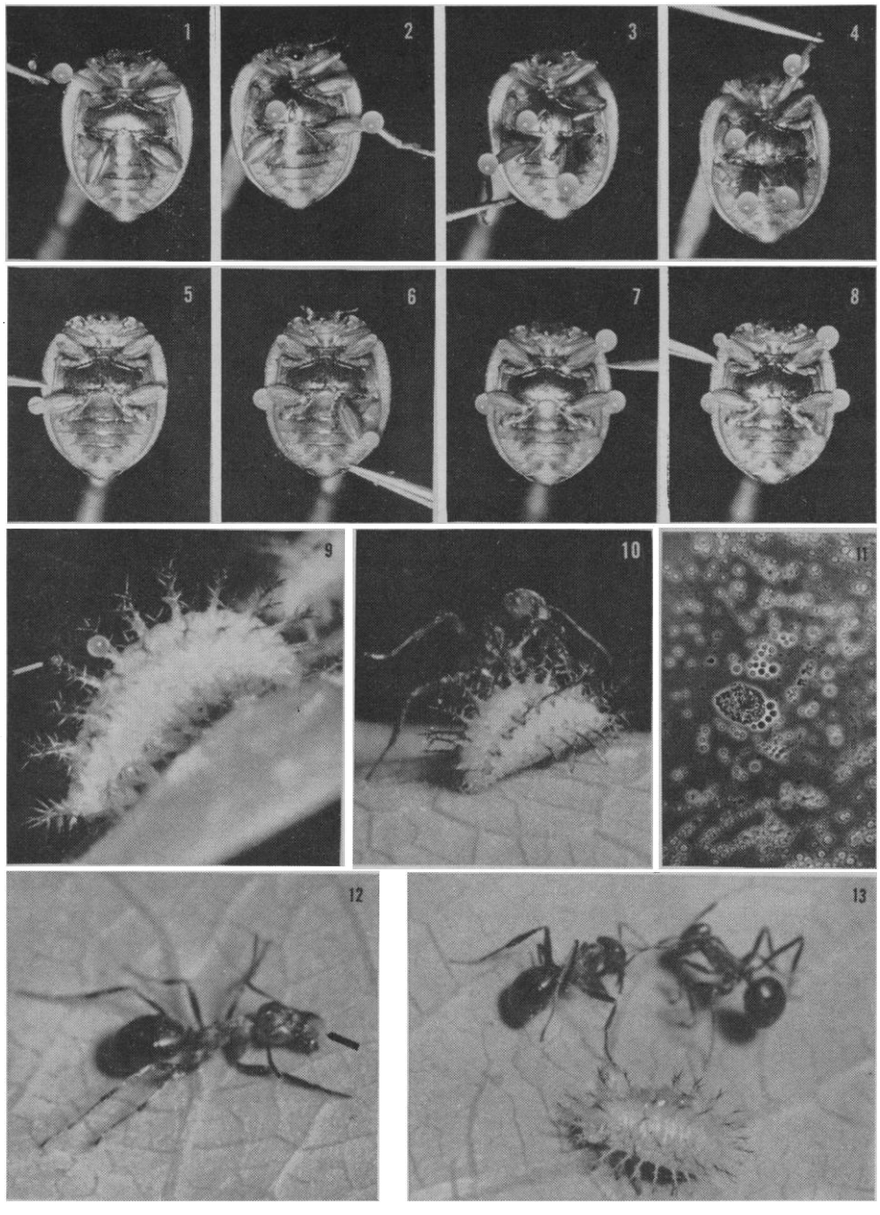
Abstract. Special refinements of the bleeding mechanism of the Mexican bean beetle, *Epilachna varivestis* Mulsant, are described, and the defensive effectiveness of the mechanism against ants is demonstrated. Ants may have provided a major selective force in the evolution of the mechanism.

A variety of insects and other terrestrial arthropods have the peculiar habit of discharging small droplets of blood from one or more points on their body surface when they are handled or otherwise molested. This autohemorrhage or "reflex bleeding" as it is often called, is generally acknowledged to be a mechanism of defense against predators. The purpose of this report (*1*) is to describe some hitherto unnoticed adaptive features of this mechanism as it occurs in adults and larvae of the Mexican bean beetle, *Epilachna varivestis* Mulsant.

In adult *Epilachna*, as in apparently all coccinellid beetles that show reflex bleeding, the release of blood is exclusively from the tibio-femoral joints of the legs (*2*). In order to facilitate observation of the bleeding response, individual beetles were affixed to rods [by a technique used previously with other insects and described elsewhere (*3*)], and were subjected to localized traumatic stimuli, applied either by pinching individual appendages with forceps, or by touching different body

regions and the elytra with the point of a hot needle. Bleeding was the almost invariable response: the only stimuli which were ineffective were those applied to the dorsum of the abdomen beneath the elytra (this is, of course, a region not likely to be initially traumatized by a predator).

Of special interest was the finding that the legs do not all bleed simultaneously, but do so individually (Figs. 1–4). As a rule, only the particular leg closest to the stimulus responds at a given time (Figs. 5–8). The leg is even sometimes rotated in such a way that its blood-laden knee joint is brought closest to the point traumatized (for example, Fig. 6). When stimuli are repeatedly applied to the same site, there occurs first an enlargement of the initial drop formed on the nearest leg, followed by a spread of the bleeding response to other legs, first to those of the same side and then to those of the opposite side. The droplets sometimes flow onto the lateroventral margins of the body; in ambulatory specimens this is a common consequence of leg movements.



Figs. 1–4. Four consecutive bleedings elicited by pinching in succession the four corresponding legs. Figs. 5–8. Same as preceding, but stimuli applied as shown to elytral and abdominal margins. Fig. 9. Two droplets of blood (arrow to smaller drop) oozing from ruptured larval spines. Fig. 10. *Formica* worker about to bite and rupture larval spines. Fig. 11. Smear of blood released at knee joint of adult (phase contrast). Fig. 12. *Formica*, after attack on larva, dragging its body and blood-laden mouth parts (arrow). Fig. 13. Two ants, contaminated with larval blood, stuck together by their antennae.

Simultaneous application of several localized stimuli results in a synchronous discharge from a corresponding number of legs, involving invariably only those closest to each stimulus. Broadly generalized stimulation, as when the animals are handled, results in simultaneous or almost simultaneous discharge from all six legs. The blood coagulates rapidly on exposure to air, hardening to a crust within minutes. The crust does not hinder the beetle in any apparent way, and eventually always flakes off completely during the normal course of activity.

The amount of blood that the beetles can stand losing is considerable; no noticeable ill effects result from a loss of six droplets, one from each leg. Some of our beetles did eventually die, but this may well have been due to the more or less extensive cautery induced by the hot needle.

In the larvae of *Epilachna*, bleeding is also easily induced, but it cannot be considered autohemorrhagic in a strict sense, since it occurs only at the actual sites of injury. The entire back and sides of the soft-bodied larva are beset with a dense armature of erect branched spines. These spines are hollow and brittle, and when prodded rupture readily, resulting in instantaneous emergence of a small droplet of blood at the break (Fig. 9). Like the adults, the larvae can also survive considerable hemorrhage: specimens that were shorn of all their spines went on to pupate normally.

The distasteful properties of a variety of coccinellid beetles have been demonstrated, at least with regard to some vertebrate predators (*4*). The localized nature of the bleeding response suggests that it might also serve with particular effect against smaller arthropod predators that are likely to inflict local rather than general injury when they first attack. Observations on arthropod predators are rare, however, and such as do exist are sketchy at best (*5*). This prompted us to set up a series of laboratory encounters between adults and larvae of *Epilachna* and the ant *Formica exsectoides* Forel.

The adult beetles were introduced directly into an artificial nest of the ants; they were attacked instantly, the ants converging upon them in groups, and biting them. Sometimes the beetles were seized by an appendage, but more

frequently—since they "feigned death" and tucked their legs under the body —they were grasped by the elytral margins. No sooner had an ant bitten than the beetle responded by bleeding from the nearest knee joint. The droplet of blood invariably spread onto the assailant, contaminating its antennae, and usually oozing between the gaping mouthparts. The ant immediately released its hold, backed away abruptly, and then began intensive cleansing activities, brushing its antennae with the forelegs, or walking along slowly, flailing its legs and dragging its blood-drenched mouth parts along the substrate (Fig. 12). [This peculiar dragging behavior has been noticed before in this ant in comparable encounters involving arthropods with defensive secretions (*6*)].

More pronounced signs of distress became apparent as the blood coagulated, becoming increasingly viscous and sticky. It was then not uncommon to see an ant with its antennae stuck together, with a leg stuck to an antenna, or with the mouth parts gummed up and virtually immobilized. Groups of ants would become stuck to one another (Fig. 13). Recovery is eventually always complete, but it may take an ant several minutes to over an hour to clear itself from the remaining flakes of clot. A few tests were also made by releasing ants and beetles onto the leaves of the beetle's food plant. The results differed only in that they pointed out clearly the protective value of "death-feigning"; when attacked, the beetles often simply let themselves drop from the leaves.

The experiments with larvae were all done on leaves. The ants attempted to bite, but would usually only succeed in breaking one or more of the larval spines. The droplets of blood released were instantly repellent to the ants, and affected them in the same way as did the blood of the adults. Unlike the adults, the larvae never dropped from leaves.

Evidently, the bleeding mechanism of *Epilachna* is a means of defense admirably suited for use against ants. It is certainly conceivable that the mechanism might be similarly effective against other arthropod enemies, but ants may well be one of the most important groups of predators—if not *the* most important group—responsible for the evolution of the mechanism. Most coccinellids are carnivorous, and the habit shared by so many of them of feeding on aphids and coccids may well be primitive for the family (*7*). Though the tribe to which *Epilachna* belongs (Epilachnini) is predominantly herbivorous, it is "regarded as a relatively late and specialized offshoot of the Coccinellid stock . . ." (*8*). It seems that its hemorrhagic defense mechanism is an evolutionary legacy from its Homoptera-feeding forebears, whose exposure to the well-known aggressive tendencies of Homoptera-tending ants (*9*) provided the selective force that evoked the adaptation in the first place. It is interesting in this connection to note that the larvae of certain lycaenid butterflies that also feed on Homoptera tended by ants are protected from attack by secreting a substance that is not repellent but attractive to the ants (*10*).

In the past there has been some controversy as to whether the liquid exudate of adult coccinellids is indeed blood, rather than the product of special glands (*11*). Our own observations tend to confirm the prevalent view that it is blood. The liquid has all the diagnostic features of blood (Fig. 12): the same cells are present, and so are the tiny spherules known to be typical of coccinellid blood (*12*). One might add also that the size of the droplet released at the knee joint often distinctly exceeds the estimated volume of the leg that produces it. Histological studies (*13*) reveal no glandular reservoirs of appropriate capacity.

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References and Notes

1. This study was supported by grant No. E-2908 from the U.S. Public Health Service. It is the fifth paper of our series on defense mechanisms of arthropods.
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* Predoctoral fellow, National Science Foundation.

17 April 1961

Localization of Porphyrin Fluorescence in Planarians

Abstract. Two species of planarians were studied by fluorescence microscopy. Red fluorescence of uroporphyrin was observed localized in the epidermal rhabdites and subepidermal rhabdite-containing gland cells. Fluorescence was observed in isolated rhabdites of homogenates, but was not seen in rhabdites of the living animal. The identity of rhabdites was established by their location, shape, size, and acidophilic staining properties.

The occurrence of uroporphyrin in the planarian, *Dugesia dorotocephala,* has been described previously (*1*). This finding was based on analyses of acid extracts of the planarian. In that study, general porphyrin fluorescence of the acid-treated animal was observed; in the present study, however, higher intensity of ultraviolet light permitted the observation of fluorescence within definite morphological structures.

Detection of fluorescence was made microscopically; a Leitz HB 200 lamp was the source of ultraviolet light. A Leitz 2-mm BG-12 exciter filter was used, and a 2.5-mm OG-1 barrier filter was placed in the ocular. Observations and photographs were made with a dark-field condenser.

The two species of planarians studied were *Dugesia dorotocephala* and *D. tigrina.* Living animals were placed in water between a slide and cover slip, and exposed to ultraviolet light under the microscope. Red fluorescence observed in the living animal was restricted to a few irregular structures within cells lining the digestive tract, which probably were food particles or products of digestion. However, after treatment with a variety of chemical substances, a brilliant and extensive red fluorescence was observed in the epidermis and subepidermis of these animals.