review

Insect pheromones

FRED E. REGNIER and JOHN H. LAW

Department of Biochemistry, University of Chicago, Chicago, Illinois 60637

ABSTRACT The evidence for intraspecies chemical communication in insects is reviewed, with emphasis on those studies where known organic compounds have been implicated. These signal-carrying chemicals are known as pheromones. There are two distinct types of pheromones, releasers and primers. Releaser pheromones initiate immediate behavioral responses in insects upon reception, while primer pheromones cause physiological changes in an animal that ultimately result in a behavior response.

Chemically identified releaser pheromones are of three basic types: those which cause sexual attraction, alarm behavior, and recruitment. Sex pheromones release the entire repertoire of sexual behavior. Thus a male insect may be attracted to and attempt to copulate with an inanimate object that has sex pheromone on it. It appears that most insects are rather sensitive and selective for the sex pheromone of their species. Insects show far less sensitivity and chemospecificity for alarm pheromones. Alarm selectivity is based more on volatility than on unique structural features. Recruiting pheromones are used primarily in marking trails to food sources. Terrestrial insects lay continuous odor trails, whereas bees and other airborne insects apply the substances at discrete intervals.

It appears that a complex pheromone system is used by the queen bee in the control of worker behavior. One well-established component of this system is a fatty acid, 9-ketodecenoic acid, produced by the queen and distributed among the workers. This compound prevents the development of ovaries in the workers and inhibits their queen-rearing activities. In addition, the same compound is used by virgin queen bees as a sex attractant.

KEY WORDS insect attractants · sex attractants · pheromones · releasers · primers · behavior · chemical communication · terpenes · fatty alcohols · alarm · repellents · recruitment · territorial marking · odor

ALL LIVING THINGS are sources of volatile compounds which result from the metabolic activity of the organism. Slight genetic, dietary, and environmental differences make it improbable that any two organisms produce the same blend of volatile organic compounds. This probably accounts for the fact that many animals are able to identify their young or members of their own group in large assemblages of other individuals. Groups of odorants are often species-specific and indicate sex or other intraspecies information. It is not surprising, then, that in the complex interactions of animals with their environment they have come to depend to a large extent on olfactory perception for survival. Olfactory communication is probably one of the oldest and, in some cases, the most efficient means of communication employed by animals. The power of olfactory communication has been witnessed by anyone who has seen how male dogs locate a bitch in heat or how the odor of a virgin female moth will draw males from great distances.

Karlson and Butenandt have proposed the name "pheromone" for the chemical compounds that enable members of the same species to communicate with each other (1). The term pheromone was derived from the Greek "pherein" (to carry) and "horman" (to excite, stimulate). Pheromones and hormones are distinctly different. Whereas hormones are produced in the endocrine glands and released internally to act upon a target tissue within the organism, pheromones are produced and discharged from glands with external ducts. Pheromones function by influencing other members of the same species, not the individual that produced them.

Pheromones affect the central nervous system in two different ways. One class of compounds causes an immediate behavioral response upon reception, as in the case of the sex substances mentioned above. The second group of compounds has a delayed effect on behavior. A typical example is the blockage of ovoimplantation in female mice that have been exposed to the odor of an alien male immediately after mating (2). These delayed responses are the result of physiological changes induced by the pheromones. Chemical stimuli initiating immedi-

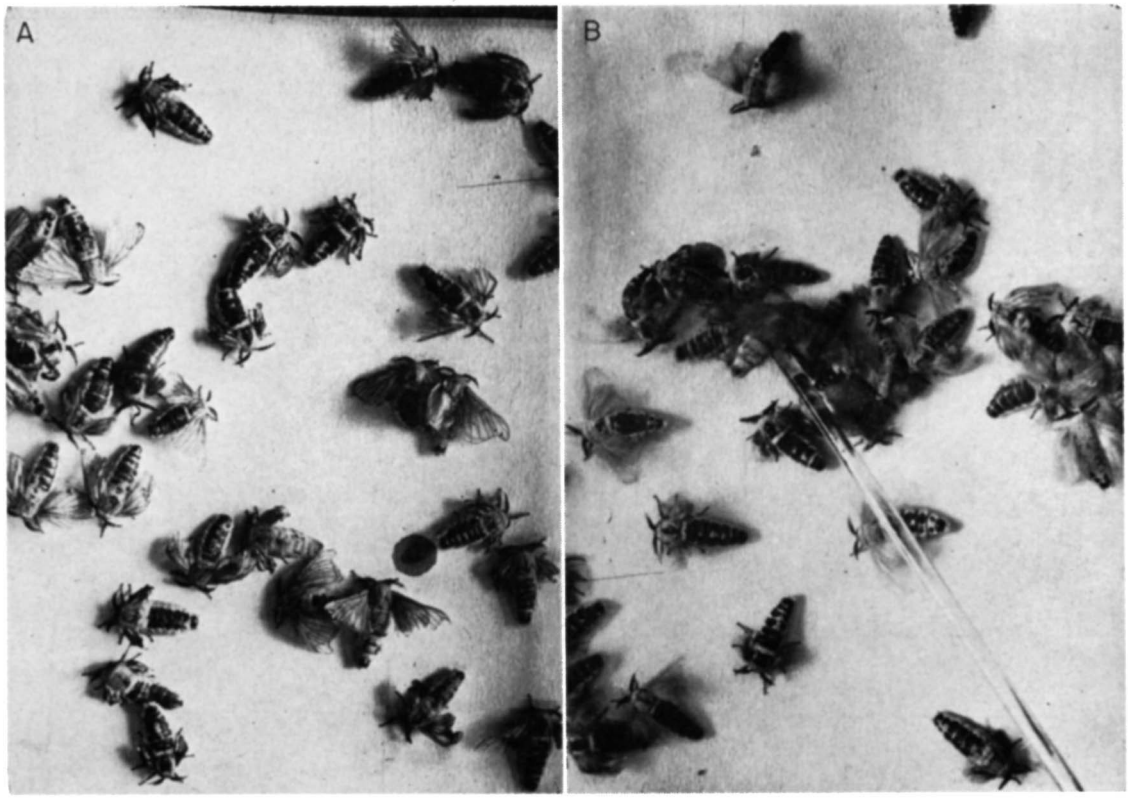


FIG. 1. Behavioral response of *Bombyx mori* males upon reception of the sex pheromone. A, animals at rest; B, glass rod with female attractant is placed into the midst of males.

ate behavioral responses are referred to as *releasers* while those inducing delayed responses are referred to as *primers* (3).

The very nature of chemical communication between isolated animals requires that pheromones be volatile and thus be subject to the laws of gaseous diffusion. Pheromones are in most cases released from the exocrine glands as liquids that evaporate into the surrounding air and form a cloud of vapor about the signalling animal (4). The distance through which a pheromone may transmit a message is a function of the volatility of the compound, its stability in air, its rate of diffusion, olfactory efficiency of the receiver, and, of course, wind currents. Long-distance communication of a mile or more must be mediated by the use of stable compounds with high vapor pressures. The molecular weights of pheromones usually do not exceed 250. A number of pheromones have been found to be lipids, since these compounds have the appropriate chemical and physical properties and apparently react well with chemoreceptors.

Here we shall discuss those systems that have been studied most extensively and in which a definite chemical-behavioral relationship has been established. Types of chemical communication will be categorized according to similarities of the behavioral responses that are initiated.

SEX PHEROMONES

The most thoroughly documented cases of long-range chemical communication are those of sex substances used in signalling a mating partner. For example, female moths release chemical substances into the air to signal their availability, and thereby attract males over long distances (5, 6). In one remarkable case, females of the emperor moth were able to attract males up to 11 km away (6). Males usually perceive the female sex substances by means of their antennae. Removing the antennae or coating them eliminates all sexual response from the males (6).

In 1939 Adolph Butenandt and his colleagues undertook the isolation and identification of the substance responsible for attraction in the commercial silkworm moth, *Bombyx mori*. After 20 yr of extensive work, 12 mg of a derivative of the compound was extracted from half a million virgin females and the active pheromone was identified in 1959 (7). Shortly after emergence from the cocoon the adult female moth releases the sex pheromone into the air by the eversion of the abdominal sacs in which the compound is produced (1, 6). Males are immediately attracted to the female where their excitement increases and leads to the characteristic wing-fluttering (Fig. 1). A simple bioassay was devised in which glass rods were dipped in dilute pentane solutions of the substance and

held 1 cm from the antennae of the male. A positive response consisted of wing-flapping, and a sex attractant unit was defined as the amount of substance that gave a positive response in 50% of the males (7, 8).

The active substance, which was given the name bombykol, was recognized as an alcohol, so the final purification was carried out by successive esterifications with succinic anhydride and 4′-nitroazobenzene carboxyl chloride (9). On the basis of the azo spectral band in the 4′-nitroazobenzene carboxylate ester, the molecular weight of this derivative was calculated to be 475 ± 15. The natural attractant with full biological activity could be regenerated from this ester by saponification. Both ultraviolet and infrared spectrometry indicated the presence of conjugated double bonds in bombykol. The infrared analysis also indicated that the conjugated double bonds probably were in a *cis–trans* arrangement and that the compound was a primary alcohol. Hydrogenation of bombykol yielded cetyl alcohol and therefore demonstrated the presence of a 16-carbon aliphatic skeleton (10). The double bonds in the carbon chain were located by the oxidative cleavage of 1 mg of bombykol with $KMnO_4$. Butyric, oxalic, and ω-hydroxydecanoic acids were isolated as the only degradation products; they accounted for the total carbon skeleton and showed the double bonds to be at the 10 and 12 positions. Thus bombykol was shown to be 10,12-hexadecadien-1-ol. However, this still left the possibility that bombykol might be one of several geometrical isomers.

The structure of bombykol was proved by a combination of synthesis and biological testing (8). *trans*-10-*cis*-12-Hexadecadien-1-ol was 10^9 times more active than the other isomers; it was the only isomer with activity equivalent to natural bombykol (6). Any uncertainty about the structures of the synthetic isomers was eliminated by three independent synthesis.

Shortly after the purification of bombykol, Jacobson, Beroza, and Jones (11, 12) isolated and characterized the sex attractant of the gypsy moth. The last abdominal segments from 500,000 females were used in the isolation of the sex pheromone. The infrared spectrum of the purified attractant (termed "gyptol") indicated the presence of a primary hydroxy group, a secondary acetoxyl group, and a *cis* double bond. Quantitative catalytic hydrogenation of gyptol confirmed the presence of a single double bond. Oxidation of the natural attractant with periodate–permanganate reagent gave 3-acetoxy-1-nonanoic acid and a viscous oil, which was further oxidized to pimelic acid. Gyptol was therefore proposed to be 10-acetoxy-*cis*-7-hexadecen-1-ol. That this compound is the natural attractant was confirmed by the synthesis of material with activity equivalent to that of the natural attractant.

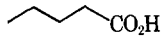
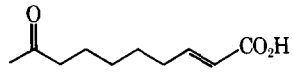
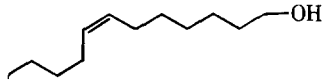
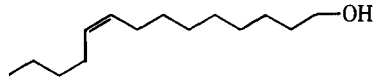
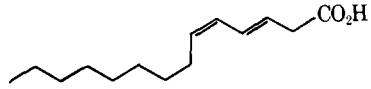
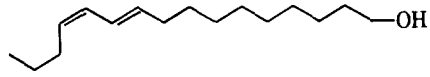
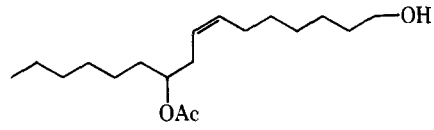
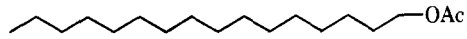
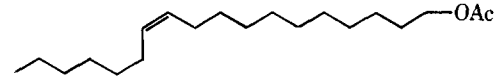
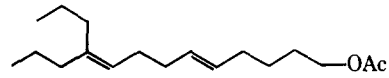
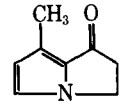
In the past few years a series of sex pheromones has been isolated and characterized (Table 1). Several of the pheromones that have been identified are simple straight-chain aliphatic compounds. It is probable that these are formed biosynthetically in the same fashion as fatty acids. For example, it has been shown in Butenandt's laboratory (20) that the fatty acids extracted from *B. mori* are inactive as sex attractants, but that reduction with $LiAlH_4$ yields a preparation with pheromone activity. These results imply that an acid with the bombykol chain is present in the insect and that this acid may be converted to bombykol by reduction of the carboxyl group.

There have been numerous reports of males producing aphrodisiacs (21), but these reports have not been confirmed by the use of synthetic chemicals in biological assays. Male butterflies of the subfamily *Lycorea* possess a pair of extrusible, odoriferous, brushlike structures on the posterior of their abdomens. These odor-producing structures have been named "hair pencils." During mating the males extrude the "hair pencils" while in aerial pursuit of the female. The male induces the female to alight on available herbage by brushing the "hair pencils" against her antennae. "Hair penciling" the female's antennae continues until she is acquiescent, at which time copulation occurs (22). Meinwald, Meinwald, Wheeler, Eisner, and Brower (18) have found three compounds of the "hair pencils," a pyrrolizidine and two aliphatic esters (Table 1). The biological function of these compounds is not known. However, the manner in which the "hair pencils" are used suggests that these compounds are used in mating.

Chemoreception of sexual odorants by male insects has been demonstrated to be highly efficient and selective. Synthetic bombykol gives a positive response in 50% of the challenges at a concentration of only 10^{-12} μg/ml of pentane when assayed by the technique previously described (7, 8). Boeckh, Kaissling, and Schneider (23) have shown that *B. mori* males will respond to air streams containing as little as 200 molecules of bombykol per cm^3. Bombykol is thus one of the most biologically active substances known to man. This remarkable level of activity may be typical of the long-range attractants, which must be highly effective because of the large volume of air through which they diffuse before reaching the olfactory receptors of the male. Other sex pheromones, shown in Table 2, that have high biological activity include gyptol, and the sex substance of the fall army worm. Unfortunately, the attractancy of the compounds is not directly comparable because of differences in assay techniques.

The selectivity of males for their sex pheromones is as extraordinary as their sensitivity. A single change in the stereochemistry of one of the double bonds in bombykol will decrease the biological activity a billionfold (8). Of the four possible geometrical isomers, only one of the bombykol isomers has any significant biological activity.

TABLE 1 INSECT SEX PHEROMONES

Insect	Compound	Structure	Reference
Sugar beet wireworm (*Limonius californicus*)	Valeric acid		13
Honeybee (*Apis mellifera*)	9-Keto-2-decenoic acid		14
Cabbage looper (*Trichoplusia ni*)	*cis*-7-Dodecen-1-ol		15
Fall army worm (*Laphygma frugiperda*)	*cis*-9-Tetradecen-1-ol		16
Black carpet beetle (*Attagenus megatoma*)	*trans*-3-*cis*-5-Tetradecadienoic acid		17
Silk worm moth (*Bombyx mori*)	*trans*-10-*cis*-12-Hexadecadien-1-ol		7
Gypsy moth (*Porthetria dispar*)	*d*-10-Acetoxy-*cis*-7-hexadecen-1-ol		11
Male butterfly (*Lycorea ceres ceres*)	Cetyl acetate		18
Male butterfly (*Lycorea ceres ceres*)	*cis*-Vaccenyl acetate		18
Pink bollworm (*Pectinophora gossypiella*)	10-Propyl-*trans*-5,9-tridecadienyl acetate (Propylure)		19
Male butterfly (*Lycorea ceres ceres*)	2,3-Dihydro-7-methyl-1 H-pyrrolizidin-1-one		18

In the case of synthetic gyptol, only the *cis* isomers have biological activity equivalent to that of the natural attractant (21). However, the *d*- and *l*-isomers are equally active. It has been found that a synthetic homologue of gyptol known as gyplure (*d*-12-acetoxy-*cis*-9-octadecen-1-ol), which is readily prepared from ricinoleic acid, has biological activity approaching that of the natural pheromone in both laboratory and field tests (24). The *trans* isomer of gyplure has little activity. The same specificity is exhibited by the fall army worm for *cis*-9-tetradecen-1-ol; only the *cis* isomer is active (16).

The possibility that pheromones may be used in the control of insect populations has drawn an unusual amount of publicity. Many workers have proposed the use of attractant in drawing males to an area where they may be exterminated. In fact, Wood et al., (25) have shown by using synthetic pheromones that bark beetles may be drawn into traps. However, the use of a synthetic pheromone in conjunction with an insecticide or chemosterilant has not been demonstrated. A second technique of control is the saturation of a mating area with such high concentrations of sex pheromone that olfactory orientation between the sexes is inhibited. Gaston, Shorey, and Saario (26) have recently used this technique in field tests on the cabbage looper.

ALARM PHEROMONES

Alarm pheromones are used primarily by social animals to warn other members in the colony of impending danger. The behavior of most animals upon reception of an alarm signal is basically the same. They initially orient osmotactically to the source at low pheromone concentration and at high concentration go into frenzied activity, occasionally attacking the pheromone source. This may easily be demonstrated in ants by placing the crushed body of a worker near the nest entrance. In most cases those workers passing within a few centimeters of

TABLE 2 COMPARATIVE ATTRACTANCY OF SOME NATURAL AND SYNTHETIC COMPOUNDS AS SEX ATTRACTANTS

Insect	Compound	Attractancy*	Reference
Silkworm moth (*Bombyx mori*)	10-*trans*-12-*trans*-Hexadecadien-1-ol	1 μg/ml	8
	10-*trans*-12-*cis*-Hexadecadien-1-ol	10^{-12} μg/ml	8
	10-*cis*-12-*trans*-Hexadecadien-1-ol	10^{-3} μg/ml	8
	10-*cis*-12-*cis*-Hexadecadien-1-ol	10 μg/ml	8
	Bombykol (natural)	10^{-10} μg/ml	8
Gypsy moth (*Porthetria dispar*)	*d,l*-10-Acetoxy-*cis*-7-hexadecen-1-ol	10^{-12} μg	21
	d-10-Acetoxy-*cis*-7-hexadecen-1-ol	10^{-12} μg	21
	l-10-Acetoxy-*cis*-7-hexadecen-1-ol	10^{-12} μg	21
	Gyptol (natural)	10^{-12} μg	21
	d-12-Acetoxy-*cis*-9-octadecen-1-ol	10^{-12} μg	21
	d-12-Acetoxy-*trans*-9-octadecen-1-ol	10^{4} μg	21
	d-14-Acetoxy-*cis*-11-eicosen-1-ol	10^{-2} μg	21
Fall army worm moth (*Laphygma frugiperda*)	*cis*-9-Tetradecen-1-ol	3×10^{-6} μg	16

* Attractancy in *P. dispar* and *L. frugiperda* is expressed as the minimum amount of material that is effective in attracting males. Attractancy in *B. mori* is expressed as that concentration of material in pentane on a glass rod that will attract 50% of the males tested.

the injured worker will respond with alarm behavior. All of the alarm pheromones at present identified are of insect origin. The behavioral response thresholds for alarm substances vary from 10^{8} to 10^{11} (27, 28) molecules of odorant per cm^3 of air. The response thresholds for alarm substances are probably 10^{7} to 10^{10} times higher than sex pheromones such as bombykol and gyptol.

The discrimination of structural features in alarm pheromones is considerably less than for sex pheromones. Blum, Warter, and Traynham (29) have shown, with the ant *Iridomyrmex pruinosis*, that the alarm response to the natural pheromone 2-heptanone is mimicked by many other acyclic ketones of comparable volatility. The same effect is seen in the reaction of the ant, *Acanthomyops claviger*, to its alarm pheromones. This animal is known to use citral, citronellal, undecane, tridecane, and 2-tridecanone as alarm substances (27, 30). When these compounds and their analogues were tested behaviorally, olfactory efficiency varied with molecular weight rather than structure. In this animal, the C_{10}–C_{13} hydrocarbons produce optimal signalling because, apparently on the basis of molecular weight alone, they combine moderate olfactory efficiency with moderate vapor pressure. It is thus possible for the animal to broadcast in the centimeter range when the compounds are present in microgram quantities.

Other insects also produce medleys of alarm pheromones. The honeybee produces 2-heptanone in the mandibular glands (31) and a series of other compounds in the sting apparatus. Isoamyl acetate, the principal alarm pheromone of the sting apparatus (32), is used to signal other workers to sting in the same place, thus amplifying the defense capabilities of the group. Compounds that have been shown to be alarm pheromones are shown in Table 3.

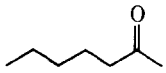
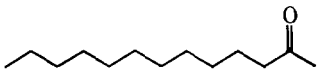
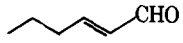
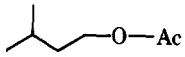
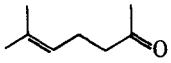
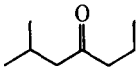
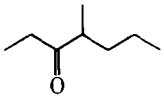
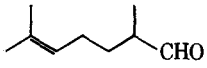
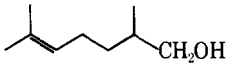
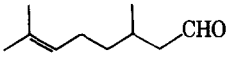
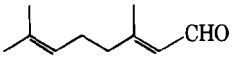
It is now apparent that alarm pheromones, in addition to signalling danger, may also function as defensive substances (27). *Acanthomyops claviger* has two organs that secrete compounds with this dual role: the mandibular and Dufour's glands (Fig. 2). The mandibular gland produces large concentrations of terpenes which may function as defensive repellents. Dufour's gland is an accessory organ to the formic acid-producing venom gland. During the act of stinging, formic acid and hydrocarbons are discharged simultaneously from these glands in fine droplets. The hydrocarbons act as spreading agents for formic acid in addition to being alarm pheromones. When one considers that the members of this subfamily produce from 0.1 to 2% of their body weight in mandibular and Dufour's gland substances and from 1 to 20% of their body weight in 50% formic acid (44), it is easily seen that these ants can be formidable warriors.

RECRUITMENT PHEROMONES

Recruitment by chemical communication appears to be widespread in social insects and has been demonstrated in bees, termites, and ants. Trail-marking substances have been the most widely studied of the recruiting pheromones. Trail pheromones are used by animals as navigational aids in directing other members of the colony to a distant location, varying from hundreds of meters in bees to meters in terrestrial insects. The reasons for orienting members of the colony to a distant point may vary. In most cases, trails are laid by foraging workers as they return from a food source. These trails are then used by other foragers (45–48). In other cases, however, trails may be laid to recruit workers for slave raids, colony emigration, or the repair of a breach in the nest wall (46).

Different types of trail marking are found in terrestrial insects and flying insects. The terrestrial insects appear to lay a continuous or nearly continuous trail between points. Wilson (45) has shown that the fire ant (*Solenopsis saevissima*) drags its sting and lays a trail in a manner similar to a pen inking a line. If the food source is of good quality, other workers choose to reinforce this trail and a highway several centimeters wide may be formed. Walsh,

TABLE 3 ALARM PHEROMONES

Compound	Structure	Species,	Response Threshold (*molecules/cm*^{3})	Reference
Heptan-2-one		*Iridomyrmex pruinosis, Apis mellifera* *Conomyrma pyramica, Atta texana*		33, 31 34, 28
Tridecan-2-one		*Acanthomyops claviger, Lasius umbratus*	3×10^{10}	27, 35
Undecane		*Acanthomyops claviger, Lasius umbratus*	10^{11}–10^{12}	27, 35
Tridecane		*Acanthomyops claviger*		27
2-*trans*-Hexan-1-al		*Crematogaster africana*		36
Isoamyl acetate		*Apis mellifera*		32
2-Methyl-2-hepten-6-one		*Tapinoma nigerrimum, Iridomyrmex detectus* *I. conifer, I. nitidiceps*		37, 38 39, 40
2-Methylheptan-4-one		*Tapinoma nigerrimum*		37
4-Methylheptan-3-one		*Pogonomyrmex barbatus, P. badius* *P. californicus, P. occidentalis* *Atta texana*	 2.7×10^8	41, 41 41, 41 28
2,6-Dimethyl-5-hepten-1-al		*Acanthomyops claviger*		27
2,6-Dimethyl-5-hepten-1-ol		*Acanthomyops claviger*	10^{12}	27
Citronellal		*Acanthomyops claviger*	10^{11}	27, 30
Citral		*Acanthomyops claviger, Atta sexdens*	7×10^{10}	27, 30, 42
α-Pinene		*Nasutitermes exitiosus*		43

* All of the insects referred to are ants, with the exception of *Apis mellifera* (honeybee) and *Nasutitermes exitiosus* (termite).

Law, and Wilson (49) isolated 250 μg of this pheromone from 200,000 workers but did not determine its structure.

Wilson has proposed that the vapors emanating from the volatile trail laid by *S. saevissima* form a corridor of pheromone through which workers move (4). As the lateral distance from the liquid trail increases, a point is reached at which the animal can no longer sense the pheromone. As the liquid source is depleted by evaporation, the odor corridor contracts until the pheromone concentration is lower at all points than the threshold concentration. The time required for this fade-out in *S. saevissima* is about 100 sec (3). Other ants, however, lay trails which last for days (50).

Moore (51) has isolated and partially characterized a termite trail pheromone which is common to several species of *Nasutitermes*. The ultraviolet spectrum showed only end absorption below 220 mμ and the infrared spectrum included strong bands at 3070, 1640, and 880 cm^{-1}, which suggested one or more α-substituted vinyl groups. Catalytic hydrogenation shifted the molecular weight from 272 to 280 (shown by mass spectrometry). On the basis of these data, Moore proposed that this pheromone is a monocyclic diterpene hydrocarbon with four double bonds having an empirical formula of $C_{20}H_{32}$.

Airborne insects, on the other hand, leave patches of odorants to mark their hive or sources of food (52, 53).

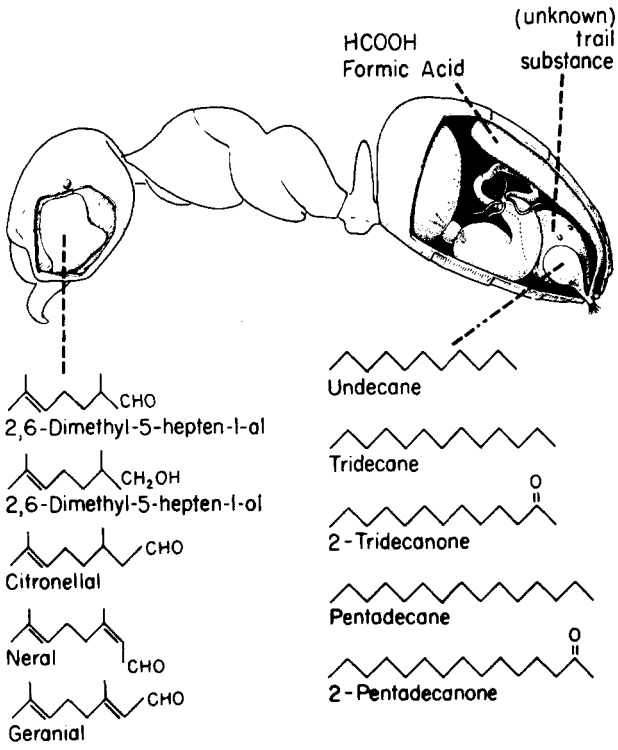


FIG. 2. Volatile substances found in the exocrine gland of the ant, *Acanthomyops claviger*.

Bees apparently mark the nest and rich food finds with the Nassanoff gland contents. Geraniol, citral (geranial and neral), geranoic acid, and nerolic acid (Table 4) have been identified as constituents of the secretion of this gland and have all been shown to be attractive to other bees (54, 55). The principal constituent of this galnd is geraniol, which is produced in amounts up to 1.5 μg per bee. The stingless bees of the genus *Trigona* have extended this system by leaving a trail of odor patches on the ground and vegetation at 5- to 15-meter intervals between the hive and a food source (53). These patches are then reinforced by returning foragers until a system of guide posts is established.

The feces and frass produced by male bark beetles (*Ips confusus*) that have been feeding on fir trees contain a volatile attractant which causes a mass infestation of the area by both sexes (56). The attractant has been found in the hindgut and Malpighian tubes of males but does not occur in the wood on which they have been feeding. Pheromone activity has never been found in female tissue or their frass. Males begin producing the attractant within a few hours after they infest a fir log. Production increases through the next 96 hr and then gradually decreases through the 14th day. A detectable response from females is produced by benzene extracts equivalent to 3×10^{-8} g of frass. Silverstein, Rodin, and Wood (57) have identified three terpene alcohols (Table 4) that account for a part of the total activity of the frass. These compounds were inactive when tested individually, but elicited an attractive response when mixed. Recruitment in the laboratory was achieved by mixing 1 μg of (−)-2-methyl-6-octen-4-ol with either 0.01 μg (+)-*cis*-verbenol or 1 μg of (−)-2-methyl-6-methylene-2,7-octadien-4-ol. Since these compounds account for only a part of the total recruiting activity of the frass, there are apparently other recruiting substances. The biological function of these pheromones, aside from simple recruitment, is not known. However, the fact that the pheromones are produced only by one sex tends to implicate them in reproduction.

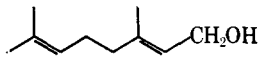
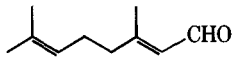
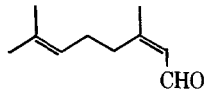
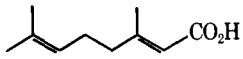
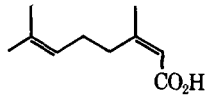
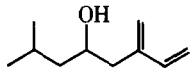
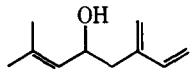
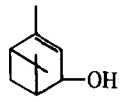
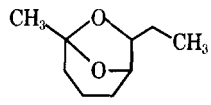
A similar type of recruitment exists in the western pine beetle (*Dentrotonus brevicomis*). The recruiting substance in the frass produced by female *D. brevicomis* boring in ponderosa pine also initiates a mass attack on the tree (58). This compound has been identified as the cyclic ketal *exo*-7-ethyl-5-methyl-6,8-dioxabicyclo(3.2.1)octane and has been given the trivial name brevicomin (59). Since the synthetic compound attracts both sexes and has not been reported to release sexual behavior on the part of either the male or the female, it may not strictly be classified as a sex pheromone. Determination of the exact biological status of this compound awaits further study.

PRIMER PHEROMONES

In some cases single substances can serve a complicated variety of functions. An excellent example is the role of 9-keto-2-decenoic acid in the control of behavior in the honeybee society. As indicated in Table 1, this compound is a sex pheromone, since it is released by the virgin queen bee and serves to lure drones to the receptive female during mating flights (14). In a number of cases, mated female insects cease to produce their sex pheromone upon mating (60), but this is not the case with the honeybee queen. When the newly mated queen returns to her hive, she continued to produce ketodecenoic acid, which has important primer functions of an inhibitory nature (61, 62).

The honeybee society is composed primarily of female insects. Drones appear only in summer for reproductive purposes; they contribute nothing else to the honeybee society. Queen bees can arise from the same fertile egg as do workers. The differences are not genetic but arise from the special treatment given queen larvae by workers. Under *normal* conditions a hive will have only one functional queen, but may have 30,000 to 80,000 workers at the height of summer (63). Introduction of a second fertile or even a virgin queen will lead to a death fight between the queens. If the queen is removed from the hive, workers act quickly to produce a new queen. This is done by building enlarged chambers (queen cells) which are filled with a food substance (royal jelly) secreted from the mandibu-

TABLE 4 RECRUITING PHEROMONES

Insect	Compound	Structure	Reference
Honeybee (*Apis mellifera*)	Geraniol		55
Honeybee (*Apis mellifera*)	Geranial		56
Honeybee (*Apis mellifera*)	Neral		56
Honeybee (*Apis mellifera*)	Geranoic acid		55
Honeybee (*Apis mellifera*)	Nerolic acid		55
Bark beetle (*Ips confusus*)	2-Methyl-6-methylene-7-octen-4-ol		58
Bark beetle (*Ips confusus*)	2-Methyl-6-methylene-2,7-octadien-4-ol		58
Bark beetle (*Ips confusus*)	*cis*-Verbenol		58
Beetle (*Dendrotonus brevocomis*)	Brevocomin		60

lar gland of young worker bees. A single young larva is placed into the queen cell. Feeding on a large amount of royal jelly causes differentiation to the queen type of adult female. In this way the honeybee colony can rear a number of virgin queens. The survivor of a series of death fights will become the new mother queen of the hive.

A second effect of the removal of the queen is enlargement of the usually atrophied worker ovaries. This takes place over a period of weeks until workers can actually begin to lay eggs. Such eggs usually give rise only to drones.

Both queen-rearing behavior and worker egg production are inhibited by 9-keto-2-decenoic acid secreted by the queen (64, 65). Inhibition of these activities with synthetic ketodecenoic acid requires rather large doses, and there is evidence that the queen produces synergists which potentiate the activity of ketodecenoic acid (65). Johnston, Law, and Weaver (66) have studied the metabolism of labeled ketodecenoic acid by worker bees and find that it is metabolized to three inactive acids (Fig. 3). They have further postulated a "pheromone cycle," in which inactive metabolites such as compounds 2, 3, and 4 (Fig. 3) are fed back to the queen who would then transform them into active compounds. In fact, these compounds are found in the mandibular glands of queens along with ketodecenoic acid (67). The queen is thought to use compound 3 in controlling the behavior of swarming bees (68, 69).

ANALYSIS OF PHEROMONAL COMMUNICATION

Many of the studies described here are purely qualitative and descriptive, dealing primarily with identification of pheromones and the effects of various organic compounds on behavior. Fundamental studies of the importance of a signalling odorant in the life of an animal necessitate a more quantitative analysis of the communication system. Since the signal vehicle in chemical communication is an organic compound, the spatial pattern of a message is determined by the transmission characteristics of the compound in air. The distance through which a chemical message may be transmitted in still air is a function of the volatility of the compound, its rate of diffusion, its stability in air, and the olfactory efficiency of the receiver. Bossert and Wilson (4) have derived an equation which predicts the diffusion behavior of a volatile odorant on the basis of these parameters. Pheromone concentra-

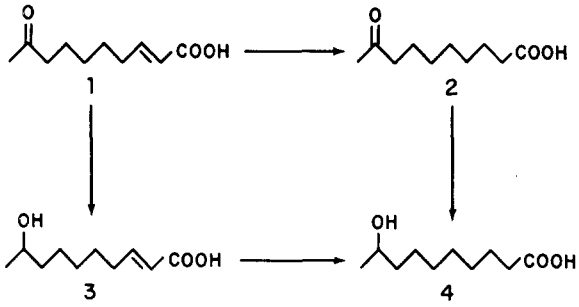


FIG. 3. Metabolism of 9-keto-2-decenoic acid.

tion at various distances from a point source as a function of time may be calculated from the formula

$$U(r,t) = (Q/2D\pi r)\ \text{erfc}\ \{r/(4Dt)^{1/2}\}$$

where the concentration $U(r,t)$ is measured in molecules/cm^3, and Q is the emission rate of vapor from the source in molecules/sec; D, the diffusion coefficient of the substance in air in cm^2/sec; r, the distance in cm from the emission source; t, the time in seconds from the beginning of emission; and erfc, the complementary error function.

If an alarm pheromone is released in a group of resting ant workers, alarm is seen to spread outward from the source in a wave as the pheromone diffuses. Actually, the pheromone diffuses outward at a rate faster than the proliferation of alarm seen in the workers. The workers do not go into alarm behavior until the concentration of pheromone at their antennae is high enough to release alarm behavior. This concentration, in molecules/cm^3 at the chemoreceptors, is called the behavioral threshold concentration (K). If the value $U(r,t)$ in the diffusion equation cited above is made equal to K, the behavioral threshold concentration may be determined experimentally.

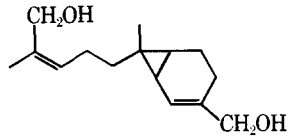
When a liquid pheromone in a glass capillary tube is placed near a group of animals, the responding animals become in effect measuring devices for the estimation of threshold concentrations. The time (t) from the presentation of the pheromone to the onset of the response and the distance (r) from the emission source to the sensory organ of the animal may be inserted directly into the diffusion equation. Q, the emission rate of the odorant in molecules/sec, is measured by observing the decrease per unit time in volume of the liquid pheromone in the capillary with the aid of an ocular micrometer. The diffusion coefficient (D) is either obtained from standard tables or measured experimentally. Using these values in the diffusion equation provides a practical means for obtaining behavioral threshold concentrations (K).[1]

[1] Wilson, E. O., W. H. Bossert, and F. E. Regnier. Manuscript in preparation.

The accurate determination of behavioral threshold concentrations of pheromones is a prerequisite to the assessment of relative effectiveness of pheromones, synergism, diurnal variation in receptivity, and the design of pheromonal control systems.

OTHER PHEROMONE SYSTEMS

Pheromonal communication is by no means confined to insects. Well-documented cases of chemical communication are to be found in both higher and lower organisms. In the water mold *Allomyces*, unopened gametangia produce "sirenin," a strong attractant to male gametes (70). Sirenin is active at a concentration of 10^{-10} M and has been shown (71) to have the following structure



This attractant is the first structurally characterized plant sex pheromone. The slime mold, *Dictyostelium discoideum*, also produces an aggregating substance (72). This chemical stimulant, called acrasin, causes aggregation of the mold cells to form a multicellular organism.

There are a few chemical and behavioral studies on the releaser pheromones of higher animals. Identification of sexual partners has been shown to be mediated by odors in salamanders (73), fish (74), and snakes (75). While olfactory communication is not crucial to mating in many animals, it probably provides valuable information that facilitates mating. Male rabbits use their chin gland secretions to mark a network of objects and thus label territories (76). This territorial marking may serve as an advertisement to sexually receptive females and discourage encroachment by other males. The use of scent glands, urine, and feces to mark territories and trails is a common practice among animals (77). The mongolian gerbil marks objects with the secretion of a midventral sebaceous gland which is under androgen control (78). It is possible that the oily secretion from this gland acts as a pheromone to signal territorial possession. Trails left by an estrous black-tailed deer are used by the sexually active buck to locate the female (79).

Several pheromones that modify reproductive physiology in mice have been recognized. The most spectacular of these primer effects is the blockage of pregnancy in recently mated females by the odor of a strange male (80). These alien male odors cause many of the newly mated females to return to estrus within a week. Groups of isolated females produce pheromone(s) that increase the length of their estrous cycle (81) while pheromone(s) from a male negate this effect in addition to shortening

and synchronizing the estrous cycle (82). The general importance of primer pheromones in higher animals is not known.

It is not suprising that sexual discrimination and recognition of various stages of the estrous cycle in higher animals may be achieved by olfaction. Hormonally induced physiological changes are the result of changes in body chemistry and therefore should significantly alter the olfactory "signature" of an animal. In fact, it is possible that metabolites of the horomones themselves possess sufficient volatility to be used in olfactory communication (83). Given the proper olfactory sensing device, it is probable that these various physiological states could be recognized in most mammals.

When one considers a biological phenomenon which occurs widely amongst animal species, the question of whether it extends to primates, including man, inevitably arises. Unfortunately, there is essentially no information about the occurrence of pheromonal systems in man. It seems likely that such systems may be present, and it is even possible that man has generally lost awareness of them because he has developed other effective communication systems. This remains an intriguing area for future investigation.

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