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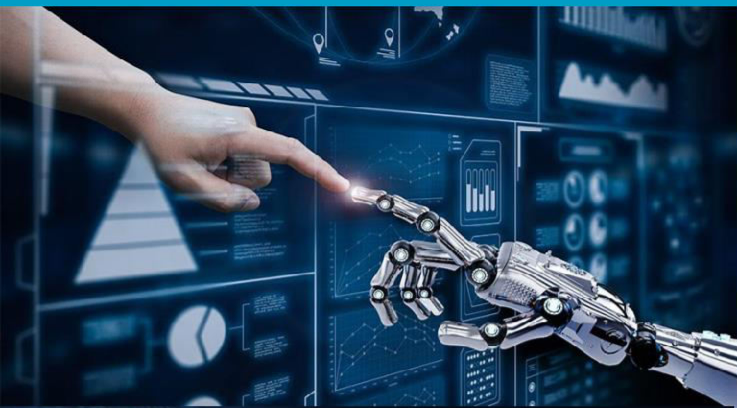

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Pheromones- Chemical Structure and Significance

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ABSTRACT: The first pheromone was identified in 1953. This pheromone, called BOMBYKOL is secreted by female moths and carried a "come to me" signal to males. This signal can travel large distances and is effective at low concentrations.

The mammalian pheromone system was more difficult to understand because mammals, far from having the simple stereotyped behaviour of insects, have complex and independent behaviour. Mammals were found to detect pheromones through an organ in the nose called the VNO, which is similar in function to the olfactory membranes but connects to the hypothalamus.

Scientists were beginning to speculate whether a pheromone system existed in humans. In the 70's Martha McClintock noticed that females living in close proximity underwent a changing of phase of their menstrual cycles causing them to ovulate at similar times.

KEYWORDS-pheromones, chemical, structure, significance, mammals

I. INTRODUCTION

Aggregation is one of the most remarkable behaviours in the animal kingdom. Classically, aggregation behaviour has been viewed as evolutionarily advantageous, whereby individuals gain the benefits of protection, mate choice, and resource exploitation1. In gregarious insects, aggregation pheromones facilitate mate choice, group foraging, and collective gathering2. Aggregation pheromones gather conspecifics near the pheromone source, either by attracting them from a distance (attractant activity) or inducing passing conspecifics to remain at the pheromone source (arrestant activity)3. These pheromones can comprise a sole compound or multiple compounds. In sole-compound pheromones, a single compound plays the role of both attractant and arrestant;4 in multiple-compound pheromones, some compounds act as attractants and others act as arrestants5. Aggregation pheromones have been identified in various gregarious insects including cockroaches, stink bugs, bed bugs, locusts, fruit flies, bark beetles, longhorn beetles, and bees2,6. Social insects can also demonstrate self-assemblage; however, few studies have explored the proximate mechanisms of this aggregation behaviour.[1,2,3]

Termites are eusocial insects that occupy dead wood (their food source and habitat) at high densities. Termite colonies with a large number of siblings develop a remarkable caste-based division of labour; the worker caste collectively displays coordinated behaviours such as migration, nest construction, foraging, and recruitment8. Termites are typically classified into three categories based on their nesting habits: one-piece nesters (species which nest in and feed on a single piece of wood), multiple-piece nesters (species which nest in multiple wood pieces connected by underground tunnels and aboveground shelter tubes), and separate-piece nesters (species whose nests are physically separated from their food resources)9,10. In one-piece and multiple-piece nesters, the royal chamber typically acts as a central locus for foraging and feeding behaviours; the colony expands the nest area by feeding and foraging outward from the chamber, which is typically located deep in an inhabited wood piece. Therefore, workers and soldiers are densely distributed in the central and foraging areas11. When foraging workers discover a new food source, they gather their nestmates to that area; they first exploit the new foraging area, and later colonise it. Termites aggregate to a high density during this process because they require allogrooming and trophallaxis to exchange nutrients, gut symbionts, and immune substances with each other, which enables them to survive in oligotrophic and microbe-rich environments8. Thus, workers are susceptible to the Allee effect;12 rapidly formed yet long-lasting aggregation in newly colonised wood is essential to the ecological success of one-piece and multiple-piece nesting termites.

Reticulitermes termites are multiple-piece nesters with a sophisticated pheromone communication system13,14,15,16,17,18. Previous studies have suggested the existence of a chemical signal associated with worker aggregation, but this compound was not previously identified. Labial gland extracts derived from workers of *Reticulitermes santonensis* and *Schedorhinotermes lamanianus* have been shown to elicit gnawing and feeding

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behaviours, which result in the aggregation of feeding workers19. Other studies have shown that extracts of worker *Reticulitermes* termites have attractant activities to other workers20; the volatile components, 3-octanone and 3-octanol, play a role in corpse recognition cues and can induce aggregation-like conditions for corpse retrieval and cannibalism21. Moreover, termites have been shown to secrete a trail pheromone to lead and recruit passing nestmates to novel food locations, although this pheromone has no arrestant activity22. These studies indicate that chemicals trigger temporal aggregation. However, the main chemical components of the aggregation pheromone in *Reticulitermes*, which induces assembly on the pheromone source over an extended duration, remains unknown.[4,5,6]

We compared the attraction activity of hexane extract fractions from worker *Reticulitermes speratus* and identified the pheromone components in active fractions. We then explored the optimal component ratio, dose dependency, and the persistence of aggregation activity of this pheromone.

We identified pheromone components using a two-choice bioassay with multiple steps and gas-chromatographic analyses. In the two-choice bioassay, two pieces of filter paper were placed on the bottom of a plastic dish: a sample paper and a solvent control. In each bioassay, we compared the aggregation ratios (i.e., ratio of total number of workers on sample paper to total number of workers on solvent papers) in each treatment and also compared the proportions of workers on sample papers among treatments after 5 min, with longer times used in the later tests of dose-dependency.
Initially, crude worker extract was fractionated into neutral, acidic, and basic fractions; the neutral fraction was further chromatographed into five consecutive fractions, eluted with *n*-hexane, three mixtures of diethyl ether (DEE) in *n*-hexane (10%, 30%, 50%), and DEE, respectively . When comparing total number of workers on the sample papers with total number of workers on the solvent papers in each treatment, the acidic, neutral, hexane, 10% DEE, and 50% DEE fractions attracted significantly more workers, relative to solvent papers, within 5 min (binomial test with Bonferroni correction, $P < 0.01$. Comparing the proportion of workers on sample papers among treatments, there were no significant difference among these fractions and the crude extract (a generalised linear mixed model [GLMM] followed by Tukey HSD test, $P < 0.05$). The main active components of the aggregation pheromone were therefore separated into the acidic, hexane, 10% DEE, and 50% DEE fractions.

To identify candidate pheromone components, the neutral and acidic fractions from four colonies were analysed using gas chromatography-mass spectrometry (GC-MS). In total, 29 compounds were commonly detected in all fractions from all colonies. The hexane fractions contained hydrocarbons, including nine straight-chain hydrocarbons [*n*-dodecane, *n*-tridecane, *n*-tetradecane, *n*-hexadecane, *n*-tricosane (C23), *n*-tetracosane, *n*-pentacosane (C25), *n*-heptacosane (C27), and *n*-nonacosane], 2-phenylundecane (2PhC11), and six methyl-branched long-chain alkanes. All straight-chain hydrocarbons and 2PhC11 were identified by comparing retention times and mass spectra with commercial standards of *n*-alkanes and a synthesised standard of (±)-2PhC11. Although 2PhC11 is an optically active aromatic compound, enantioselective analysis indicated that the hexane fractions contained both (+)-(*S*)-2PhC11 and (−)-(*R*)-2PhC11 in a relative abundance ratio of approximately 2:1. The 10% DEE fractions also contained six hydrocarbons, including C25 and C27. Importantly, 10% DEE fractions contained butylated hydroxytoluene and bis(2-ethylhexyl) adipate; however, butylated hydroxytoluene is a stabiliser contained in the diethyl ether solvent, while bis(2-ethylhexyl) adipate was assumed to be a contaminant. We therefore concluded that the aggregation activity of the 10% DEE fraction was explained by the presence of long-chain hydrocarbons (i.e., C25 and C27).

The 50% DEE fractions contained myristic acid (MA), vaccenic acid, palmitic acid (PA), and cholesterol (Ch), while the acid fractions contained oleic acid (OA) and PA. Comparative GC analyses of methyl ester derivatives identified the vaccenic acid as the *trans*-isomer (tVA) (*cis*-isomer: $t_R = 17.94$ min, *trans*-isomer: $t_R = 17.25$ min, natural vaccenic acid from 50% DEE fractions: $t_R = 17.15$ min). Collectively, these results suggested that the aggregation pheromone comprised straight short-chain hydrocarbons, an aromatic hydrocarbon, long-chain hydrocarbons, fatty acids, and/or cholesterol.[7,8,9]

II.DISCUSSION

A pheromone (from Ancient Greek φέρω *(phérō)* 'to bear', and *hormone*) is a secreted or excreted chemical factor that triggers a social response in members of the same species. Pheromones are chemicals capable of acting like hormones outside the body of the secreting individual, to affect the behavior of the receiving individuals.[1] There are *alarm pheromones*, *food trail pheromones*, *sex pheromones*, and many others that affect behavior or physiology. Pheromones are used by many organisms, from basic unicellular prokaryotes to complex multicellular eukaryotes.[2] Their use among insects has been particularly well documented. In addition, some vertebrates, plants and ciliates communicate by using pheromones. The ecological functions and evolution of pheromones are a major topic of research in the field of chemical ecology.[10,11,12]

Categorization by function

Aggregation

Aggregation of bug nymphs

Aggregation of the water springtail *Podura aquatica*

Aggregation pheromones function in mate choice, overcoming host resistance by mass attack, and defense against predators. A group of individuals at one location is referred to as an aggregation, whether consisting of one sex or both sexes. Male-produced sex attractants have been called aggregation pheromones, because they usually result in the arrival of both sexes at a calling site and increase the density of conspecifics surrounding the pheromone source. Most sex pheromones are produced by the females; only a small percentage of sex attractants are produced by males.[7] Aggregation pheromones have been found in members of the Coleoptera, Collembola,[8] Diptera, Hemiptera, Dictyoptera, and Orthoptera. In recent decades, aggregation pheromones have proven useful in the management of many pests, such as the boll weevil (*Anthonomus grandis*), the pea and bean weevil (*Sitona lineatus*, and stored product weevils (e.g. *Sitophilus zeamais*, *Sitophilus granarius*, and *Sitophilus oryzae*). Aggregation pheromones are among the most ecologically selective pest suppression methods. They are non-toxic and effective at very low concentrations.[9]

Alarm

Some species release a volatile substance when attacked by a predator that can trigger flight (in aphids) or aggression (in ants, bees, termites, and wasps)[10][11][12][13][14] in members of the same species. For example, *Vespula squamosa* use alarm pheromones to alert others to a threat.[15] In *Polistes exclamans*, alarm pheromones are also used as an alert to incoming predators.[16] Pheromones also exist in plants: Certain plants emit alarm pheromones when grazed upon, resulting in tannin production in neighboring plants.[17] These tannins make the plants less appetizing to herbivores.[17] An alarm pheromone has been documented in a mammalian species. Alarmed pronghorn, *Antilocapra americana* flair their white rump hair and exposes two highly odoriferous glands that releases a compound described having the odor "reminiscent of buttered popcorn". This sends a message to other pronghorns by both sight and smell about a present danger. This scent has been observed by humans 20 to 30 meters downwind from alarmed animals. The major odour compound identified from this gland is 2-pyrrolidinone.[18]

Epideictic

Epideictic pheromones are different from territory pheromones, when it comes to insects. Fabre observed and noted how "females who lay their eggs in these fruits deposit these mysterious substances in the vicinity of their clutch to signal to other females of the same species they should clutch elsewhere." It may be helpful to note that the word epideictic, having to do with display or show (from the Greek 'deixis'), has a different but related meaning in rhetoric, the human art of persuasion by means of words.

Territorial

Laid down in the environment, territorial pheromones mark the boundaries and identity of an organism's territory. In cats and dogs, these hormones are present in the urine, which they deposit on landmarks serving to mark the perimeter of the claimed territory. In social seabirds, the preen gland is used to mark nests, nuptial gifts, and territory boundaries with behavior formerly described as 'displacement activity'.[19]

Trail

Social insects commonly use trail pheromones. For example, ants mark their paths with pheromones consisting of volatile hydrocarbons. Certain ants lay down an initial trail of pheromones as they return to the nest with food. This trail attracts other ants and serves as a guide.[20] As long as the food source remains available, visiting ants will continuously renew the pheromone trail. The pheromone requires continuous renewal because it evaporates quickly. When the food supply begins to dwindle, the trail-making ceases. Pharaoh ants (*Monomorium pharaonis*) mark trails that no longer lead to food with a repellent pheromone, which causes avoidance behaviour in ants.[21] Repellent trail markers may help ants to undertake more efficient collective exploration.[22] The army ant *Eciton burchellii* provides an example of using pheromones to mark and maintain foraging paths. When species of wasps such as *Polybia sericea* found new nests, they use pheromones to lead the rest of the colony to the new nesting site.[13,14,15]

Gregarious caterpillars, such as the forest tent caterpillar, lay down pheromone trails that are used to achieve group movement.[23]

Sex

In animals, sex pheromones indicate the availability of the female for breeding. Male animals may also emit pheromones that convey information about their species and genotype.

At the microscopic level, a number of bacterial species (e.g. *Bacillus subtilis*, *Streptococcus pneumoniae*, *Bacillus cereus*) release specific chemicals into the surrounding media to induce the "competent" state in neighboring bacteria.[24] Competence is a physiological state that allows bacterial cells to take up DNA from other cells and incorporate this DNA into their own genome, a sexual process called transformation.

Among eukaryotic microorganisms, pheromones promote sexual interaction in numerous species.[25] These species include the yeast *Saccharomyces cerevisiae*, the filamentous fungi *Neurospora crassa* and Mucor mucedo, the water mold *Achlya ambisexualis*, the aquatic fungus *Allomyces macrogynus*, the slime mold *Dictyostelium discoideum*, the ciliate protozoan *Blepharisma japonicum* and the multicellular green algae *Volvox carteri*. In addition, male copepods can follow a three-dimensional pheromone trail left by a swimming female, and male gametes of many animals use a pheromone to help find a female gamete for fertilization.[26]

Many well-studied insect species, such as the ant *Leptothorax acervorum*, the moths *Helicoverpa zea* and *Agrotis ipsilon*, the bee *Xylocopa sonorina,* the frog Pseudophryne bibronii, and the butterfly Edith's checkerspot release sex pheromones to attract a mate, and some lepidopterans (moths and butterflies) can detect a potential mate from as far away as 10 km (6.2 mi).[27][28] Some insects, such as ghost moths, use pheromones during lek mating.[29] Traps containing pheromones are used by farmers to detect and monitor insect populations in orchards. In addition, *Colias eurytheme* butterflies release pheromones, an olfactory cue important for mate selection.[30]

The effect of Hz-2V virus infection on the reproductive physiology and behavior of female *Helicoverpa zea* moths is that in the absence of males they exhibited calling behavior and called as often but for shorter periods on average than control females. Even after these contacts virus-infected females made many frequent contacts with males and continued to call; they were found to produce five to seven times more pheromone and attracted twice as many males as did control females in flight tunnel experiments.[31]

Pheromones are also utilized by bee and wasp species. Some pheromones can be used to suppress the sexual behavior of other individuals allowing for a reproductive monopoly – the wasp *R. marginata* uses this.[32] With regard to the *Bombus hyperboreus* species, males, otherwise known as drones, patrol circuits of scent marks (pheromones) to find queens.[33] In particular, pheromones for the *Bombus hyperboreus,* include octadecenol, 2,3-dihydro-6-transfarnesol, citronellol, and geranylcitronellol.[34]

Sea urchins release pheromones into the surrounding water, sending a chemical message that triggers other urchins in the colony to eject their sex cells simultaneously.[15,16,17]

In plants, some homosporous ferns release a chemical called antheridiogen, which affects sex expression. This is very similar to pheromones.

Other

This classification, based on the effects on behavior, remains artificial. Pheromones fill many additional functions.

- Nasonov pheromones (worker bees)
- Royal pheromones (bees)
- Calming (appeasement) pheromones (mammals)
- Necromones, given off by a deceased and decomposing organism; consisting of oleic and linoleic acids, they allow crustaceans and hexapods to identify the presence of dead conspecifics.[35]
- Suckling: TAA is present in rabbit milk and seems to play a role of pheromone inducing suckling in the newborn rabbit.[36]

III.RESULTS

No study has led to the isolation of true human sex pheromones, though various researchers have investigated the possibility of their existence.[1][2] Sex pheromones are chemical (olfactory) signals, pheromones, released by an organism to attract an individual, encourage it to mate with it, or perform some other function closely related with sexual reproduction. While humans are highly dependent upon visual cues, when in proximity, smells also play a role in sociosexual behaviors. An inherent difficulty in studying human pheromones is the need for cleanliness and odorlessness in human participants.[3] Experiments have focused on three classes of putative human pheromones: axillary steroids, vaginal aliphatic acids, and stimulators of the vomeronasal organ.

Axillary steroids are produced by the testicles, ovaries, apocrine glands and adrenal glands.[4] These chemicals are not biologically active until puberty when sex steroids influence their activity.[5] The activity change during puberty suggest that humans communicate through odors.[4] Several axillary steroids have been described as possible human pheromones: androstadienol, androstadienone, androstenone, androstenol, and androsterone.

Androstenol is the putative female pheromone.[5] In a 1978 study by Kirk-Smith, people wearing surgical masks treated with androstenol or untreated were shown pictures of people, animals and buildings and asked to rate the pictures on attractiveness.[6] Individuals with their masks treated with androstenol rated their photographs as being "warmer" and "more friendly".[6] The best-known case study involves the synchronization of menstrual cycles among women based on unconscious odor cues, the *McClintock effect*, named after the primary investigator, Martha McClintock, of the University of Chicago.[7][8] A group of women were exposed to a whiff of perspiration from other women. Depending on the time in the month the sweat was collected (before, during, or after ovulation), there was an association with the recipient woman's menstrual cycle to speed up or slow down. The 1971 study proposed two types of pheromone involved: "One, produced prior to ovulation, shortens the ovarian cycle; and the second, produced just at ovulation, lengthens the cycle". However, recent studies and reviews of the methodology have called the validity of her results into question.[9][10] A 2013 meta-review of existing studies showed that the syncing of ovarian cycles likely did not exist.[11]

Androstenone is postulated to be secreted only by men as an attractant for women and is also thought to affect their mood positively. It seems to have different effects on women, depending on where a female is in her menstrual cycle, with the highest sensitivity to it during ovulation.[5] In 1983, study participants exposed to androstenone were shown to undergo changes in skin conductance.[12] Androstenone has been found to be perceived as more pleasant to women at a woman's time of ovulation. It is hypothesized that this may be a way for a male to detect an ovulating female who would be more willing to be involved in sexual interaction[18,19]

IV.CONCLUSION

Sex pheromones are pheromones released by an organism to attract an individual of the same species, encourage them to mate with them, or perform some other function closely related with sexual reproduction. Sex pheromones specifically focus on indicating females for breeding, attracting the opposite sex, and conveying information on species, age, sex and genotype. Non-volatile pheromones, or cuticular contact pheromones, are more closely related to social insects as they are usually detected by direct contact with chemoreceptors on the antennae or feet of insects.

Insect sex pheromones have found uses in monitoring and trapping of pest insects.

Sex pheromones have found applications in pest monitoring and pest control. For monitoring, pheromone traps are used to attract and catch a sample of pest insects to determine whether control measures are needed. For control, much larger quantities of a sex pheromone are released to disrupt the mating of a pest species. This can be either by releasing enough pheromone to prevent males from finding females, effectively drowning out their signals, or by mass trapping, attracting and removing pests directly.[14] For example, research on the control of the spruce bud moth (*Zeiraphera* *canadensis)* has focused on the use of the pheromone E-9-tetradecenyl-acetate, a chemical the spruce bud moth releases during mating[20]

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