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ORIGIN OF COURTSHIP AND SEX PHEROMONES OF THE ORIENTAL FRUIT MOTH AND A DISCUSSION OF THE ROLE OF PHYTOCHEMICALS IN THE EVOLUTION OF LEPIDOPTERAN MALE SCENTS

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ABSTRACT

The sexual communication system of the oriental fruit moth is comprised of a long-distance female-emitted sex pheromone that attracts males, and a shorter-range male-emitted courtship pheromone that attracts females. Typical of lepidopterous communication, the female-emitted sex pheromone components appear to be comprised of fatty acid derivatives. Sustained upwind flight by the male is optimally evoked only if three pheromone components are emitted in the following proportions: 6% (*E*)-8-dodecenyl acetate and 3% (*Z*)-8-dodecenyl alcohol in (*Z*)-8-dodecenyl acetate. Males use a combination of optomotor anemotaxis and self-steered counterturning in order to progress upwind to a female or a synthetic point source. If the pheromone lacks the filamentous fine structure of a normal point-source plume, as when a uniform fog or cloud of pheromone is generated, then males do not progress upwind, but rather cast widely to and fro as if they had just lost pheromone. Also typical of the Lepidoptera, the courtship pheromone contains compounds which may possibly be taken up by males in intact form from plants, sequestered, and then emitted during an elaborate display of eversible scent brushes to gain acceptance from females for copulation. The active components are ethyl *trans*-cinnamate and methyl 2-epijasmonate. Females respond to a displaying male by walking upwind to him from *ca.* 2 cm away. This display may have evolved by means of female-choice sexual selection in which a female preference and preferred male trait become linked as a result of the mating advantage conferred upon male offspring of discriminating females. Male scent-disseminating structures and courtship pheromones in other Lepidoptera may have also evolved by this mechanism. An alternative hypothesis for male Lepidoptera that sequester from plants compounds that have defensive value is that females assess males' displays for evidence, in the form of odorous metabolites, that males are defensively well-endowed. There is evidence that for both palatable and noxious species the fitness-related initiator of the female preference for odor-endowed males was interspecific or inter-demic mating mistakes.

Key Words: Sex pheromone, phytochemicals, courtship, hairpencils, Lepidoptera, pyrrolizidine alkaloids, cardenolides, *Grapholita molesta,* sexual selection.

INTRODUCTION

Much has been learned in recent years concerning pheromonal communication among male and female butterflies and moths. The most prevalent form of pheromonal communication in the Lepidoptera, and that for which there has already been much practical benefit gained for agriculture, is sexual communication involving female emission of sex pheromones. By studying moth sex pheromone communication, profound insights have also been gained into the processes of speciation and evolution. In addition, male Lepidoptera also sometimes emit chemical signals of their own in the form of courtship pheromones during displays of scent-disseminating structures called hairpencils when they are near the female. These induce the female to accept the male by remaining quiescent or else by a more active response such as attraction or turning her body so that he can grasp her with clasping organs on the end of his abdomen (Baker, 1985). Male assemblages called leks (Bradbury, 1981) also occur (Willis and Birch, 1982; Wunderer *et al.*, 1986), apparently as a result of males responding to each other's courtship pheromone emissions. The pooled effect of the emissions may increase the chances of attracting females and gaining a copulation (Baker, 1985; Wagner, 1985).

One species of moth, the oriental fruit moth, *Grapholita molesta* (Busck), has been studied more extensively than any other over the years regarding the pheromone emitted by females, the male's orientation responses during upwind flight, the temporal and chemical characteristics that optimize stimulation of the antennae and subsequent behavior, and the aspects of the male courtship pheromone and the behavioral characteristics of the male display that result in successful mating. Insights have been gained into the evolution of new species and into the mode of selection that has occurred by studying *G. molesta.* In my presentation today at what has been in my opinion an outstanding Symposium on Phytochemical Ecology, hosted graciously and with great skill by Professor C.-H. Chou and other members of Academia Sinica, I will attempt to summarize the current knowledge concerning pheromonal communication in this species and relate it to what we know about other Lepidoptera. In keeping with the emphasis on Phytochemical Ecology, I will then discuss aspects of the possible evolution of courtship pheromones, which seem to be made from minimally altered, plant-derived compounds, in contrast to moth sex pheromones that are synthesized *de novo* from acetate. My emphasis in research has always been a behavioral one, and so I hope I may be forgiven if, in contrast to many of the other papers presented here, my contribution seems to emphasize the effects of chemicals more than their structrues.

THE SEX PHEROMONE OF *GRAPHOLITA MOLESTA*

Typical of the Lepidoptera, sexual communication in the oriental fruit moth is initiated by the female, who a few hours before dusk, raises her wings and abdomen and releases four compounds from a gland on her extended abdominal tip. The blend of three of these compounds elicit upwind flight, landing, and courtship display in males: (*Z*)-8-dodecenyl acetate (Z8-12:Ac) (Roelofs *et al.*, 1969), (*E*)-8-dodecenyl acetate (E8-12:Ac), and (Z)-8-dodecenyl alcohol (Z8-12:OH) (Cardé *et al.*, 1979). The fourth compound, dodecan-1-ol (12:OH), seems to increase males' reactions only if Z8-12:OH is at lower than normal levels (Baker and Cardé, 1979a). Other examples of extra, apparently redundant components emitted by females of other species abound, the most well-investigated example being the cabbage looper *Trichoplusia ni* (Linn *et al.*, 1984). As in *G. molesta,* the extra compounds in the pheromone blend of *T. ni* do not function in evoking behavior in the male unless some of the others are missing.

There are at least two good explanations as to why there are such extra compounds in pheromone blends of moths. First is the possibility that they serve as a buffer against the pheromonal background noise in the locality where the female is calling. Other species using the same compounds as their major pheromone components could possibly cause habituation of sensory pathways in male *T. ni* specific for those components, but the presence of others that can fill in for the "missing" habituated compound pathways and evoke the complete behavior would ensure optimal communication (Baker, T. C.; Haynes, K. F., unpublished). A second explanation states that these extra compounds are important in interspecific communication. They may cause antagonistic reactions in other, perhaps congeneric males that otherwise would have responded to the other portion of the blend because the two species share it (Linn *et al.*, 1984).

The acetates and alcohols used by *G. molesta* are again typical of the Lepidoptera, the females of which employ fatty acid-derived compounds for pheromonal communication (Bjostad and Roelofs, 1983, 1986; Bjostad *et al.*, 1984, 1985, 1987). The males, on the other hand, generally employ compounds that appear to be minimally altered compounds directly derived from plants (for reviews see Boppré, 1978, 1986; Eisner and Meinwald, 1987; Baker, 1985). Female Lepidoptera from the majority of families use straight-chain structures 10 to 18 carbons in length with an even number of carbon atoms (Bjostad *et al.*, 1987; Tamaki, 1985). These molecules have a functional group on the end of the molecule that makes it either an aldehyde, alcohol, or acetate. They also usually have either one, two, or three double bonds at various positions on the molecule in either the *Z* or *E* geometric configuration.

Some of the compounds used by the remainder of the families are branched and unbranched straight-chain hydrocarbons with no functional group, sometimes saturated but usually multiply olefinic at the 1, 3, 6, 9, 12, or 15 positions in the *Z* configuration, or with an epoxy group instead of a double bond at these positions (Tamaki, 1985). It is now clear that the great diversity of the structures in the tortricids, noctuids, and pyralids are biosynthesized by means of a surprisingly few biochemical reactins and corresponding enzymes. The main chain, up to 16 or 18 carbons long, is synthesized *de novo* from acetate. Various combinations of chain shortening and desaturation occur by mainly a Δ-11, but also Δ10, E9, and perhaps even a Δ8, desaturase, to create nearly all of the compounds used by these families (Roelofs and Brown, 1982; Löfstedt and Bengtsson, 1988). Also, the sequence in which these two types of enzymes act creates extra possibilities for pheromone compounds, including multiply unsaturated compounds with conjugated double bonds. Chain elongation following desaturation has also been implicated and adds to the possibilities for pheromone structures (Bjostad *et al.*, 1987). The final step in pheromone biosynthesis involves reduction of the acids to the alcohol, followed either by acetylation, oxidation to an aldehyde (Teal and Tumlinson, 1986), or merely emission of the alcohol itself from the gland as a pheromone component.

The first three compounds in *G. molesta* appear to act in concert to elicit upwind flight to the female (Baker and Cardé, 1979a). Again, this is typical of the Lepidoptera; males of several species exhibit the lowest upwind flight behavioral thresholds to blends of all the components compared to partial blends or individual components (Linn *et al.*, 1986, 1987). In the field, the complete blend of *G. molesta* components was effective in evoking wing fanning behavior in males at twice the distance from the source at which the same dosage of even the best incomplete blends was operative (Linn *et al.*, 1987). Thus it is not consistent with the current experimental evidence to view attraction of males to females as occurring as a hierarchical sequence of responses to first one component far downwind, then another component closer to the source, as the male approaches the female. Even though this scenario is intuitively appealing, or even one involving simple blends far away and more complex ones closer to the source, there is no support for it thus far. The behavioral lack of support for sequential activity of sex pheromone components does not preclude the possibility that only the receptors specific to the major component might fire at some distance far downwind, even though the complete blend is being emitted from the source (e.g., Rumbo, 1983; Baker, 1986). The behavioral data only remove the possibility that such firing from one cell type is sufficient to evoke behavior. We must be careful to distinguish between the neuronal detection of only one compound in a blend at great distances, and the behavioral response to that single type of neuronal activity at that distance, which is another matter entirely (Baker, 1988).

The blend of three *G. molesta* pheromone components switches on two major programs of behavior in flying males, as well as stimulating them to take flight if they are resting: a program of counterturning that reverses their direction across the windline approximately 7 times per second (Kuenen and Baker, 1983; Baker, 1986) and optomotor anemotaxis (Kennedy, 1940, 1986; Kennedy and Marsh, 1974; Marsh *et al.*, 1978), which allows them to react to wind and progress against it toward the pheromone source according to the apparent movement of images across their eyes (Fig. 1). Thus one of the primary reactions to the pheromone odor is a visual one, and the optomotor-based reaction to wind-induced drift polarizes the counterturns in the upwind direction (Baker *et al.*, 1984), resulting in a zigzagging flight track that is typical of many male moths as they respond to sex pheromones (Fig. 1).

We now know that a plume of the *G. molesta* pheromone blend, as for other moths, consists of intermittent filaments of pheromone interspersed with holes of virtually clean air, as first suggested by earlier studies with smoke (Wright, 1958) and ionized air (Murlis and Jones, 1981). In the field and in laboratory wind tunnels, we measured this intermittency with an isolated *G. molesta* antenna by the electroantennogram (EAG) technique, and found that male antennae receive filaments at a frequency of from 0.3–2 per second in a wind of 0.7 m/s in which they are known to respond by upwind flight (Baker and Haynes, 1989). It is the intermittency from the plume that causes the male's upwind flight to be sustained. Otherwise, as in an experimentally generated fog or cloud of pheromone the flight abruptly switches to cessation of upwind progress while maintaining narrow zigzagging, and then cross-wind casting flight (Willis and Baker, 1984; Baker *et al.*, 1985). This is similar to what was earlier found by Kennedy *et al.* (1980, 1981) for another

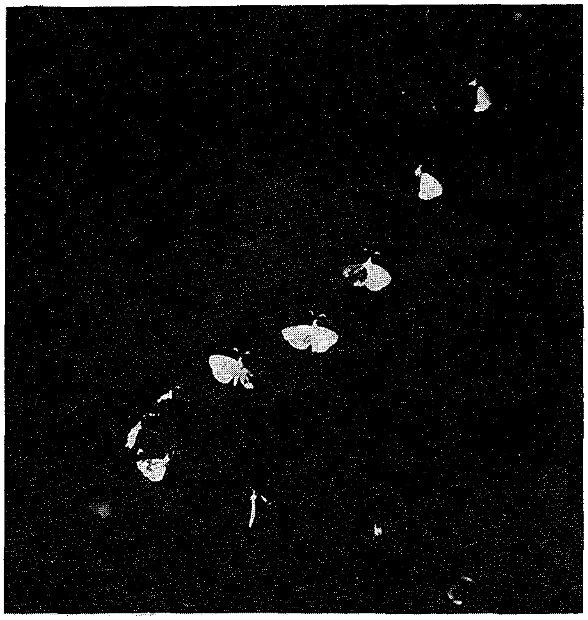

Fig. 1.
A male oriental fruit moth, viewed from above, flying in zigzag fashion upwind in response to the blend of three pheromone components emitted by the female. Wind direction (and pheromone) is directly from the top of the photograph. Note the severe rolling of the moth at the apex of the turn at the left, demonstrating the maneuver used to perform a counterturn. Also note the discrepancy between the male's anatomical heading and its resultant path (track) as a result of the force of the wind. This creates a small amount of transverse image movement across the male's eyes (in this case from right to left), which is apparently used in combination with front-to-back longitudinal image movement in order to steer and progress upwind, a process called optomotor anemotaxis (Kennedy, 1940; Baker, 1988).

tortricid species, *Adoxophyes orana*. If the cloud is pulsed, however, and thus made intermittent, upwind flight can be sustained (Baker *et al.*, 1985). In a normal plume from a point source, it is thought that contacts with the filaments and clean air between them causes reiterative reactions in the counterturning and optomotor anemotactic programs that result in sustained upwind progress (Baker and Haynes, 1987). The angle of orientation with respect to the wind can change very quickly in *G. molesta* following contact with pheromone or clean air, within one reversal back across the wind (0.15 s). This is well under the average interval between contacts with pheromone, which is 0.5/s, so there probably is often lots of time for behavior to change between contacts with filaments.

The upwind progress in a plume will stop and change to casting flight across wind if the concentration is too high or the composition of the blend is slightly off (Baker *et al.*, 1981a; Baker and Roelofs, 1981; Linn and Roelofs, 1983), especially if the air temperature is 20° instead of 26° (Linn *et al.*, 1988). We know that if the chilled receptor neurons on the *G. molesta* male antennae sensitive to Z8-12:Ac are challenged by pulses of the pheromone blend at a frequency of greater than two per second, their bursts of firing in response to the pulsing become attenuated due to adaptation (Baker *et al.*, 1988). However, the warmer neurons do not adapt as easily, and are able to more accurately register the ups and downs of the pulsed signal for a longer time (Baker *et al.*, 1988). Interestingly, males cease flying upwind in the cold at high concentrations of the optimal blend, perhaps due to the chilling-facilitated adaptation of the neurons, which cannot register the onset and offset of filaments any longer as the male flies up the plume. The behavioral data also show that in cool air and high concentrations of pheromone, the males now prefer a different blend of components having a lower percentage of the E8-12:Ac than at higher temperatures and lower concentrations (Baker *et al.*, 1988). This is again consistent with the observed adaptation of the neurons specific for the Z8-12:Ac. If these neurons were preferentially being adapted relative to the E8-12:Ac-sensitive neurons that are exposed to a 20-fold lower concentration (only 6% E in the blend), then the blend that previously had been optimal might now appear to be too rich in E8-12:Ac. In order to register optimally in the male's higher olfactory pathways, it now would require a blend that is lean in E8-12:Ac (e.g., 2% E8-12:Ac) (Baker *et al.*, 1988).

Differential adaptation of antennal neurons on antennae of the turnip moth, *Agrotis segetum*, also was observed, but this time directly in a pheromone plume. Neurons sensitive to the major component became adapted in plumes of the pheromone blend at concentrations that are behaviorally excessive, whereas neurons sensitive to a secondary component did not adapt as frequently. The cessation of upwind flight could be explained by a shift in the ratio of neuronal input that now would be too rich in activity from the cells sensitive to the minor component (Baker *et al.*, 1988).

COURTSHIP IN *GRAPHOLITA MOLESTA*

Following sustained upwind flight, when a *G. molesta* male arrives at the female, he performs an elaborate hairpencil display, directed towards and performed one or two centimeters from her (Fig. 2). Displays consist of multiple rhythmic extrusions and retractions of the white hairpencil organs and claspers at the end of the abdomen (Baker and Cardé, 1979b). The paired caudal hairpencils consist of a tuft of about 93 hollow scales with honey-combed porous walls that are typical of structures designed continually to secrete, store, and disperse volatile material (George and Mullins, 1980). The lumen of each scale is continuous with an underlying reservoir within a fibrous space which in turn is bounded by microvilli of an enveloping trichogen cell. A blend of four volatile chemicals are propelled by wind from the male's vibrating wings over a female: ethyl *trans*-cinnamate, methyl 2-epijasmonate, methyl jasmonate, and (*R*)-(−)mellein) (Baker *et al.*, 1981b). The female is attracted to the hairpencils and when she contacts the male's abdomen she stops walking. The male immediately turns quickly and attempts copulation (Baker and Cardé, 1979b).

Only the ethyl *trans*-cinnamate and the methyl 2-epijasmonate have been shown to be pheromone components, attracting females from 2 cm away. Interestingly, evidence shows that the cinnamate can be sequestered from the larval diet, or adults also likely can imbibe it from natural sources such as rotting Japanese pears (Nishida *et al.*, 1985). Jasmonic acid and methyl jasmonate are common constituents of plants, found in apples and other fruits (Meyer, 1985). Males deficient in ethyl *trans*-cinnamate and allowed to imbibe this compound from sugar water as adults were

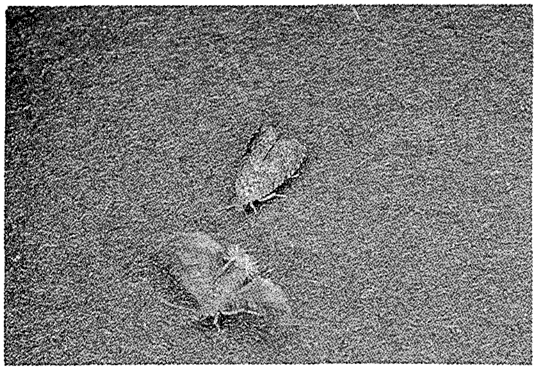

Fig. 2. A male oriental fruit moth rhythmically extruding and retracting his hairpencils near a female, who is walking toward the male in response to the courtship pheromone emitted during this display (Baker and Cardé, 1979b; Baker *et al.*, 1981).

shown to be more successful in courtship than males that drank sugar water alone (Löfstedt *et al.*, 1989). Interestingly, the dependence on these compounds has perhaps waned after years of laboratory breeding in small mating boxes (Löfstedt *et al.*, 1989).

When only a single male is near the female, the only way that he is able to successfully mate is to display and induce a female to touch his abdomen. However, the situation is quite different if two or more males arrive near a female at about the same time (Baker, 1983). If a male is displaying when a second male approaches, the latter male may attempt to copulate (often successfully) with the female without first displaying. Such late-arriving males appear to "sneak" in for a copulatory attempt under the cover of the first male's chemical barrage. Other alterations in the normal male courtship sequence also occur, including late-arriving males being attracted to first-arrivals' displays, and the touches they deliver to the first males' abdomens causing misdirected copulatory attempts by displaying males and also reduced mating success. Two males may display simultaneously toward one female, whereupon she will "choose" one male over the other by touching his hairpencils. Simultaneous displaying occurs infrequently, but even for sequentially arriving males, females sometimes choose late arrivals after not responding to earlier males' displays. Females fail to respond to males that are prevented experimentally from everting their hairpencils, even though the rest of their courtship sequence is otherwise unaltered.

ORIGIN OF COURTSHIP PHEROMONES IN THE LEPIDOPTERA

Baker and Cardé (1979b) viewed the courtship behavior of *G. molesta* as having evolved primarily by means of female-choice sexual selection. They were the first to link behavioral data from moths with Darwin's (1898) ideas about sexual selection in order to explain the evolution of scent structures and displays in the Lepidoptera. They hypothesized that a female preference and the preferred trait, the odor from male moth hairpencil structures, would become linked to result in amplified emissions and exaggerated organs. If the displays and female discrimination were heritable, as they seem to be for some species (Phelan and Baker, 1986a), females that choose males with "better" displays would gain because their sons would be at a mating advantage amongst the discriminating females in the next generation (Darwin, 1898; Fisher, 1958; O'Donald, 1962, 1967). As the limiting sex, females can exercise discrimination in selecting mates, considering their larger parental investment in potential offspring. However, for highly directional, "runaway" sexual selection to occur and drive this discrimination and the display together to an extreme level, a situation must occur in nature in which some males gain more matings at the expense of others (Fisher, 1958; O'Donald, 1962, 1967). Protandry (early seasonal emergence of male adults), or a skewed sex ratio with a predominance of males, are two conditions that could result in a proportion of the male population being excluded from mating, but in *G. molesta* a third situation effectively creates a shortage of females during the mating period: most females mate only once, whereas males can mate an average of more than once per evening (Dustan, 1964).

The fitness-related feature of the odor that would cause the original female preference for the *G. molesta* male odor (perhaps originating during clasper extension in ordinary courtship) would be the protection against interspecific mating mistakes with sympatric and synchronic congeners (Baker and Cardé, 1979a). This might occur if there was incomplete specificity in the female-emitted sex pheromone systems of *G. molesta* and another species, and such cross-attraction of males to females of the wrong species does sometimes occur with at least one species in New York state, *Grapholita prunivora* (Baker and Cardé, 1979a, b). *G. prunivora* males also perform a display of abdominal hairpencils toward their females, but the rhythm is much faster and females are not attracted. Males must back into the females while displaying, forcing them to stop walking forward so that a copulatory attempt can be made (Baker and Cardé, unpublished observations). Cross-specific courtship is readily evoked between males and females of these two species due to the similarity in sex pheromone blends. A blend deficient in Z8-12:OH attracts *G. prunivora* as well as a few *G. molesta,* and adding more of this alcohol to the blend effectively prevents *G. prunivora* males from being attracted all the way to the source (Baker and Cardé, 1979a). Cross-specific courtships are not successful. Displaying *G. molesta* males wait for the touch that never comes from the unattracted females of *G. prunivora,* and *G. prunivora* males disturb *G. molesta* females by continually bumping and backing into them rather than waiting for them to be attracted (Baker and Cardé, unpublished observations).

In the rest of the Lepidoptera, as in *G. molesta,* the scents that are truly courtship pheromones are nearly always closely structurally related to compounds peculiar to certain plants, and unlike female-emitted sex pheromones, depend directly on the ingestion of these compounds (e.g., Edgar and Culvenor, 1974; Meinwald *et al.,* 1969; Pliske and Eisner, 1969; Schneider *et al.,* 1982; Conner *et al.,* 1981; Krasnoff and Roelofs, 1989a; Nishida *et al.,* 1985). One logical reason for the tendency for male scents to consist of minimally altered plant products is that in the initial steps of the evolution of the pheromones, these are the compounds for which females would have already-existing receptors and behavioral response systems. That is, if olfaction played a significant role during host-finding and acceptance, then females would already have a sensory system sensitive to such compounds (Edgar *et al.,* 1974). The only thing lacking would be the initiation of a preference by females for this odor during mating in addition to during host-finding. The fitness-related trigger to cause such a male-odor-based mating preference to arise could be interspecific mating mistakes (Baker and Cardé, 1979b; Phelan and Baker, 1987) or perhaps defense (Baker and Cardé, 1979b). Concerning mating mistakes, were males to ingest the same foodplant to which females were most olfactorily sensitive (as is likely for conspecifics or members of the same deme), then the re-emission of those same plant compounds by males during mating would be very likely if their tissues had retained some of the same compounds (Edgar *et al.,* 1974).

Such a link is known in some species. In *G. molesta,* gravid females in the field are known to be attracted from a distance to one of the hairpencil constituents, ethyl *trans*-cinnamate (Nishida *et al.,* 1985), and cinnamic acid is a common allelopathic constituent of plants (N. Fischer, this symposium). In one species of moth, *Cisseps fulvicollis,* females as well as males are attracted to hydroxydanaidal baits as well as to plant parts containing pyrrolizidine alkaloids (PA's). Hydroxydanaidal is found in the male coremata and is a possible courtship pheromone. These results thus suggest that the compound used in the location of such plants and in courtship are one and the same (Krasnoff and Dussourd, 1989). The display of odor by the male, as well as the degree of preference for it, would be driven to extremes by "runaway" sexual selection (Baker and Cardé, 1979b; Fisher, 1958). With the link to host-plant compounds, there is the possibility that extreme sexual preference may significantly influence female host-plant preference. Likewise, selective pressures that weaken female host-plant discrimination will likely lower the requirements for courtship displays of plant-specific compounds and hence contribute to the vestigiality of the displays, first behaviorally and chemically, then morphologically (Krasnoff and Roelofs, 1989b). This link should not be surprising in the context of female-choice sexual selection and the above discussion; the plants on which male larvae fed and sequestered odorous compounds were chosen by females that may have discriminated for those same odors before ovipositing. It is now well known that host-plant feeding by larval males influences both the quantity and sometimes the quality of the PA-related odor in male coremata and hairpencils (Conner *et al.*, 1981; Schneider *et al.*, 1982; Wunderer *et al.*, 1986; Krasnoff and Roelofs, 1989a; Conner *et al.*, 1989; Krasnoff *et al.*, 1987).

A second evolutionary scenario for the evolution of hairpencils organs and scents has been advanced for those species, such as are found in the Danainae of the Nymphalidae and the Arctiinae of the Arctiidae, which are apparently defended by sequestered, distasteful compounds. Adults (and larvae) of many of these species are aposematically colored, indicative of their distastefulness. This hypothesis involves the use of these scents by females as ways to "assess" a male's ability to sequester such defensive compounds (and hence of his defensive fitness) (Conner *et al.*, 1981; Eisner, 1980; Eisner and Meinwald, 1987; Dussourd *et al.*, 1988), or to serve as a "measure" of his nuptial gift of defensive alkaloids to be transferred via his ejaculate during mating (Eisner and Meinwald, 1987; Dussourd *et al.*, 1988). I suggest here that both of these hypotheses are inherently untestable and therefore unfalsifiable due to their teleological nature; they posit a motive on the part of the female. However, there remains a legitimate question as to whether females discriminate for males that emit more or higher-quality courtship pheromone, and also whether some of the compounds ingested by the male do aid in defending both the parents and their offspring and therefore add to the benefit accrued by discriminating females and by optimally odorous males (Conner *et al.*, 1981; Eisner, 1980; Eisner and Meinwald, 1987; Dussourd *et al.*, 1988). Such an extra fitness-related feature might enhance and amplify still further both the display of odor and the female discrimination for it that has evolved owing to the courtship value itself in the context of sexual selection (Fisher, 1958; Baker and Cardé, 1979b; Baker, 1985; Phelan and Baker, 1986a, 1987). Possibly, the defensive advantage might even serve as the fitness-related initiator of sexual selection (Baker and Cardé, 1979b). Thus these possibilities deserve further examination.

There are many questions concerning the hypothesis that male contribution to defense was a primary force behind the evolution of corematal and hairpencil emissions in PA-based lepidopteran systems. Why, for instance, are adult females of some species not found feeding on PA-containing plants as often as are males and hence augmenting their chemical defense of their own bodies and of their eggs? For instance, Pliske (1975) reported that only adult male *Danaus gilippus* feed on the available *Crotalaria, Eupatorium, Heliotropium,* and *Senecio* plants that are rich in PA's. It has been shown that *D. gilippus* adult males need to obtain PA's in order to manufacture danaidone, their courtship pheromone, without which they are only about 20% as successful at mating as danaidone-emitting males (Pliske and Eisner, 1969). His observations resulted in well over 400 males and not a single female observed feeding on such plants.

It should behoove *D. gilippus* females to feed on these compounds directly from the plants rather than take the unnecessary risk of waiting for a perhaps insufficiently endowed male to give them to her. The risk of predation while flying about and finding such plants should be minimal, because females and males both are already defensively protected by the cardenolides they have ingested as larvae (Brower and Brower, 1964; Brower, 1968). Also, these butterflies benefit by being conspicuous, because bird predators learn quickly to avoid all members of the population after feeding on one of them and becoming ill from the cardenolides (Brower and Brower, 1964). In addition, if PA's are an augmentation of the already-existing cardenolide defense (Eisner, 1980; Eisner and Meinwald, 1987), it would seem that females, who carry the eggs, are more in need of ensuring that they have this readily available extra protection than are males. It is curious that they do not take every opportunity to do so.

In Pliske's study (1975) only in the Arctiidae were some species found whose females visited PA-containing plants more often than males. It is interesting that one notable exception to the adult feeding was *Utethesia ornatrix,* which were abundant at lights but were never once found visiting the PA plant baits (Pliske, 1975). The larvae of this species feed on species of *Crotalaria,* a PA-rich plant genus, and thus the adults already have acquired significant PA-associated defensive characteristics resulting from sequestration of these compounds as larvae (Eisner and Meinwald, 1987). However, depending on the part of the plant larvae feed upon, the adult males may have lower or higher titers of hydroxydanaidal and the associated PA's (Conner *et al.,* 1989). If feeding on the wrong part of the plant as a larva could possibly translate into reduced defense of a male's offspring (Conner *et al.,* 1989), it is surprising that males do not try to supplement their titer of hydroxydanaidal with adult visitations to PA-containing plants to ensure maximal numbers of matings. It is also surprising that females "care " at all (my quotes) as to whether males have PA's to pass to them (as "assessed" by hydroxydanaidal emission levels), because they are already well-provisioned with PA's by their own feeding as larvae, resulting in sufficient protection of themselves and their eggs (Dussourd *et al.,* 1988). In fact, data from a recent study (Dussourd *et al.,* 1988) show that the PA's transferred by males to females in their ejaculate did not enhance the defense of *U. ornatrix* eggs unless the female was completely devoid of PA's herself, a situation that apparently cannot occur in nature because they are obligate feeders on *Crotalaria* spp., all of which have at least some level of PA's in their tissues (Conner *et al.,* 1989). Again as seen for cardenolide-endowed species, it appears that the odor itself, which can be peculiarly derived from PA's, is most important, and *U. ornatrix* males already have at least *some* level of it due to larval feeding.

Interestingly, in another species, *Pyrrharctia isabella,* a value for passage of PA's via the ejaculate to the female has now been demonstrated, but it lies not in defense but in the pheromonal competence of the sons (Krasnoff and Roelofs, 1989a). This species is only a facultative feeder on PA-containing plants, and thus under field conditions there may be adult females that are lacking PA's in their body tissues. When PA-deprived females were mated with PA-fed males, the coremata of their male offspring were able to evoke the ultrasonic click from females that indicated they had detected the pheromone, hydroxydanaidal; male offspring from parents that had both been deprived of feeding on PA's were unable to evoke this response (Krasnoff and Roelofs, 1989a). Thus the evidence in moths thus far implicates the nuptial transfer of PA's as having evolutionary significance due to this transfer's transovarial *pheromonal* value, and not to its defensive value. Such a *direct* inheritance of male signalling competence, in this case the *very chemicals* themselves from the father through the mother's egg, could not have been envisaged by Darwin (1898) or Fisher (1958).

These findings (Krasnoff and Roelofs, 1989a) support still further the hypothesis that whatever the fitness-related factor triggering the initiation of a female preference, the evolution of male lepidopteran pheromones proceeded primarily by the inheritance of the mating advantage by sons, gained from the chemicals' pheromonal effect (Baker and Cardé, 1979b). Such transovarial transfer of pheromonal competence should be looked for in species, such as in the Danainae, which practice male-biased adult feeding on PA-containing plants, the adult females being devoid of PA's initially. This will perhaps reveal that the true value of PA acquisition by males and discrimination of it by females is in the direct transfer of the chemicals to their sons (in addition to the genetic transfer of the behavior) to ensure mating competence in the next generation.

Another interesting question stemming from the defense hypothesis involves cardenolides; if male hairpencil displays have evolved to indicate defensive capability due to protective chemicals to be passed to the female, why are cardenolides or their metabolites not used? Cardenolides are perhaps the most powerful and best-studied plant-derived defensive compounds in insects, yet the male danaids that feed on them curiously have not found a need, or a way, to display to females how much they have ingested. The ability of *D. gilippus* butterflies to sequester such compounds from their *Asclepias* host plants as larvae has been shown to be nearly as good (77%) as that of monarchs (Brower *et al.*, 1982) and they are protected by them (Brower and Brower, 1964), yet males instead visit other plants in order to acquire PA's for manufacturing danaidone. The possible lack of volatility of potential cardenolide metabolites compared to those of PA's might be a factor were it not for the fact that the display and transfer of danaidone to females' olfactory pathways is essentially performed by contact chemoreception; cuticular dust particles loaded with danaidone are sprinkled onto female antennae (Pliske and Eisner, 1969). It would seem that possibly even the lowest-volatility cardenolide metabolites could be easily transferred by means of the existing system, but they are not.

Interestingly, the male bias in adult feeding on PA-containing plants that is prevalent in the Lepidoptera extends also to monarch butterflies, *Danaus plexippus,* although the overall frequency of visitation is very low compared to that of *D. gilippus* (Pliske, 1975). The male hairpencils of the monarch are known to be entirely lacking in danaidone, the PA-derived courtship pheromone used by *D. gilippus* and *Lycorea ceres* (Boppré, 1978; Meinwald and Meinwald, 1966; Meinwald *et al.*, 1969; Edgar *et al.*, 1971, 1973). Monarch males do not need this compound in order to have a high success rate in mating, although it is often overlooked that 33% of males in Pliske's (1976) study of monarch courtship gave a hairpencil display and evoked settling by females without the need of a forcible takedown. The chemicals, if any, involved in this percentage of males able to induce female quiescence are not known, but again, as for *D. gilippus,* it would seem to be more important for males to display evidence of defensive ability gained from ingestion of cardenolides rather than PA's. Again, if females were in need of supplementing their defense through PA's, they would do better to go out and get them themselves. Danger to females in performing such feeding should be minimal, since they are already protected by cardenolides, either directly or by Müllerian or auto-mimicry (Brower and Brower, 1964; Brower, 1968).

As first discussed by Boppré (1978), mimicry may in fact be the key to understanding the use of PA-derived metabolites during courtship of these day-flying, aposematically colored insects, although he did not include runaway sexual selection as part of the evolutionary pathway. The adaptive response to interspecific mating mistakes (Baker and Cardé, 1979b; Phelan and Baker, 1987) by members of a mimicry complex that all rely primarily on vision to make their long-distance approach to females could trigger a female preference to arise that is based on odor. This would allow the individuals to maintain their visually similar appearance (Boppré, 1978) for purposes of defense based on cardenolides (Edgar *et al.*, 1974), while sexual selection could drive PA -based courtship systems to extremes (Baker and Cardé, 1979b). The mimicry that proliferates in such systems involving distasteful Lepidoptera, whether visual or possibly even sonic in nocturnal Lepidoptera, thus would promote mating mistakes if these modalities were also used for mate location or courtship, as they both are known to be (Brower *et al.*, 1965; Conner, 1987). Of course in nocturnal moths, the real analog to long-distance visual cross-attraction in a butterfly mimicry complex is cross-attraction due to incomplete specificity of female sex pheromones. Significant levels of pheromonal cross-attraction among congeners is in fact associated with courtship use of hairpencil structures and associated scents in palatable Lepidoptera (Phelan and Baker, 1986b, 1987).

It would thus seem that the most likely *primary* reason for adult males of most species of Lepidoptera to be more predisposed than females to visiting plants rich in PA's is merely to obtain the asssociated odor itself, consisting of danaidone, hydroxydanaidal, or danaidal, for their own mating success and hence also that of their sons, whether this competence is genetically or directly (transovarially) passed along. Thus the wide array of male courtship pheromones and scent-disseminating structures, whether based on defense-related compounds or on compounds of no known defensive value, all can be explained by the model invoking an adaptive response to interspecific mating mistakes followed by runaway female choice sexual selection (Baker and Cardé, 1979b; Phelan and Baker, 1987). The acquisition of such compounds from plants would be no different from the known uptake of floral odors by male euglossine bees, which later display these fragrances and form leks by attracting other males, and eventually females (Boppré, 1984; Dodson, 1975; Dressler, 1982). These floral compounds have no known defensive value, yet are actively sought after and used by males. The evolution of danaine butterflies away from larval feeding on PA-containing plants (Edgar *et al.*, 1974) is not inconsistent with this evolutionary scenario. Edgar *et al.* (1974) viewed the PA-derived volatiles as functioning only to evoke alightment of females due to their (former) host-odor behavior-mediating properties, and not necessarily as providing defense or a token stimulus of defense, which would still be provided by cardenolides (Edgar *et al.*, 1974).

For male butterflies, *secondarily* there may be an extra benefit conferred directly to males in the form of self-defense, as evidenced by males of some species everting their brushes upon handling (Müller, 1878). In the butterflies, this behavior only occurs in a few genera (e.g., *Lycorea, Ituna, Euploea*) (Brower and Brower, 1964; Chow and Tsai, 1988), but curiously not in *Danaus* (Brower and Brower, 1964). During the course of this symposium, the participants had the opportunity to visit the beautiful Kenting National Park in the southernmost part of Taiwan. Here, Professor Y. S. Chow demonstrated the way in which males of several danaine (*Euploeae*) butterfly species display their hairpencils in an apparently defensive mode when they are handled (see photos in Chow and Tsai, 1988). It is not known which, if any, of these butterflies emit compounds sequestered from plants as part of their courtship displays, or whether the males also acquire extra defensive protection from their displays during handling. We are attempting to develop a cooperative research project between our research group and groups from Taiwan in order to address these and other questions.

CONCLUSION

Many questions concerning lepidopteran pheromone communication have been answered by research on the oriental fruit moth. Many others remain, but should provide powerful new insights when they are explored and answered in the future with new lines of experimentation. Concerning the evolution of the lepidopteran courtship pheromones, an intriguing link is now emerging between female host-plant acceptance and runaway sexual selection that is a potentially powerful key to understanding the process of speciation. If female discrimination (sensory and behavioral thresholds) for host-related chemical cues can be significantly influenced by highly directional sexual selection for these same host-plant related cues during courtship, then host preference and acceptance may be channeled deeply by sexual preference. This would be a profound interaction indeed, and it seems to me that further inquiry along this line should yield important and interesting results.

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REFERENCES

Baker, T. C. 1983. Variations in male oriental fruit moth courtship patterns due to male competition. *Experientia,* **39,** 112–114.

Baker, T. C. 1985. Chemical control of behavior. In "Comprehensive Insect Physiology, Biochemistry, and Pharmacology", Volume 9 (Kerkut, G. A.; Gilbert, L. S., Eds.); Pergamon Press, New York, pp. 621–672.

Baker, T. C. 1986. Pheromone-modulated movements of flying moths. In "Mechanisms in Insect Olfaction" (Payne, T. L., Kennedy, C. E; Birch, M. C., Eds.); Clarendon Press, Oxford, pp. 39–48.

Baker, T. C. 1989. Pheromones and flight behaviour. In "Insect Flight" (Goldsworthy, G. G.; Wheeler, C., Eds.); CRC Press, Boca Raton, FL, pp. 231–255.

Baker, T. C.; Cardé, R. T. 1979a. Analysis of pheromone-mediated behaviors in male *Grapholitha molesta,* the oriental fruit moth (Lepidoptera: Tortricidae). *Environ. Entomol.,* **8,** 956–968.

Baker, T. C.; Cardé, R. T. 1979b. Courtship behavior of the Oriental fruit moth (*Grapholitha molesta*): experimental analysis and consideration of the role of sexual selection in the evolution of courtship pheromones in the Lepidoptera. *Ann. Entomol. Soc. Am.,* **72,** 173–188.

Baker, T. C.; Hansson, B. S.; Löfstedt, C.; Löfqvist, J. 1988. Adaptation of antennal neurons in moths is associated with cessation of pheromone-mediated upwind flight. *Proc. Nat. Acad. Sci. USA,* **85,** 9826-9830.

Baker, T. C.; Haynes, K. F. 1987. Manoeuvres used by flying male oriental fruit moths to relocate a sex pheromone plume in an experimentally shifted wind-field. *Physiol. Entomol.,* **12,** 263-279.

Baker, T. C.; Haynes, K. F. 1989. Field and laboratory electroantennographic measurements of pheromone plume structure correlated with oriental fruit moth behaviour. *Physiol. Entomol.,* **14,** 1-2

Baker, T. C.; Meyer, W.; Roelofs, W. L. 1981a. Sex pheromone dosage and blend specificity of response by Oriental fruit moth males. *Entomol. Exp. Appl.,* **30,** 269-279.

Baker, T. C.; Nishida, R.; Roelofs, W. L. 1981b. Close-range attraction of female oriental fruit moths to herbal scent of male hairpencils. *Science,* **214,** 1359-1361.

Baker, T. C.; Roelofs, W. L. 1981. Initiation and termination of Oriental fruit moth male response to pheromone concentrations in the field. *Environ. Entomol.,* **10,** 211-218.

Baker, T. C.; Willis, M. A.; Haynes, K. F.; Phelan, P. L. 1985. A pulsed cloud of pheromone elicits upwind flight in male moths. *Physiol. Entomol.,* **10,** 257-265.

Baker, T. C.; Willis, M. A.; Phelan, P. L. 1984. Optomotor anemotaxis polarizes self-steered zigzagging in flying moths. *Physiol. Entomol.,* **9,** 365-376.

Bjostad, L. B.; Linn, C. E.; Du, J. W.; Roelofs, W. L. 1984. Identification of new sex pheromone components in *Trichoplusia ni,* predicted from biosynthetic precursors. *J. Chem. Ecol.,* **10,** 1309-1323.

Bjostad, L. B.; Linn, C. E.; Du, J. W.; Roelofs, W. L. 1985. Identification of new sex pheromone components in *Trichoplusia ni* and *Argyrotaenia velutinana,* predicted from biosynthetic precursors. In "Semiochemicals: Flavors and Pheromones" (Acree, T. E.; Soderlund, D. M., Eds.); American Chemical Society, Washington, D.C., pp. 223-237.

Bjostad, L. B.; Roelofs, W. L. 1983. Sex pheromone biosynthesis in *Trichoplusia ni*: key steps involve Δ11 desaturation and chain shortening. *Science,* **220,** 1387-1389.

Bjostad, L. B.; Roelofs, W. L. 1986. Sex pheromone biosynthesis in the redbanded leafroller moth studied by mass-labeling with stable isotopes and analysis with mass spectrometry. *J. Chem. Ecol.,* **12,** 431-450.

Bjostad, L. B.; Wolf, W. A.; Roelofs, W. L. 1987. Pheromone biosynthesis in lepidopterans: desaturation and chain shortening, In "Pheromone Biochemistry" (Prestwich, G. D.; Blomquist, G. L., Eds.); Academic Press, New York, pp. 77-120.

Boppré, M. 1978. Chemical communication, plant relationships, and mimicry in the evolution of danaid butterflies. *Entomol. Exp. Appl.,* **24,** 264-277.

Boppré, M. 1984. Redefining "pharmacophagy". *J. Chem. Ecol.,* **10,** 1151-1156.

Boppré, M. 1986. Insects pharmacophagously utilizing defensive plant chemicals (pyrrolizidine alkaloids), *Naturwissenschaften,* **73,** 17-26.

Bradbury, J. W. 1981. The evolution of leks. In "Natural Selection and Social Behavior" (Alexander, R. D.; Tinkle, D. W., Eds.); Chapman and Hall, London, pp. 139-169.

Brower, L. P. 1968. Ecological chemistry. *Sci. Am.,* **220,** No. 2, 22-29.

Brower, L. P.; Brower, J. Van Zandt. 1964. Birds, butterflies, and plant poisons: a study in ecological chemistry. *Zoologica,* **49,** 137-159.

Brower, L. P.; Brower, J. V. Z.; Cranston, F. P. 1965. Courtship behavior of the queen butterfly, *Danaus gilippus berenice. Zoologica,* **50,** 1-39.

Brower, L. P.; Seiber, J. N.; Nelson, C. J.; Lynch, S. P.; Tuskes, P. M. 1982. Plant-determined variation in the cardenolide content, thin-layer chromatography profiles, and emetic potency of monarch butterflies, *Danaus plexippus* reared on the milkweed, *Asclepias eriocarpa* in California. *J. Chem. Ecol.,* 8, 579-633.

Cardé, A. M.; Baker, T. C.; Cardé, R. T. 1979. Identification of a four-component sex pheromone of the female oriental fruit moth, *Grapholita molesta* (Lepidoptera: Tortricidae). *J. Chem. Ecol.,* **5,** 423-427.

Chow, Y. S.; Tsai, R. S. 1988. Recent studies of insect chemical communication in Taiwan. *Symp. Insect Biochem. Physiol.,* 69-82.

Conner, W. E. 1987. Ultrasound: its role in the courtship of the arctiid moth, *Cyania tenera. Experientia,* **43,** 1029-1031.

Conner, W. E.; Eisner, T.; Vander Meer, R. K.; Guerrero, A.; Meinwald, J. 1981. Precopulatory sexual interaction in an arctiid moth (*Utethesia ornatrix*): role of a pheromone derived from dietary alkaloids. *Behav. Ecol.*

Sociobiol., **9**, 227-235.

Conner, W. E.; Roach, B.; Benedict, E.; Meinwald, J.; Eisner, T. 1989. Courtship pheromone production and body size as correlates of larval diet in males of the arctiid moth, *Utethesia ornatrix*. *J. Chem. Ecol.* (in press).

Darwin, C. 1898. "The Descent of Man and Selection in Relation to Sex." Revised 2nd ed., D. Appleton and Co., New York, 688 pp. First published in 1871.

Dodson, C. H. 1975. Coevolution of orchids and bees. In "Coevolution of Animals and Plants" (Gilbert, L. E.; Raven, P. H., Eds.); University of Texas Press, Austin, pp. 91-99.

Dressler, R. L. 1982. Biology of the orchid bees (Euglossini). *Annu. Rev. Ecol. System.*, **13,** 373-394.

Dussourd, D. E.; Ubik, K.; Harvis, C.; Resch, J.; Meinwald, J.; Eisner, T. 1988. Biporental defense endowment of eggs with acquired plant alkaloid in a moth (*Utethesia ornatrix*). *Proc. Nat. Acad. Sci. USA*, **85,** 5992-5996.

Dustan, G. G. 1964. Mating behaviour of the oriental fruit moth, *Grapholitha molesta* (Busck) (Lepidoptera: Olethreutidae). *Can. Entomol.*, **22,** 377-405.

Edgar, J. A.; Culvenor, C. C. J. 1974. Pyrrolizidine ester alkaloid in danaid butterflies. *Nature,* **248,** 614-616.

Edgar, J. A.; Culvenor, C. C. J.; Robinson, G. S. 1973. Hairpencil dihydropyrrolizine derivatives of Danainae from the New Hebrides. *J. Aust. Entomol. Soc.*, **12,** 144-150.

Edgar, J. A.; Culvenor, C. C. J.; Smith, L. W. 1971. Dihydropyrrolizine derivatives in the "hairpencil" secretions of danaid butterflies. *Experientia,* **27,**761-762.

Edgar, J. A.; Culvenor, C. C. J.; Pliske, T. E. 1974. Coevolution of danaid butterflies with their host plants. *Nature* (London), **259,** 646-648.

Eisner, T. 1980. Chemistry, defense, and survival: case studies and selected topics. In "Insect Biology and the Future" (Locke, M.; Smith, D. S., Eds.); Academic Press, New York, pp. 847-878.

Eisner, T.; Meinwald, J. 1987. Alkaloid-derived pheromones and sexual selection in Lepidoptera. In "Pheromone Biochemistry" (Prestwich, G. D.; Blomquist, G. L., Eds.); Academic Press, New York, pp. 251-269.

Fisher, R. A. 1958. "The Genetical Theory of Natural Selection" 2nd revised ed., Dover, New York. First published in 1930.

George, J. A.; Mullins, J. 1980. Hairpencils on males of the oriental fruit moth, *Grapholitha molesta* (Busck) (Lepidoptera: Tortricidae). *Proc. Entomol. Soc. Ont.*, **111,** 21-31.

Kennedy, J. S. 1940. The visual responses of flying mosquitoes. *Proc. Zool. Soc. London, A,* **109,** 221-242.

Kennedy, J. S. 1986. Some current issues in orientation to odour sources. In "Mechanisms in Insect Olfaction" (Payne, T. L.; Birch, M. C.; Kennedy, C., Eds.); Clarendon Press, Oxford, pp. 11-25.

Kennedy J. S.; Ludlow, A. R.; Sanders, C. J. 1980. Guidance system used in moth sex attraction. *Nature,* **295,** 475-477.

Kennedy, J. S.; Ludlow, A., R.; Sanders, C. J. 1981. Guidance of flying male moths by wind-borne sex pheromone. *Physiol. Entomol.*, **6,** 395-412.

Kennedy, J. S.; Marsh, D. 1974. Pheromone-regulated anemotaxis in flying moths. *Science,* **184,** 999-1001.

Krasnoff, S. B.; Bjostad, L. B.; Roelofs, W. L. 1987. Quantitative and qualitative variation in male pheromones of *Phragmatobia fuliginosa* and *Pyrrharctia isabella* (Lepidoptera: Arctiidae). *J. Chem. Ecol.*, **13,** 807-822.

Krasnoff, S. B.; Dussourd, D. E. 1989. Dihydropyrrolizidine attractants for arctiid moths that visit plants containing pyrrolizidine alkaloids. *J. Chem. Ecol.* (In press.)

Krasnoff, S. B.; Roelofs, W. L. 1989a. Quantitative and qualitative effects of larval diet on male scent secretions of *Estigmene acrea, Phragmatobia fuliginosa* and *Pyrrharctia isabella* (Lepidoptera: Arctiidae). *J. Chem. Ecol.*, **15,** 1077-1093.

Krasnoff, S. B.; Roelofs, W. L. 1989b. The courtship behavior of *Phragmatobia fuliginosa* and *Pyrrharctia isabella* (Lepidoptera: Arctiidae) with special reference to the role of male pheromones. *Zool. J. Linn. Soc.*, (in press).

Kuenen, L. P. S.; Baker, T. C. 1983. A non-anemotactic mechanism used in pheromone source location by flying moths. *Physiol. Entomol.*, **8,** 277-289.

Linn, C. E.; Bjostad, L. B.; Du, J. W.; Roelofs, W. L. 1984. Redundancy in a chemical signal: behavioral responses of male *Trichoplusia ni* to a 6-component pheromone blend. *J. Chem. Ecol.*, **10,** 1635-1658.

Linn, C. E., Jr.; Campbell, M. G.; Roelofs,W. L. 1986. Male moth sensitivity to multicomponent pheromone: critical role of female-released blend in determining the functional role of components and active space of the pheromone. *J. Chem. Ecol.*, **12,** 659-668.

Linn, C. E., Jr.; Campbell, M. G.; Roelofs, W. L. 1987. Pheromone components and active spaces: what do moths smell and where do they smell it? *Science,* **237,** 650-652.

Linn, C. E., Jr.; Campbell, M. G.; Roelofs,W. L. 1988. Temperature modulation of behavioural thresholds controlling male moth sex pheromone response specificity. *Physiol. Entomol.*, **13**, 59-67.

Linn, C. E.; Roelofs, W. L. 1983. Effect of varying proportions of the alcohol component on sex pheromone blend discrimination in male oriental fruit moths. *Physiol. Entomol.*, **8**, 291-306.

Löfstedt, C.; Bengtsson, M. 1988. Sex pheromone biosynthesis of (*E,E*)-8,10-dodecadienol in codling moth *Cydia pomonella* involves E9 desaturation. *J. Chem. Ecol.*, **14**, 903-915.

Löfstedt, C. L.; Vickers, N. J.; Roelofs, W. L.; Baker, T. C. 1989. Diet-related courtship success in the oriental fruit moth, *Grapholita molesta* (Busck) (Lepidoptera:Tortricidae). *Oikos*, (In press.)

Marsh, D.; Kennedy, J. S.; Ludlow, A. R. 1978. An analysis of anemotactic zigzagging flight in male moths stimulated by pheromone. *Physiol. Entomol.*, **3**, 221-240.

Meinwald, J.; Meinwald, Y. C. 1966. Structure and synthesis of the major components in the hairpencil secretion of a male butterfly, *Lycorea ceres ceres* (Cramer). *J. Am. Chem. Soc.*, **88**, 1305-1310.

Meinwald, J.; Meinwald, Y. C.; Mazzocchi, P. H. 1969. Sex pheromone of the queen butterfly: chemistry. *Science*, **164**, 1174-1175.

Meyer, A.; Miersch, O.; Büttner, C.; Dathe, W.; Sembdner, G. 1984. Occurrence of the plant growth regulator jasmonic acid in plants. *J. Plant Growth Regul.*, **3**, 1-8.

Müller, F. 1878. Notes on Brazilian entomology. *Trans. Entomol. Soc. London*, 211-223.

Murlis, J.; Jones, C. D. 1981. Fine-scale structure of odour plumes in relation to insect orientation to distant pheromone and other attractant sources. *Physiol. Entomol.*, **6**, 71-86.

Nishida, R.; Fukami, H.; Baker, T. C.; Roelofs, W. L.; Acree, T. E. 1985. Oriental fruit moth pheromone: attraction of females by an herbal essence. In "Semiochemistry: Flavors and Pheromones" (Acree, T. C.; Soderlund, D. M., Eds.): Walter de Gruyter & Co., Berlin, pp. 47-63.

O'Donald, P. 1962. The theory of sexual selection. *Heredity*, **17**, 541-552.

O'Donald, P. 1967. A general model of sexual and natural selection. *Heredity*, **22**, 499-518.

Phelan, P. L.; Baker, T. C. 1986a. Male-size-related courtship success and intersexual selection in the tobacco moth, *Ephestia elutella*. *Experientia*, **42**, 1291-1293.

Phelan, P. L.; Baker, T. C. 1986b. Cross-attraction of five species of stored-product Phycitinae (Lepidoptera: Pyralidae) in a wind tunnel. *Environ. Entomol.*, **15**, 369-372.

Phelan, P. L.; Baker, T. C. 1987. Evolution of male pheromones in moths: reproductive isolation through sexual selection? *Science*, **235**, 205-207.

Phelan, P. L.; Silk, P. J.; Northcott, C. J.; Tan, S. H.; Baker, T. C. 1986. Chemical identification and behavioral characterization of male wing pheromone of *Ephestia elutella* (Pyralidae). *J. Chem. Ecol.*, **12**, 135-146.

Pliske, T. E. 1975. Attraction of Lepidoptera to plants containing pyrrolizidine alkaloids. *Environ. Entomol.*, **4**, 455-473.

Pliske, T. E. 1976. Courtship behavior of the monarch butterfly, *Danaus plexippus* L. *Ann. Entomol. Soc. Am.*, **68**, 143-151.

Pliske, T. E.; Eisner, T. 1969. Sex pheromone of the queen butterfly: Biology. *Science*, **164**, 1170-1172.

Roelofs, W. L.; Brown, R. L. 1982. Pheromones and evolutionary relationships of Tortricidae. *Annu. Rev. Ecol. System.*, **13**, 395-422.

Roelofs, W. C.; Comeau, A.; Selle, R. 1969. Sex pheromone of the oriental fruit moth. *Nature*, **224**, 723.

Rumbo, E. R. 1983. Differences between single cell responses to different components of the sex pheromone in males of the light-brown apple moth (*Epiphyas postvittana*). *Physiol. Entomol.*, **8**, 195-201.

Schneider, D.; Boppré, M.; Zweig, J.; Horsley, S. B.; Bell, T. W.; Meinwald, J.; Hansen, K.; Diehl, E. W. 1982. Scent organ development in *Creatonotus* moths: regulation by pyrrolizidine alkaloids. *Science*, **215**, 1264-1265.

Tamaki, Y. 1985. Sex pheromones. In "Comprehensive Insect Physiology, Biochemistry, and Pharmacology", Volume 9 (Kerkut, G. A.; Gilbert, L. S., Eds.); Pergamon Press, Ltd., New York, pp. 145-191.

Teal, P. E. A.; Tumlinson, J. H. 1986. Terminal steps in pheromone biosynthesis by *Heliothis virescens* and *H. zea*. *J. Chem. Ecol.*, **12**, 353-366.

Wagner, D. 1985. Lek behavior in lepidoptera with emphasis on *Hepialus* (Hepialidae): Reevaluation of the enhanced conspicuousness hypothesis. Paper presented at the Annual Meeting of the Entomol. Soc. Am., Hollywood FL, Dec. 8-12.

Willis, M. A.; Baker, T. C. 1984. Effects of intermittent and continuous pheromone stimulation on the flight behaviour of the oriental fruit moth, *Grapholita molesta. Physiol. Entomol.*, **9**, 341-358.

Willis, M. A.; Birch, M. C. 1982. Male lek formation and female calling in a population of the arctiid moth *Estigmene acrea. Science,* **218**, 168-170.

Wright, R. H. 1958. The olfactory guidance of flying insects. *Canad. Entomol.*, **90**, 81-89.

Wunderer, H.; Hansen, K.; Bell, T. W.; Schneider, D.; Meinwald, J. 1986. Sex pheromone of two Asian moths (*Creatonotus transiens, C. gangis*: Lepidoptera: Arctiidae): behavior, morphology, chemistry and electrophysiology. *Exp. Biol.*, **46**, 11-22.

QUESTIONS, ANSWERS, AND COMMENTS

Question: Dr. Lovett to Dr. Baker. Concerning your statement about Lepidoptera females producing fatty acid derivatives for their sex pheromones and males producing plant-related compounds: is this true for all insect orders or just for Lepidoptera?

Answer: Thus far, for the Lepidoptera in general this pattern is persisting. However, the only other order that we have similarly extensive information about is the Coleoptera, and here things apper to be somewhat different. Some of the families, such as bark beetles, have male-based as well as female-based communication systems that involve a mixture of host plant volatiles plus host volatiles that have been slightly altered into pheromone components. Other families have female-based pheromone systems that appear to be derived from fatty acid precursors, and others have male-based systems involving complex compounds apparently not derived from plants.

Comment by Dr. Baker: To begin with, lepidopterous pheromone components have been established by toxicological tests to be completely nontoxic; it is difficult to even measure an LD_{50} for them since they are metabolized so easily by mammalian systems because of their close relationship to common fatty acids. Esterases, oxidases, and epoxidases in insect tissues are known to convert the behaviorally active pheromone molecules to inactive forms very quickly, with half-lives (depending on the tissues) on the order of milliseconds to minutes. In the environment, pheromone molecules emitted at high concentrations from synthetic lures can sometimes be adsorbed onto plants and be re-emitted for hours, as evidenced by the persistent behavioral responses of male moths attracted to the plants long after the lure has been removed. There is some evidence that a pheromone emitted at the usual low rates by insects may last for a few minutes on plant surfaces, after which it is dissipated by volatilization and degradation. Active pheromone molecules, probably only at the picogram or femtogram levels, are also known to persist on human skin and clothing, especially leather articles, for days or weeks, presumably because they are protected from enzymatic degradation on such surfaces. Evidence of their re-emission again comes from behavioral responses of male moths attracted to the (human) emitter at inopportune moments, often to the amusement of onlookers.

Question: Dr. Zenda Munro to Dr. Baker: The GC-EAD should give an accurate determination for compounds, but from your presentation it looks like this may not always be the case. Is there any reason why an EAG signal cannot be always obtained for behaviorally active compounds?

Answer? In most cass, the GC-EAD will accurately detect the compound or compounds of major behavioral importance. However, for some minor components, there may not be enough receptors on the antenna devoted to the detection of these compounds to generate a signal that we researchers can see after electronic amplification. In the moth brain, on the other hand, sufficient amplification of the signal from these relatively rare receptors does in fact occur due to convergence of thousands of such receptors, and the animal has no problem in adding the signal from these minor component receptors to the signal from the total blend. An example of this comes from work by Van Der Pers and Löfstedt (1986) on *Agrotis segetum,* the turnip moth. Only one or two sensory hairs out of every hundred on male antennae contain sensory neurons that are sensitive to a minor but behaviorally important pheromone component, (*Z*)-9-tetradecenyl acetate, whereas the rest of the hairs contain cells sensitive to the two major components. These two components evoke significant EAG's and good GC-EAD signals, whereas (*Z*)-9-tetradecenyl acetate does not. Normally, the GC-EAD technique is very good during the first stages of pheromone identification in which even the most abundant components of the blend need to be isolated and identified. After that, however, behaviorally important components that were undetectable by GC-EAD may still need to be identified by means of good old-fashioned recombination of GC or HPLC fractions coupled with behaviorally discriminating assays such as those that measure upwind flight in wind tunnels.