

Tanning of Grasshopper Eggs by an Exocrine Secretion

Abstract. Eggs of the grasshopper, *Romalea microptera*, are laid in a tight cluster, encased within a matrix of hardened froth. When first laid, the eggs are yellow and soft, and the froth is white and lathery. During subsequent hours, eggs and froth undergo a tanning process, as a result of which they harden and darken. An exocrine secretion, supplied by a pair of glands situated beside the gonopore, is mixed with the froth as the eggs are laid, and serves to promote tanning. Similar glands are found in some, but not all grasshoppers.

Certain adult female grasshoppers have long been known (1) to possess a pair of exocrine glands near the ventral tip of the abdomen, beside the gonopore (Figs. 1, 2B). Each gland is essentially a sac-like invagination of the body wall, and consists of a membranous cuticular lining associated with a glandular epithelium (2, 3). A short muscle connects the gland to an internal skeletal process (Fig. 1). Nothing was known about the function of these glands. Claims to the effect that they might be the source of sex attractants (2) or other pheromones (4) were advanced without supporting evidence. The suggestion that they might serve for defense (5) seemed unlikely because of their restriction to a single adult sex. This report deals with the function of the glands in the

large Southern lubber grasshopper, *Romalea microptera* (Fig. 2A).

The secretion is yellowish-brown and odorless, and forms a thin layer of waxy consistency adherent to the cuticular lining of the gland (Fig. 2E). The first clue to its function stemmed from the observation that, whereas mating, defecation, and other activities of the adult female do not result in a visible loss of stored secretion, the act of oviposition leaves the glands totally depleted.

Oviposition in *Romalea* involves the same sequence of events as in other grasshoppers that bury their eggs in batches or "pods" (6). By digging with her ovipositor, the gravid female works her abdomen downward into the soil (Fig. 2C) and scrapes out a chamber at the bottom of the burrow. A viscous liquid, stemming from the lateral oviducts, is then passed out through the gonopore and, as a result of a persistent opening and closing action of the ovipositor, is worked into a white lathery froth (Fig. 2F). The eggs are then released one at a time, and deposited in the froth. After about 50 eggs have been laid, the female caps the batch (Fig. 2D) with additional froth, extricates herself, and walks away.

When first laid, the eggs are bright yellow and the shell is soft and resilient. They subsequently undergo a tanning process, and in a matter of one to several hours become hardened and dark brown. The froth also darkens, although not as intensely as the eggs, and becomes hard and water-insoluble. A fresh batch of eggs falls apart when handled or when submerged in water, but the tanned pod, rigidly encased within the hardened froth, holds together intact.

The disappearance of the secretion at oviposition suggested that it might be incorporated into the egg pod itself. To test this supposition, crystals of dye (methylene blue) were added to the stored secretion in the glands of gravid females (7). The froth of

the egg pods subsequently laid was distinctly blue, indicating that the dye, and hence probably the secretion, had been mixed with the liquid effluent from which the froth was made (8).

Additional experiments, aimed at defining the function of the secretion, were carried out on isolated eggs, dissected from the ovaries of gravid females. When such eggs were spotted or streaked with secretion, the affected regions tanned within an hour, often within minutes. Untreated regions also tanned, but the first signs of darkening were usually delayed for hours (Fig. 2G).

The secretion also hastens the tanning of the froth. When liquid from the lateral oviducts of gravid females was transferred to a vial and stirred with a glass rod, it could be quickly worked into a lather that was indistinguishable from the natural froth. If secretion was added to the liquid prior to stirring the resulting froth "set" and began to darken within an hour; by this time, similar froth prepared without secretion was still lathery and white.

The following experiment served to demonstrate that the small amount of secretion actually available to the ovipositing female is sufficient to cause a substantial acceleration of the tanning process. The full complement of mature eggs from the two ovaries of a gravid female, together with the fluid from the lateral oviducts, was divided equally between two vials. Secretion scraped from one of the glands of the female was added to one vial, and none was added to the other. Both vials were stirred, until a proper froth had formed around the eggs. Six hours later the batch without secretion was only beginning to tan. The other had already attained the color and consistency of a mature pod (Fig. 2H). This experiment was done repeatedly, with consistent results.

Contrary to expectation, egg pods laid by females from which the glands had been dissected away (1 to 3 days beforehand) tanned at a normal rate. It was found that blood from the partially healed sites of operation, and particularly the older clots of melanized blood from around the margins of the wound, accelerated tanning as effectively as the secretion (9). It seemed probable, therefore, that material from the wounds, inevitably rinsed away by the froth and incorporated into the pods,

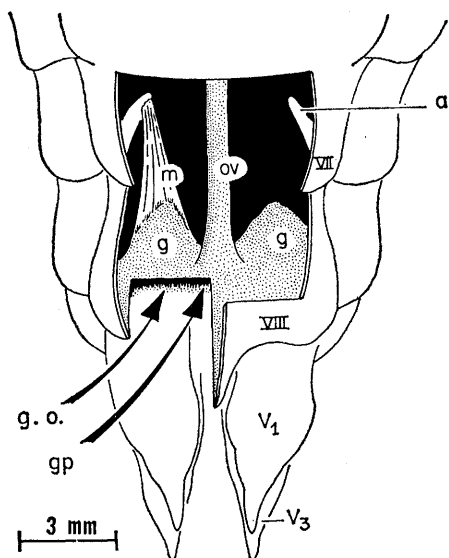


Fig. 1. Ventral view of tip of abdomen of female *Romalea*, dissected open so as to expose the two glands (g) beside the median oviduct (ov). The muscle (m) that connects each gland to a skeletal process (a) is shown in right gland only. Arrows point to slit-like opening of gland (g. o.), and to the gonopore (gp) through which the eggs emerge. The ventral exoskeletal plates (sternites) are designated by roman numerals, according to convention; V₁ and V₃ are the first and third valves of ovipositor.

had acted as a functional substitute for the secretion (10).

The fact that blood from sites of injury can mimic the action of the secretion is in itself meaningful. Insect blood, when exposed to air or a foreign surface, undergoes a process of melanization, in which tyrosine-derived quinones (11), and perhaps some proteins, polymerize to form darkened clots (12). Fundamentally similar tanning processes, mediated by comparable enzyme systems, bring about the

hardening and darkening of the insect exoskeleton after molting (12), and also, interestingly, the tanning of certain insect eggs (13). It is therefore not surprising that wound-associated melanization and egg tanning in *Romalea* should appear to involve biochemically related processes. Whether the active factor in the secretion, as well as its counterpart from wounds, is actually enzymatic in nature (consisting perhaps of one or more phenol oxidases, or their proenzymes) remains

to be confirmed. We do know that the secretion is composed largely of protein (14), and that it is inactivated by heat treatment at 100°C for 20 minutes. The occurrence of an integumentary exocrine secretion of enzymatic character would not be without precedent in insects (15).

A mechanism that provides for rapid tanning of the egg pod could serve a variety of adaptive functions. Tanning of eggs, like tanning of the exoskeleton, may protect against water loss

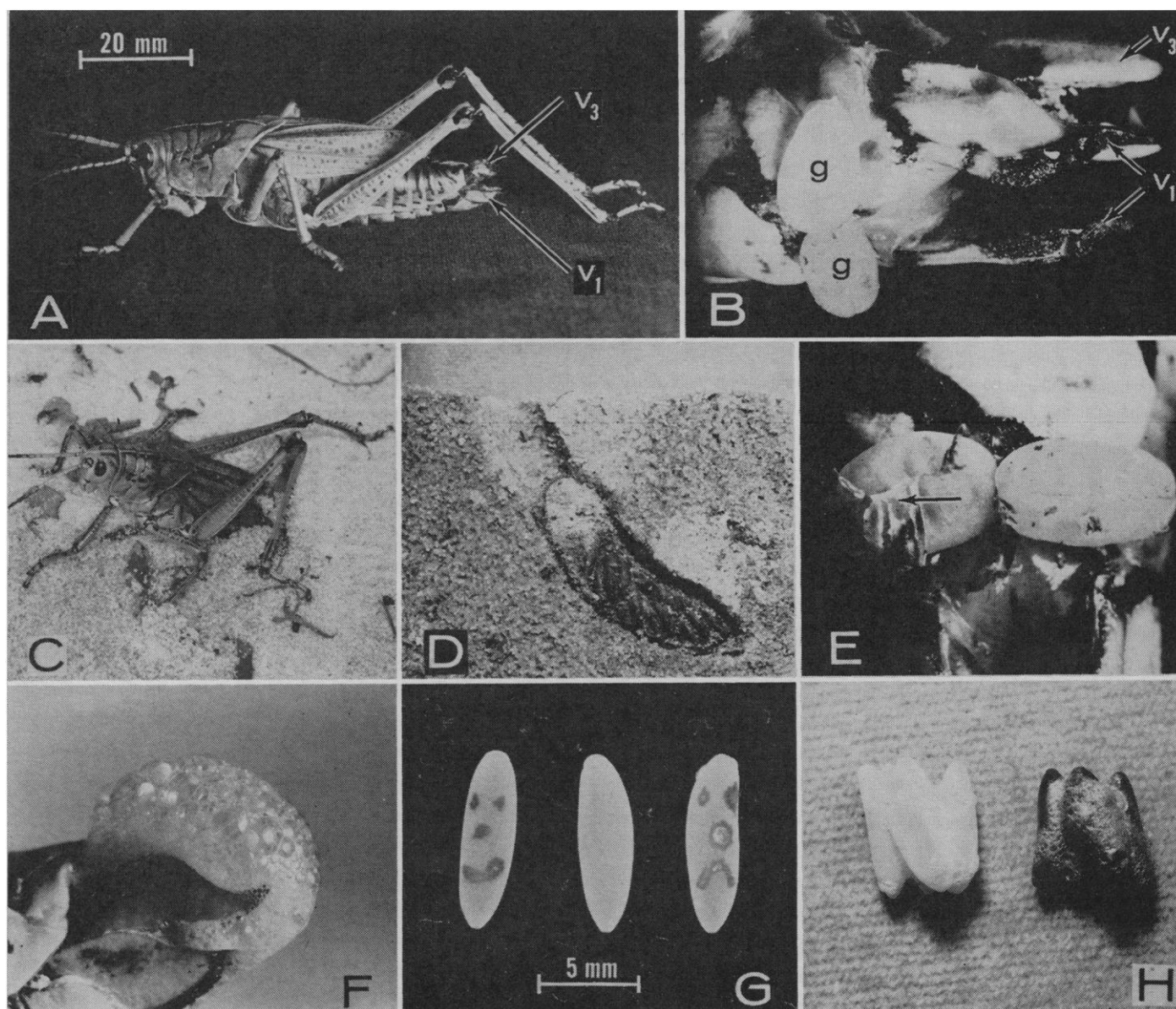


Fig. 2. (A) *Romalea microptera*, female. Arrows point to first (V_1) and third (V_3) valves of ovipositor. (B) Ventrolateral view of tip of abdomen of female. The two glands (g) have been artificially everted by massive injection of fluid into the abdomen. Arrows point to valves of ovipositor (labels as in A). (C) Female ovipositing in sand. (D) Profile view of burrow made by ovipositing female, showing egg pod in place. (E) The two glands (artificially everted as in B), with arrow pointing to secretion on right gland. Secretion from left gland was scraped away. (F) Tip of abdomen of female, laden with froth produced during oviposition. (G) Three eggs removed from ovary of gravid female. The outer ones have undergone tanning where they were spotted and streaked with secretion; egg in center is an untreated control. (H) Two groups of eggs from same ovary of gravid female. The ones on the right were stirred in oviductal fluid containing secretion; the ones on the left were stirred in the same fluid without secretion.

or water intake. Studies with other grasshopper eggs suggest that the froth may indeed shield the pod from excess water (16). During temporary flooding, a tanned pod might survive intact, whereas an untanned one would probably fall apart. In a submerged pod, the air trapped within the hard insoluble froth could possibly serve for respiratory purposes, as does the air carried in the "plastron" of certain aquatic insects (17). In the normally dried pod, this same trapped air could provide insulation against extreme temperatures. Severe climatic conditions are a reality in the life of *R. microptera*. In Florida, as elsewhere throughout the range of this insect in the southeastern United States, sudden torrential thunderstorms, alternating with periods of scorching heat, are the rule of the day during the summer months when the eggs are laid. Finally, accelerated tanning may also be of defensive value, since some predators might tend to ignore the pods once they have hardened.

The glands found in *Romalea* also occur in several related genera, all belonging to the subfamily Cyrtacanthacrinae (4). Species of other subfamilies (Acridinae, Oedipodinae, Pyrgomorphinae) lack the glands (4) for reasons unknown.

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7. By pulling on the ovipositor, ready access could be gained to the glands, and the crystals were inserted without contaminating the gonopore or other adjacent regions.
8. The fact that the glands can be artificially everted by forcible distention of the abdomen with injected fluid (Fig. 2B) need not be taken to indicate that evagination is the normal mechanism whereby the glands deliver their secretion during oviposition. The glands of ovipositing females pulled from their burrows were always found to be in the invaginated condition, despite the fact that the abdomen of such females was in its characteristic state (6) of sustained maximal dis-

tention. The muscle that attaches to the gland should not, therefore, be assumed to function as a retractor. Instead, its action may consist of pulling taut the normally slack glandular pouch, thereby squeezing the pasty secretion from the lumen.

9. This was established by streaking isolated eggs with secretion, and with blood or clots from wounds, and comparing the rates at which the affected regions underwent tanning.
10. Blood or clots from wounds other than the operated gland sites was also found to promote egg tanning, thus ruling out the possibility that the "wound factor" was simply residual secretion that had contaminated the gland sites. Unfortunately, the operated gland sites never healed completely with time, and it was therefore impossible to determine whether eggs laid by glandectomized females with sealed wounds might show retarded tanning.
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13. Certain cockroaches lay eggs in batches, enclosed in a hard, brown, proteinaceous capsule (ootheca). Tanning of the ootheca is effected by a structurally and functionally dissimilar pair of glands (colleterial glands) that pour into the genital chamber a mixture containing a phenol glucoside, a glucosidase,

a phenol oxidase, and protein. As a result of the interaction of these substances, the phenol glucoside is hydrolyzed and the free phenol oxidized to quinone, and the quinone tans the protein of which the capsule is made. P. C. J. Brunet and P. W. Kent, *Proc. Roy. Soc. London Ser. B* **144**, 259 (1955); P. W. Kent and P. C. J. Brunet, *Tetrahedron* **7**, 252 (1959); M. G. M. Pryor, *Proc. Roy. Soc. London Ser. B* **128**, 378 (1940); ———, P. B. Russell, A. R. Todd, *Biochem. J.* **40**, 627 (1946).

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Tetrodotoxin and Manganese Ions: Effects on Electrical Activity and Tension in *Taenia Coli* of Guinea Pig

Abstract. *Tetrodotoxin, at concentrations up to 5×10^{-6} gram per milliliter, has no effect on the spontaneous discharge in the smooth muscle of taenia coli. However, the spontaneous discharge is abolished by Mn^{++} at a concentration of 0.5 millimole per liter. The contraction induced by immersing the muscle in isotonic KCl solution is also suppressed in the presence of Mn^{++} . Because Mn^{++} is a specific suppressor of the spike induced by Ca^{++} and tetrodotoxin is an inhibitor of the spike induced by Na^{+} , we suggest that Ca^{++} is a charge carrier in the production of spike potential in the smooth muscle and that the entry of Ca^{++} through the membrane acts as a trigger for the contraction of smooth muscle.*

Holman (1) and Bülbring and Kuriyama (1) found that spike height is apparently independent of the external sodium concentration but dependent on the external calcium concentration in the taenia coli. These findings suggest that increase in permeability to Ca^{++} , rather than to Na^{+} , is involved in the spike-generating mechanism in smooth muscle. In crustacean muscle, and particularly in the giant muscle fiber of the barnacle, it has been established that Ca^{++} is the charge carrier in production of the action potential (2). Further, Hagiwara and Nakajima (3) recently found that Mn^{++} at relatively low concentration specifically inhibits the Ca^{++} -spike, while tetrodotoxin, a Na^{+} -spike inhibitor (4), was ineffective in abolishing spike electrogenesis in the muscle fiber of the barnacle. Hence we examined the effects of tetrodotoxin and Mn^{++} on the taenia coli in the hope of throwing further light

upon the role of Ca^{++} in the activity of smooth muscle.

Membrane potential and tension were recorded by means of a sucrose-gap technique, similar to that employed by Bülbring and Burnstock (5). *Taenia coli* of the guinea pig was used throughout. The experiments were performed at room temperature (25° to 30°C).

Application of tetrodotoxin at very high concentrations, up to 5×10^{-6} g/ml, in normal Locke's solution or in hypertonic solution, did not change either the resting potential of the membrane or the amplitude of the spontaneous spikes. The drug had no effect on the spontaneously occurring spikes which persisted after the muscle was immersed in Na^{+} -free solution (Bülbring and Kuriyama, 1). These results are summarized in Fig. 1. The effects of epinephrine, that is, the suppression of spontaneous discharge and the induction of hyperpolarization, were