Alarm and Defense: a Function for the Pygidial Gland of the Myrmicine Ant, *Pheidole biconstricta*[1]

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ABSTRACT

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The recently rediscovered pygidial gland of the ant subfamily Myrmicinae is greatly hypertrophied and has a defensive function in *Pheidole biconstricta* Mayr. Its contents have a volatile component that repels other ants at a distance, and a viscous component that is smeared directly on an enemy and acts as a gumming agent and irritant. Gland contents are usually released only during assaults on the gaster, trunk or head, and rarely during attacks to extremities or the waist. The gland is also a new source of alarm pheromone for the Myrmicinae.

Over 80 yr ago Charles Janet discovered in a primitive ant of the subfamily Myrmicinae small clusters of glandular cells attached to the dorsum of the last external body segment, the pygidium (Janet 1898). But only recently has the unusual development of these pygidial glands in some species, their prevalence within the Myrmicinae, and their potential importance to our knowledge of the systematics and evolution of ants in general been revealed (Hölldobler et al. 1976, Kugler 1978a). Morphologically similar glands, the "anal" glands, have long been known in the subfamily Dolichoderinae (Forel 1878), and recently such glands have been discovered in other subfamilies (Hölldobler and Haskins 1977, Hölldobler and Engel 1979). In the Dolichoderinae these glands are used in defense (Pavan 1959, Wilson and Pavan 1959) and in the ponerine *Rhytidoponera metallica* they produce a sex attractant (Hölldobler and Haskins 1977). Their functions in other ants are unknown. Within the Myrmicinae, the pygidial glands vary greatly, from microscopic clusters of cells with no reservoir (Janet 1898, Hölldobler et. al. 1976) to macroscopic organs whose reservoirs occupy a large portion of the gaster (Kugler 1978a). Here, for the 1st time, the function of this hypertrophied type of pygidial gland is reported.

Pheidole biconstricta Mayr (Fig. 1), a widespread and successful neotropical ant, is abundant in coffee plantations in the foothills of the Sierra Nevada de Santa Marta in northern Colombia, where it forms colonies of thousands of individuals in rotten logs and hollow trees. Using large trunk routes, the workers forage on the ground and in trees, often tending extrafloral nectaries and honeydew-secreting membracids or coccids. Both major and minor workers are readily identified by their strong, sweet odor when crushed.

The Secretion

Preliminary experiments quickly identified the pygidial gland as the source of the odor that to me smells much like crushed *Azteca* ants (Dolichoderinae). By gently milking the reservoir onto a slide with backlighting and viewing the contents with a dissecting microscope, I found that the fluid has 2 components. Embedded in a clear, slightly yellowish, viscous fluid are bubbles of all sizes that surface rapidly and disappear. Rate of disappearance seems directly proportional to bubble size. Most of the bubbles and the odor vanish within 2 min, but sometimes traces of both can be detected by humans several minutes longer. Meanwhile, the viscous component becomes stickier. A droplet manipulated between the tips of fine watchmaker's forceps becomes progressively more elastic. After several hours it loses some tackiness, but seems to persist indefinitely in air without hardening. If glands are voided with more mixing of their contents, such as by crushing with a pin head, the odor can be detected much longer than in the above observations, sometimes up to an hour.

The sticky fraction is insoluble in water, but readily soluble in 95% ethanol. The volatile fraction is very soluble in water. Given these properties, the 2 components should be miscible. I do not know how they are maintained in the gland, but an examination of gross morphology revealed no compartmentalization of the reservoir (Kugler 1978a). The fluid as a whole is slightly acidic, pH 5 on PHENAL® papers.

Interspecific Effects

The volatile component of the pygidial fluid serves as a repellent to other ant species (Fig. 2, 3). Note that the soldiers of *P. biconstricta,* with proportionately larger pygidial glands, have a stronger repellent potential than do the minors. In further experiments of the same design, I found that most repellent effect is lost if, before testing, the pygidial gland is crushed and allowed to evaporate until the odor disappears. If it is allowed to evaporate overnight before testing, only a 5% probability remains that the distribution of the ants in the control and pygidial gland vials will be different ($\chi^2 = 7.294$, df = 4). These results indicate that the persistent viscous fraction itself is not repugnatorial at a distance.

Nevertheless, the viscous component is an effective 2nd line of defense when applied directly to an attacker. It coats well at first, carrying with it the objectionable odor, then quickly thickens into a sticky coat that apparently retains detectable amounts of odor for some time. I saw the pygidial fluid released in some 15–20 instances in response to attacks of various arthropods of all sizes (mostly ants). The reactions of the attackers were nearly invariable; they immediately stopped and

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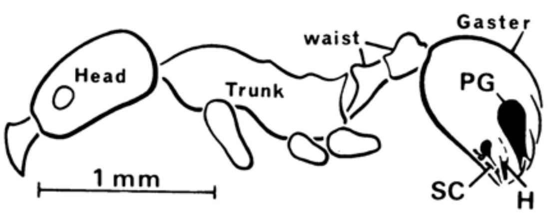

FIG. 1.—Approximate size and position of the pygidial gland (PG), hindgut (H), and the sting complex of glands (SC) in a *Pheidole biconstricta* minor worker. Larger gland of the sting complex is the poison gland and below it lies Dufour's gland.

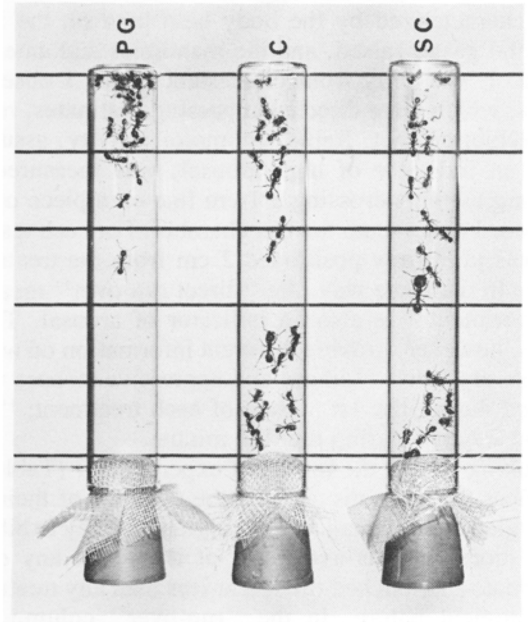

FIG. 2.—Repellent effect of pygidial fluid toward other ants. Typical test in progress. In each vial are 20 *Solenopsis* of the *geminata* species-group. On the upper stopper has been smeared the pygidial gland of one minor worker of *Pheidole biconstricta* (PG); on the bottom stopper are the sting complex and hindgut of the same ant (SC); the central vial is the control with a clean stopper (C). The vertical lines are 1 cm apart. Five min after initiating the test, the number of ants in each centimeter from each odor source was counted.

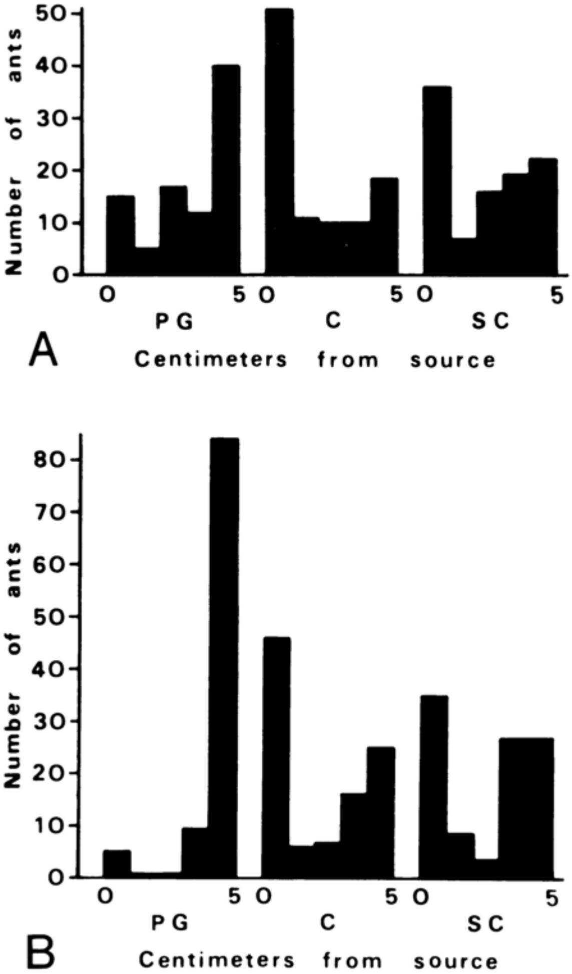

FIG. 3.—Summary distributions of *Solenopsis geminata* workers in response to squashes of glands of *Pheidole biconstricta* (see Fig. 2). Each bar is the sum of 5 tests. A. Response to glands of minor workers. B. Response to glands of soldiers.

cleaned the coated appendage or repeatedly dragged their mouthparts across the substrate in efforts to remove the fluid. In addition to the mechanical irritation, I suspect that the viscous component also serves to delay the escape of the volatile repugnatorial phase and thus increases the latter's persistence. That may explain the large variation in persistence of the volatile phase under different conditions of artificial discharge.

Depending on the site and degree of coverage, the gumming effect of the pygidial fluid can be fatal. Cold-anesthetized workers of a *Solenopsis* of the *geminata* species group were wiped to varying degrees with fresh pygidial fluid; controls were brushed on the head, antennae, and legs with water. The ants revived soon after treatment and began cleaning. Six h later 78.6% of the controls were alive, as opposed to 41.5% of those treated only on the mouthparts and antennae and 16.3% of those smeared on the mouthparts, antennae, *and* the foreleg cleaning organs (n=42, 41, and 43, respectively). The live control ants moved about normally; most of the experimental animals did not. Most mortality probably was due to the viscous component, because these ants do not seem to be fatally affected by even much higher concentrations of the volatiles than experienced in this experiment (see below). Still, it remains possible that the volatile component, when applied topically, can in some way contribute to mortality.

The pygidial fluid is thus a defensive allomone, capable of long- and short-range deterrence against other ants, the most likely enemies of *P. biconstricta* either as predators or competitors. In arranged laboratory confrontations, I have seen individual *P. biconstricta* minors repel workers of a variety of other ant species, including the *Solenopsis* used in the experiments above and a *Neivamyrmex* army ant. Members of the latter genus often raid nests of other ants for brood. Against larger ants, and once against a salticid spider, there was usually no sign of repellence until after serious harm was done to the *Pheidole,* but then both the attacker and others would avoid the injured ant. Against still larger, vertebrate foes, the defense may not work at all: a small *Phyllobates* frog ate these *Pheidole* repeatedly and unhesitatingly.

Intraspecific Effects

Pheidole biconstricta workers seem to be more susceptible to their own defensive secretion than are the *Solenopsis geminata.* They are as easily gummed up by the viscous component and are no better at removing it, and in confinement with strong concentrations of pygidial odors they actually succumbed more rapidly than did *S. geminata.* After only 3 h in a vial 4×1.5 cm separated by a screen from 30 crushed gasters, there was an avg mortality of 23.3%, compared with 1.7% for the controls (11 trials, 10–11 ants/vial per trial; results significantly different at 95% level using Wilcoxon's signed rank test). The *S. geminata* under the same conditions (but with 38–40 ants/vial for 5 trials) did not differ from the controls after 18 h (19.1 and 19.2% avg mortality).

Possible pheromonal functions of the pygidial fluid were investigated through 2 slightly differing experiments (Table 1). In each replication of each experiment 40–50 workers were trapped in a plastic petri dish connected to their nest by a tube. Six randomly ordered treatments were then presented consecutively through a hole in the lid of the chamber, with 30 min between treatments. Treatments were body parts or glands of *P. biconstricta* minors smeared onto slips of heavy paper 5×10 mm. For the control a pencil dot provided similar visual cues, but little or no odor cues. The crushed thorax served as an additional control of a biological nature. All 6 treatments were administered in each replication of the experiments. Experiment 1 was replicated 5 times; experiment 2, 6 times. All ants in any given replication could, of course, respond repeatedly to each treatment, and the numbers in Table 1 are the total numbers of *responses* of all ants given a particular treatment.

Experiment 1 dealt primarily with demonstrating either attractant or repellent properties of the treatments. To increase the instances of obvious avoidance behavior, a small droplet of sugar solution was placed on each treatment strip, 5 mm to the side of the squash. The ants were then drawn in closer to a repellent substance than they normally would venture, and would typically halt abruptly, orient toward the odor, then deflect their course. Only clear-cut cases of avoidance were recorded. As additional indicators of repellence, several indirect but less subjective measures were also recorded: contacts of the smear with antennae or mouthparts, and runs directly over the smear without stopping. Both behaviors should be minimal toward a repugnant substance. All 3 bioassays were recorded during the 1st 15 min after introducing a treatment.

In experiment 2, I measured aggression and arousal caused by the same treatments (except that no sugar solution was used). Two components of overt aggression were noted: lunges and aggressive stances. The latter were characterized by the body held high on the legs, head and gaster raised, and the mandibles and antennae spread. It was only from these stances that I observed lunges, which were directed at passing nestmates, rarely with actual contact. Rapid locomotor activity, assumed to be an indicator of high arousal, was measured by counting the ants crossing a 1-cm line on a piece of paper placed underneath the test chamber. For each test the line was uniformly positioned 2 cm from the treatment smear. In the same way, the "direct run-over" measure of experiment 1 is also an indicator of arousal. The 2 assays, however, provide different information on *where* the ants are active. Lunges and aggressive stances were counted during the 1st minute of each treatment; "running at 2 cm," during the 2nd minute.

Looking first at the results of experiment 1 (Table 1), it is clear that the ants avoided the contents of their pygidial glands more than any other glands. They exhibited much more obvious avoidance of it than of any other treatment, and touched the smear less than any treatment of biological nature. In the "run-over" column, the high values of the control and trunk are due to ants passing over these on their way to the sugar solution, and the significantly smaller response when the crushed pygidial gland is nearby shows repellence. The intermediate values for the head and Dufour's gland suggest a milder level of repugnance (head significantly different from control at 95% level). In contrast, the ants were strongly attracted to smears of the poison gland, but not in order to investigate the smear; they touched the poison gland

Table 1.—Pheromonal properties of the *Pheidole biconstricta* pygidial gland compared with other organs. Experiment 1, bioassays of avoidance or attraction. Experiment 2, bioassays of aggression and arousal. After presentation of a treatment to a chamber of 40–50 ants, each occurrence of each behavior was recorded as described in the text. Numbers are total behavioral events from all replications of the treatments, and may include multiple responses of individuals.

	Expt. 1. Avoidance/Attraction			Expt. 2. Aggression/Arousal		
Treatment	Obvious avoidance	Touch spot	Direct run-over	Aggressive stance	Lunge	Running at 2 cm
Control	3*	5	813*	2*	2*	295*
Trunk	6*	189*	819*	2*	8*	399*
Head	39*	231*	450	6*	7*	404*
Dufour's gland	30*	124*	558	3*	7*	344*
Poison gland	44*	133*	1477*	19	32	320*
Pygidial gland	188	17	299	20	43	537

* Significantly different from pygidial gland (Newman-Keuls test for randomized block design, $\alpha=0.05$).

smear no more than the trunk, head, or Dufour's gland ($\alpha=0.05$). Instead, they generally just ran over the site without stopping (column 3).

Experiment 2 (Table 1) shows that the crushed pygidial glands, like the poison glands, also increase the running activity (i.e., arousal) of the ants, but at 2 cm from the odor source, rather than directly over it. Conversely, the response to the poison gland at 2 cm is no different than toward the control. Both pygidial and poison gland squashes caused significant increases in overtly aggressive behaviors, probably a result of the mechanical disturbance of introducing the test strips into the chamber (see controls) plus the high arousal caused by the contents of these glands. The lunges and aggressive postures generally did not occur after the 1st 15 sec of the tests.

A comparison of the results of both experiments shows that the pygidial gland produces excitement and repulsion. Although the ants at first rushed toward the newly introduced test strip, they nearly always avoided touching the smear, and soon even avoided proximity to it. On the other hand, the arousal and attraction caused by the poison gland smear, together with the marked tendency of the ants to run directly over the smear without stopping, is consistent with the probable existence of a trail pheromone, which is often found in the poison gland of myrmicine ants (Wilson 1971, Maschwitz 1975).

Interestingly, the crushed heads produced no particular excitement or aggressive responses. The mandibular glands in the head usually contain the alarm pheromone in myrmicine ants (Maschwitz 1966, Blum 1969, Wilson 1971), but in *P. biconstricta* crushed heads caused no more overt aggression or arousal than the trunk, which has rarely been implicated in an alarm or defense system (Maschwitz et al. 1970, Maschwitz 1974). Some repulsion is indicated by a low direct run-over response, but not by either obvious avoidance or by direct contact measures. It appears that in this *Pheidole* the pygidial gland has replaced the mandibular glands as the main source of the alarm pheromone.

Gland Discharge and Conditions of Use

The way, and the conditions under which, the pygidial gland is discharged were studied by pinching various parts of live ants while observing their responses through a dissecting microscope. The pygidium is depressed to expose the mouth of the reservoir from under the preceding tergum (Fig. 1), then a contraction of the gaster forces the contents out as a droplet that quickly spreads over the hind segments. A strong contraction can evert the reservoir. These ants, however, do not readily discharge the pygidial gland. They do not raise the gaster and threaten intruders with droplets of defensive secretion, as do some other myrmicines with large defense glands in the gaster (e.g., *Monomorium, Crematogaster,* some *Zacryptocerus*-Kugler 1978b). Furthermore, during arranged fights with other ant species, the reservoir was not obviously discharged (as indicated by glistening fluid on the gaster) until the worker was actually bitten by another ant. Even then, attackers were sometimes repulsed without obvious discharge of the gland. By experimentally manipulating the ants, I found that the discharge stimulus is fairly specific. When only the legs were pinched with forceps, the pygidium would usually be lowered only partially, and only rarely and with repeated pinching would the contents flow out (3 cases out of 20 trials). Similar results were obtained by pinching the antennae (2 of 20) and by lightly pressing the head between the forceps (4 of 20). The gland was discharged more frequently when the waist or neck was pinched (both 10 of 20), and much more frequently when the head was crushed, or the trunk or gaster (Fig. 1) lightly squeezed (15, 16, and 19 of 20 trials, respectively). Thus, these ants seem unlikely to release at least the viscous component until there is a direct assault on the head, trunk, or gaster. The apparent partial opening of the mouth of the reservoir during leg, antenna, and waist pinching, without subsequent release of fluid, plus the observations of repulsion of other ants without obvious gland discharge, suggest that *P. biconstricta* can release the volatile component without releasing the viscous portion.

The strength and specificity of the stimuli required for complete discharge of the pygidial gland seem adaptive. These ants capture live prey by "gang pulling" on all its appendages, and frequently in their excitement, they catch and pull hard on the legs, antennae, or waists of nestmates already tugging at the prey. A low threshold of discharge response obviously would disrupt mass foraging. But the high discharge response of the gaster to even slight compression could be highly advantageous in other situations. These ants are often found on trees and shrubs tending extrafloral nectaries, scale insects, or membracids, and while tending they raise their gasters as high as possible. Of those actually licking *Inga* sp. (Mimosaceae) leaf nectaries, 73.2% (n = 56) had raised gasters, as compared to 3.8% (n = 53) of the nearby, non-tending ants. Similarly, on and around scales on coffee bushes, 82.8% of tenders (n = 29) and 12.5% of non-tenders (n = 136) had gasters up. This behavior may be for protection of the nectar source from competing ants. Raising the gaster has the effect of putting the repugnatorial gland closer to the nectar source, and makes it more likely that the gaster will be the 1st part of the body confronted by an intruder, especially a large one. Interestingly, the large and aggressive *Ectatomma tuberculatum* and *E. ruidum* that are normally some of the most conspicuous arboreal nectar feeders in the Santa Marta area were never seen foraging on the same plants with *P. biconstricta*. Gaster-raising behavior is commonly seen in other nectarivorous arboreal ants with large defense glands in the gaster, e.g., some *Zacryptocerus* species, *Crematogaster* (both Myrmicinae), and *Azteca* (Dolichoderinae) (Buren 1958, Coyle 1966). But unlike most of these, *P. biconstricta* have no obvious modification of the waist segments to permit extreme forward placement of the gaster over the body. Nor do they aggressively swarm over the plant or raise their gasters in response to mechanical disturbance of the plant. Clearly, many interesting questions remain on the chemical ecology of these common neotropical ants.

Discussion

The morphology (Kugler 1978a), the defense and alarm function, and even the odor and dual chemical properties of the *Pheidole biconstricta* (Myrmicinae) pygidial gland are strikingly similar to those of the "anal" glands of some Dolichoderinae (Forel 1878: 58–59; 1928: 81–82; other studies reviewed in Blum and Hermann 1978). Ants of these subfamilies, however, have very different external morphologies and are traditionally considered unrelated (Brown 1954, Wilson 1971, Taylor 1978). In contrast, *Rhytidoponera metallica,* a ponerine ant of the tribe believed to be directly ancestral to the Myrmicinae, produces a sex attractant in its "tergal" gland (Hölldobler and Haskins 1976).

The key to these apparently contradictory observations is that the presence of glandular cells opening onto the anterior edge of the pygidium appears to be a primitive formicid trait. They have now been found in most of the 10 or 11 subfamilies of ants, including all of the most primitive living genera (*Myrmecia, Nothomyrmecia,* nearly all Ponerinae) (Hölldobler and Engel 1979). In the advanced myrmicine, *Pheidole biconstricta,* and the advanced subfamily, Dolichoderinae, the glands are probably secondarily hypertrophied to serve a similar defensive function after the loss of a functional sting (Kugler 1978a, b). A common preadaptation, with selection for a similar function, could account for much of the observed similarity in the glands of these ants. The occurrence of a rather closely analogous viscous/volatile secretion with gumming, repellent, and alarm functions in a termite (Eisner, et al. 1976) indicates the degree of functional convergence that is possible even without a common morphological basis. The "tergal" gland of *R. metallica* can likewise be seen as homologous with anal and pygidial glands, but with a different secondarily evolved function (Hölldobler and Haskins 1977). Because of the very likely common origin of the anal, tergal and pygidial glands, I suggest we refer to them all as pygidial glands, the most morphologically precise of the 3 terms (see Kugler 1978a: 272).

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Voucher specimens of *Pheidole biconstricta* Mayr deposited in the Harvard Museum of Comparative Zoology bear the label "Kugler study 1977."

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