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3 May 1965

Biochemical Polymorphism in Ants

Abstract. *Ants of different sexes and castes produce different odorous compounds. In* Pheidole fallax *Mayr, soldier ants produce an indole base, probably skatole, whereas minor workers produce a trail substance. Males of certain species of* Lasius *and* Acanthomyops *produce mixtures of terpenes and an indole base. These mixtures are discharged during mating flights and probably are used as mating pheromones. The terpene mixtures are qualitatively similar, but each species produces a blend of distinctive proportions. Citronellol and 2,6-dimethyl-5-hepten-1-ol have been identified in the mixtures by gas chromatography and mass spectrometry.*

The various sexes and castes of individual species of social insects often produce different pheromones, and such differences are fundamental to social behavior. As a specific example, only the queen honey bee produces 9-keto-2-decenoic acid, which is a sex pheromone (*1*) and which plays a crucial role in the organization of worker behavior (*2*). Extensive observations of behavior indirectly indicate the widespread occurrence of similar phenomena (*3–5*), which we propose for convenience to term "biochemical polymorphism," since the occurrence of these different molecules must reflect differences in biochemical machinery or in the control of biochemical machinery. We now describe two new examples from the ants.

In Trinidad, West Indies, one of us (E.O.W.) noted that agitated soldiers of the myrmicine ant *Pheidole fallax* Mayr produced a fecal odor. By dissections the odor was quickly localized in the poison gland vesicle, which was also found to be peculiarly hypertrophied in the soldier caste and occupied approximately one-third of the entire abdominal cavity. The odor was characteristic of indole compounds, such as skatole. Paper chromatography of ant extracts (*6*) gave a spot that corresponded in mobility and in color with Erhlich reagent to skatole. The material also gave the Steensma test characteristic of skatole, but not of indole (*7*).

Minor workers of *P. fallax* produce no detectable amounts of the indole compound, and their poison gland vesicle is of normal proportions. On the other hand, minor workers lay odor trails, whereas soldiers do not. The trail pheromone, which is a volatile, true attractant, is produced by Dufour's gland and is disseminated through the sting. In the minor worker the Dufour's gland is exceptionally large in comparison with the same organ in workers of other myrmicine species of comparable size; it is even larger than the associated poison vesicle. In the soldier, the Dufour's gland is either greatly reduced or absent. Moreover, no trail-laying behavior on the part of soldiers was ever observed in field observations in Trinidad, and no detectable trace of the trail substance could be found in the abdomen of the soldier ant by means of the artificial-trail bioassay (*3*), although the soldiers readily follow the trails laid by minor workers. Here, then, is an instance in which one of two castes produces distinct chemical substances not produced by the other caste.

We have also observed that male ants of two members of the genus *Lasius* contain strongly odorous substances, which the workers appear to lack. Furthermore, although workers of *Acanthomyops claviger* (Roger) produce monoterpene aldehydes of strong odor (*8*), which function both as defensive substances and alarm pheromones (*4*), the males produce a mixture of compounds with an odor distinctly different from that of the workers.

Male ants of the species *Lasius neoniger* Emery, *Lasius alienus* (Förster), and *Acanthomyops claviger* (Roger) were collected at nests in the vicinity of Lexington, Massachusetts, while the ants were exhibiting preflight behavior. The crushed ants had two distinct odors. At first one could detect the sweet odor of volatile terpenes and then the fecal odor of an indole compound. Simple dissection showed that both odors originated in the head, whereas the thorax and the abdomen were without odor. The odorous substances were further localized in the reservoir of the mandibular gland, which, prior to the nuptial flights, are relatively large structures, turgid with volatile liquids. Males of *Lasius neoniger*, captured around electric lights in the evening after the mating flights, had little or no odor. Possibly these substances are discharged by the males during the flights, and they might serve as sex pheromones.

The amount of indole compound in

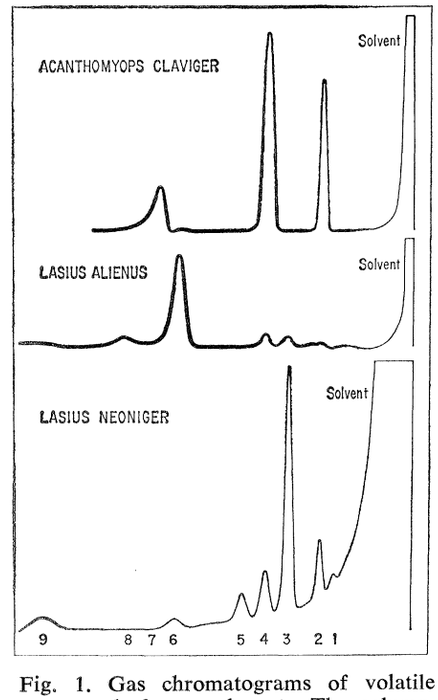

Fig. 1. Gas chromatograms of volatile compounds from male ants. The column, 1.8 m by 0.6 cm, was packed with 10 percent polydiethylene glycol succinate on Chromosorb W. The temperature was 125°C and the argon pressure, 0.7 atm (Research Specialities model 600).

the heads of male ants was even less than that found in the poison glands of *Pheidole fallax*. Paper chromatography of extracts of *Lasius neoniger* again indicated the presence of skatole.

Whole male ants or their heads were crushed in benzene, dichloromethane, or ether. A small amount of anhydrous sodium sulfate was added to remove water, and the solutions were centrifuged or filtered. Portions were withdrawn for gas-liquid chromatography. A portion corresponding to the extract of a single male ant proved sufficient for three or four chromatograms. Samples obtained from heads of *L. neoniger* collected in two successive summers and at different locations around Lexington showed strikingly similar chromatographic patterns, indicating the usefulness of this analysis as a taxonomic tool (Fig. 1). Samples of heads of *L. neoniger* males taken immediately before flight were compared with those of males captured in the evening after flight in the immediate vicinity of the source nests. After flight the amount of the volatile materials was reduced to about 10 percent of the amount present prior to flight.

The chromatograms show that the three species produce different proportions of the same basic group of compounds. Some components seem to be restricted to a single species; for example, component 7 in *A. claviger*, component 5 in *L. neoniger*, and component 8 in *L. alienus*. The distinct difference in the concentration of the components in the closely related *L. alienus* and *L. neoniger* is especially striking.

Use of a mass spectrometer equipped with a gas-liquid chromatographic inlet system (*9*) has permitted the partial or complete characterization of several of the components of these mixtures.

Component 1 has an apparent molecular ion of m/e equal to 138 (*10*), with other significant fragments at m/e equal to 123, 109, and 69. The mass spectrum of component 2 is shown in Fig. 2. The striking similarity of this spectrum with that of citronellol (M = 156) (*10*) led us to deduce that the compound was probably a lower homolog of citronellol. The mass spectrum of 2,6-dimethyl-5-hepten-1-ol prepared from commercial 2,6-dimethyl-5-heptenal (Aldrich) by reduction with sodium borohydride in isopropanol was identical to that of component 2 in extracts of both *L. neoniger* and *A. claviger*.

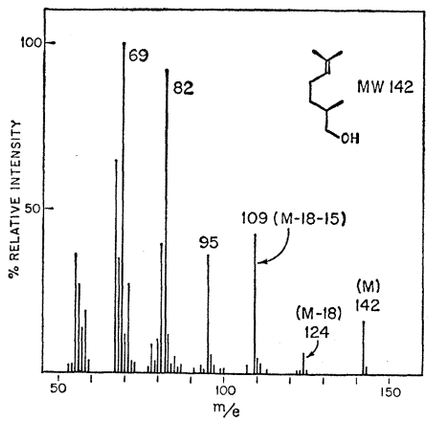

Fig. 2. Mass spectrum of component 2. Spectrums were obtained at 20 ev from an Atlas-Werke CH-4 mass spectrometer, modified to permit direct gas chromatographic introduction of samples (*9*). Spectrums were recorded in 2 to 3 seconds of each component as it emerged from the column. Temperatures: ion source 250°C, helium separators 140°C, column 116°C. The column was similar to that already described and operated at 0.7 atm with helium carrier gas.

Component 3 indicated a molecular ion of m/e equal to 156, and the spectrum was very similar to that of authentic citronellol and that reported by von Sydow (*11*) under similar conditions, except for additional peaks of moderate intensity in the lower mass range (that is, m/e = 96, 85, or 70). On the other hand, component 4 gave a spectrum virtually identical to that of citronellol and the published spectrum. Therefore, component 3 is probably an isomer of citronellol, but its structure is unclear.

Component 5 had a molecular ion of m/e equal to 198, with prominent peaks due to loss of a methyl group (m/e = 183) and elimination of the water (m/e = 180). Component 6, which had a retention time identical to that of geraniol, did not give a clear spectrum when taken from the *L. neoniger* extract. This may be caused by a facile pyrolysis of geraniol under the operating conditions, as well as by the low abundance of ions in the upper mass range, even at 20 ev (*11*). Confirmation of this assignment must therefore await larger samples of material.

Component 7 gave a molecular ion with m/e equal to 142, with additional significant peaks at m/e equal to 127 (loss of CH_3), 124 (loss of H_2O), and 69. This latter peak, which occurs in the spectra of components 1, 3, 4, and 7, may be interpreted (*11*) as arising from the energetically favorable cleavage of the bond in beta position to an isopropylidine group (Fig. 2).

We conclude, therefore, that the volatile components extractable from the heads of male ants are composed of several simple terpenes or terpene derivatives.

Further study will be necessary to determine whether these substances play a role in mating behavior in these species. If the compounds are indeed pheromones, this system will permit the exploration of some important aspects of the chemical evolution of pheromones. These three species are engaged in aerial mating activities in the same localities within overlapping time periods. A promising hypothesis is that the odorous compounds aid in species recognition and isolating mechanisms. Although the two *Lasius* species produce many of the same materials, they apparently produce the different odor components in quite different proportions. Each species is able to blend an odor distinctly its own. Such a multicomponent pheromone system in which the proportions of the substances can be varied could provide an infinite variety of odors from a few simple components, and would obviate the necessity of there being a characteristic odorous compound for each new species. The variety offered by such a system would be limited only by the degree of resolution of chemoreceptors.

The occurrence of indole or skatole along with the floral odors of terpenes is of interest in that indole and skatole are extensively used in the perfume industry as fixatives and modifiers of odor (*12*). At dilute concentration these disagreeable compounds acquire a floral odor (*13*). Skatole has also been isolated from the musk gland of the civet cat, where it occurs along with civetone (*14*); and indole is a component of the odor of several flowers (*12*). The indole base produced by male ants may play a similar role as an odor fixative.

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Desert Locusts: Sexual Maturation Delayed by Feeding on Senescent Vegetation

Abstract. *A diet of senescent* Brassica *spp. delayed sexual maturation in the desert locust. The senescent leaves were shown to be short of gibberellins, and a dietary supplement of gibberellin A_3 (1 microgram per locust per day) restored the rate of maturation to that found in animals feeding on green leaves. An external application of eugenol had a similar effect. The sexual immaturity of desert locusts during the dry season may result from the senescent condition of their desert food plants.*

The time desert locusts (*Schistocerca gregaria* Forskål) take to mature varies considerably. Under favorable conditions both in the laboratory and in the field no more than 3 or 4 weeks may elapse between fledging (imaginal moult) and egg laying. At the other extreme, no eggs may be laid for as long as 9 months after fledging, while delays of 2 to 5 months are quite common. During this period the adults retain their immature color and the ovaries show no signs of vitellogenesis.

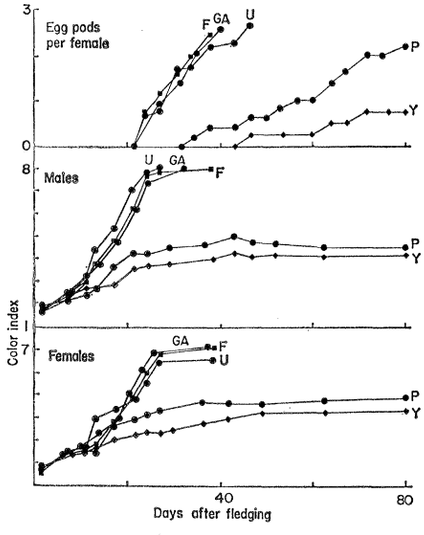

Fig. 1. Egg laying and color change in groups of desert locusts fed on *Brassica* spp. The color index changes from 1 at fledging to 7 or 8 at sexual maturity (*1*). *F*, fed on fresh green leaves; *Y*, fed on yellow, senescent leaves; *GA*, fed as *Y*, but diet supplemented with 1 μg of gibberellin A_3 per day from fledging; *P*, fed as *Y*, but diet supplemented with casein freely available to the locusts; *U*, fed as *Y*, but treated externally with 1 μl of eugenol on the 7th day after fledging.

Our recent studies (*1*) have emphasized the importance of plant monoterpenoids, such as eugenol, in triggering the onset of reproduction, and the importance of a plant hormone, the modified diterpene gibberellin, in hastening ecdysis (*2*). We have therefore investigated what part the physiological state of vegetation might play in controlling the time of maturation. Desert locust swarms frequently spend a large part of their lives in arid zones where the bush is green only for brief periods. During the dry season, when they do not breed, the locusts feed on sere and yellow vegetation. Only after the rains do they have fresh green leaves to eat. We have fed groups of desert locusts, starting about a week before the imaginal moult, on fresh green leaves of *Brassica* spp. (savoy cabbage, kale, and Brussels sprouts) and on senescent leaves of the same species (Fig. 1). Locusts fed the senescent vegetation changed color more slowly and started laying eggs several weeks later than those fed green vegetation.

Evidence is accumulating that aging of leaves is associated with a declining level of one or more of the natural plant hormones, auxins, gibberellins, or kinins (*3*, *4*). Both yellowing and senescence of attached leaves can be retarded by supplying the appropriate hormones, and the hormonal requirements for this retardation appear to be species specific (*4*, *5*). This has led to the proposal that senescence of leaves is regulated by their endogenous hormone levels (*4*). Senescence in all species of *Brassica* so far investigated can be retarded by gibberellin A_3. Hence the old leaves may well contain a relatively low level of gibberellin. A measure of the deficiency of gibberellins in some species of *Brassica* is shown in Fig. 2. It is based on the retention of chlorophyll in excised leaf discs after 2 or 3 days' incubation in darkness on filter papers moistened with either distilled water or gibberellin A_3 solution (25 or 50 μg/liter). Chlorophyll was extracted from the discs in hot 80-percent ethanol, and the optical density of the extract was measured at 665 mμ, the absorption maximum for chlorophyll *a*. Results are expressed as percentage retention of chlorophyll compared with the initial values. Because exogenous application of gibberellin A_3 delays yellowing, it seems likely that senescent *Brassica* leaves are naturally deficient in gibberellins.

When the diet of yellow leaves was supplemented with casein [an adequate protein source for locusts reared on a synthetic diet (*6*)] egg laying started earlier than it did in locusts feeding on yellow leaves alone, but the color change was no more than marginally

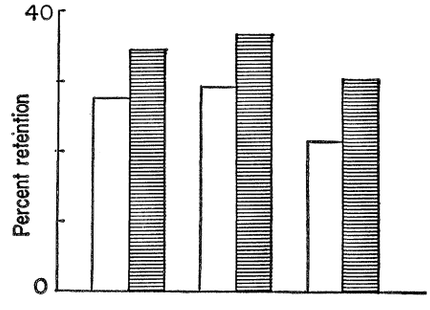

Fig. 2. Retardation of leaf senescence by gibberellin A_3 in three species of *Brassica*, measured by percentage of retention of chlorophyll in treated (cross-hatched blocks) and control (white blocks) leaf discs. Left, kale; center, Brussels sprouts; right, savoy cabbage.