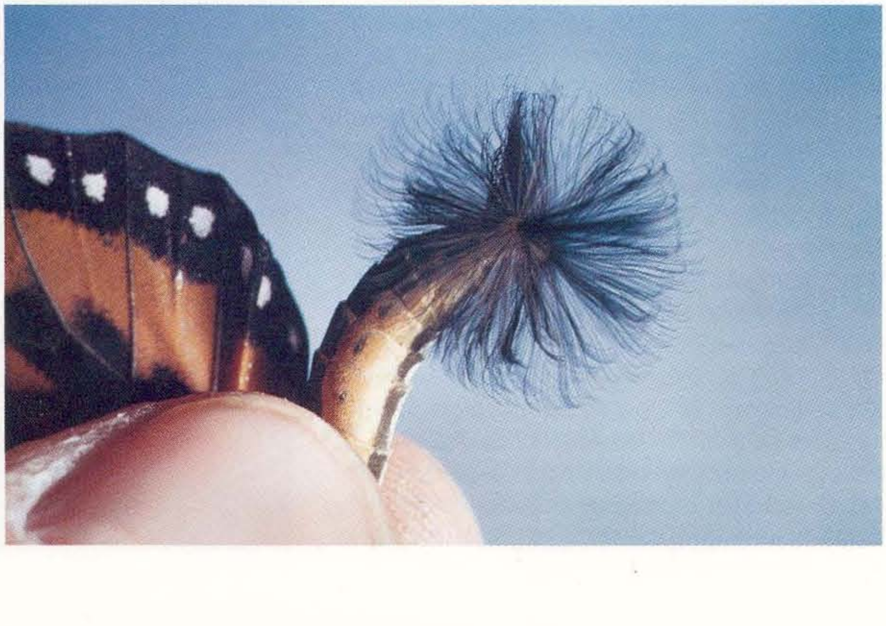
The Insect as Synthetic Chemist
Chemical Aspects of Defense, Courtship,

Above: Lycorea ceres, *male.*

Right: Everted hairpencils of Lycorea ceres, *from which danaidone (figure 11) was first isolated.*

Far right: Aimed defensive spray of a whipscorpion is made visible on phenolphthalein-impregnated indicator paper. Photographs by Thomas Eisner.



and Mate Selection

by Jerrold Meinwald

IN RESPONSE TO ATTACK, a whipscorpion, commonly known as a "vinegaroon," (*Mastigoproctus giganteus*) emits a carefully aimed defensive spray. My good friend and colleague, Thomas Eisner, and I, along with Ralph Ghent and Alistair Monro, studied this defensive mechanism many years ago. I had not taken the problem terribly seriously since the chemistry was essentially trivial: we found the secretion to consist of 85 percent acetic acid (figure 1), along with 10 percent water and 5 percent octanoic acid (2). But in spite of its organic chemical minimalism, this defensive system proved highly effective: the secretion could readily penetrate cockroach cuticle and was lethal to fly larvae. In the absence of the octanoic acid, 85 percent acetic acid is ineffective in these respects. It is clear that this primitive arachnid had hit upon an elegantly simple chemical weapon, and it is likely that this weapon has contributed to the continued success of an ancient species.

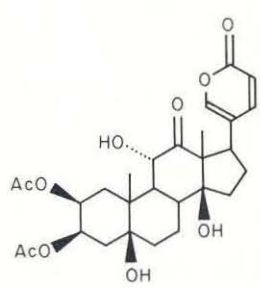
As we extended our work with insects and their relatives, it became obvious that these animals are highly skilled chemists, from whom human chemists and biologists have a great deal to learn. One recent estimate (T. L. Erwin) puts the number of insect species at about 30 million; it is going to take a considerable time to study even the smallest fraction of them! Nevertheless, since certain of our interactions with some insect species are of keen interest because of insects' roles as disease vectors and as agricultural and forest pests, there are strong practical, as well as purely scientific, motivations to learn as much about this spectacularly successful group of animals as we possibly can.

While some aspects of insect chemistry, such as the production of formic acid by certain ants, have been known since classical times, it has been only in the last three decades, with the advent of an ever-growing array of instrumental methods of analysis, that a good start has been made in elucidating the ways in which insects exploit organic chemistry. Most of our own work in this field has been concerned with defensive chemistry. However, since that is not going to be the main focus of the present paper, I will leave this subject for the moment by noting that defensive compounds that we have encountered and characterized range from the stark simplicity of hydrogen cyanide (3) to the relatively complex lucibufagins, a group

1 CH_3CO_2H

2 $CH_3(CH_2)_6CO_2H$

3 $H-C\equiv N$



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of cardiac-active steroids from fireflies, exemplified by structure 4, the chief defensive steroid found in the hemolymph of *Photinus pyralis*.

We can regard all of these compounds as *semiochemicals* (compounds that carry signals), because they convey a clear, direct message from prey to predator: *Leave me alone!* Most current discussions of chemical communication, however, have focused on pheromones, or the chemical messengers that carry information from one individual to another *within* a species, and it is this type of activity that will be my main subject.

The first pheromone to be characterized — the fruit of a heroic research effort carried out by Adolph Butenandt and his collaborators in pre- and post-World War II Germany — was bombykol (5), the sex attractant of the female silk moth *Bombyx mori*. Since the structure of bombykol was published in 1961, hundreds of other lipid-related female lepidopteran pheromones have been characterized and synthesized. While their chemical structures are often mundane, the specificity and sensitivity of the pheromone receptors present in the male's antennae have proven to be remarkable. Typically, a single female moth gives off from nanogram to microgram amounts of pheromone, eliciting a response in a male of the same species hundreds of meters downwind.

For reasons that will be apparent later, Tom Eisner and I became interested in understanding all we could about how the chemical activities of one particular species of moth, *Utetheisa ornatrix*, are related to its behavior. In the course of this work, we characterized and synthesized three C-21 polyunsaturated hydrocarbons (6, 7, and 8), which serve as female sex pheromones in this species.

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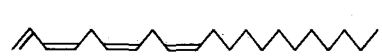
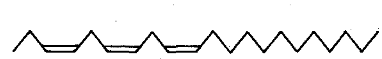
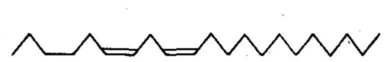
6 

7 

8 

What was most exciting about this work, however, was not the molecular structures themselves, but rather the discovery by William Conner, then a graduate student with Eisner, that the pheromone was released by a "calling" female in discrete pulses, at a frequency of about 1 Hz. Some 18 years before this observation, W. H. Bossert and E. O. Wilson at Harvard had written a fascinating theoretical paper on the possibility of animals using pulsed, aerial pheromone signals, including a consideration of the advantages that an animal might derive from the temporal modulation of a chemical signal. Although no examples of the phenomenon were known at the time, it now appears that pulsed pheromone signals may actually be of widespread significance in insect chemical communication. Using the electroantennogram technique (in which a fine electrode applied to an antennal nerve records signals generated in response to chemical stimuli), Conner and Eisner demonstrated that, at the very least, a male at a short distance from a calling female can detect sharp pulses. It is likely that this type of signal is interpreted as evidence that the female must be nearby. With synthetic pheromones available to generate experimental signals, Conner (now at Duke University) is exploring exactly what sorts of information are transmitted by pulsing females, and he is adding a hitherto unrecognized dimension to chemical communication research.

Typically, moth courtship and mating are nocturnal activities; the use of chemical sex attractants is a wonderfully appropriate adaptation in these circumstances. Butterflies, on the other hand, court during the day, and it is apparent that vision, rather than chemistry, plays an important role in the long-range attraction of males to females. Our former associate, the late Robert E. Silberglied, pointed out that butterflies have the largest spectral range of vision known for any group of animals (from below 300 nm to above 700 nm). Over a century ago, Darwin conjectured that vision plays a key role in a female butterfly's choice of mate, although no clear evidence that this is so has been presented.

About 20 years ago, we became interested in the role of chemistry in butterfly courtship. Early anatomical studies had shown that males of some butterfly species (the danaids) possess specialized organs, the *hairpencils,* which often have a characteristic odor. A study of the courtship of the Florida queen butterfly (*Danaus gilippus*) by Lincoln and Jane Van Zandt Brower, with Florence Cranston (Amherst College), showed that these specialized organs were brushed against the female's antennae during courtship. Yvonne Meinwald and I, along with James Wheeler, studied the chemistry of hairpencils of a Trinidad danaid species (*Lycorea ceres*), and found that these organs contain three major compounds, cetyl acetate (9), *cis*-vaccenyl acetate (10), and a heterocyclic ketone based on the pyrrolizidine nucleus, which we subsequently called danaidone (11).

9 $CH_3(CH_2)_{14}CH_2OAc$

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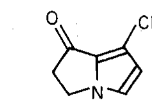
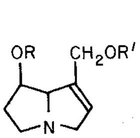
11 

Danaidone attracted our interest because it has a structure unlike that of any previously known animal metabolite. Strikingly, it bears a close resemblance to the pyrrolizidine alkaloids, a widely distributed group of plant natural products, many of which can be represented by the generalized structural formula 12.



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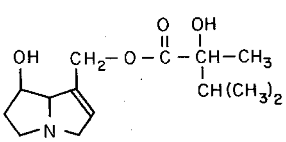

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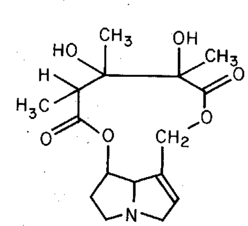
Turning to a species (the Florida queen butterfly) whose courtship behavior had already been examined and whose chemistry proved to be similar, Tom Pliske and Eisner were able to establish that danaidone is an "aphrodisiac" pheromone. Females are much more responsive to males who present the compound during "hairpencilling" than to chemically deficient males (laboratory raised) who go through the same courtship behavior. We were all puzzled, however, by the failure of males grown in captivity to produce this courtship pheromone, although we suspected that their failure was related to the lack of an unknown, plant-derived, biosynthetic precursor.

In collaboration with Dietrich Schneider (Seewiesen, West Germany), we were able to clarify this situation by studying a closely related danaid, the African monarch butterfly (*Danaus chrysippus*), which also proved to use danaidone as a pheromone. In this case, we found that adult males in the field seek out and extract material from senescent specimens of an East African plant (*Heliotropium steudneri*), thereby acquiring the pyrrolizidine alkaloid, lycopsamine (13). A series of laboratory experiments established that male African monarchs cannot produce danaidone without access either to this plant or to the alkaloid itself. Since we had not provided a source of pyrrolizidine alkaloid to the Florida queens that we raised in captivity, we can now understand why these animals lacked their pheromone.

We were now faced with an interesting contrast. Female lepidopteran pheromones are produced from ubiquitous precursors via pathways closely related to those of fatty acid biosynthesis, as brilliantly elucidated by W. L. Roelofs and L. Bjostad (Cornell). Male danaids, however, seem to require a specific type of exogenous plant alkaloid in order to produce their aphrodisiac pheromone. How can this bizarre plant/insect dependence have arisen, and what could the inability of males to function as independent pheromone synthesizers possibly signify?

More recent work on *Utetheisa ornatrix* has led Eisner, in collaboration with Bill Conner and David Dussourd, to some exciting hypotheses. These moths feed on *Crotalaria* plants as larvae and consequently ingest and sequester large amounts of pyrrolizidine alkaloids, such as monocrotaline (14). Monocrotaline protects the moths from predatory spiders and birds. We wondered whether it might also be used by the males as a pheromone precursor. Conner soon found, in some elegantly designed and executed behavioral studies, that the male's coremata (organs similar to hairpencils) play a key role in courtship. With Bob Vander Meer and Angel Guerrero, he showed that hydroxydanaidal (15), a close relative of danaidone (11) derived from dietary monocrotaline, was the active pheromone on these coremata.



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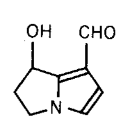

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These results demonstrated for the first time a direct chemical link between an acquired phytotoxin (14), useful in insect defense, and an insect pheromone (15). Clearly there is a certain economy in this relationship. The close chemical connection between a defensive compound and an intersexual pheromone may, however, have a still deeper significance.

Our biological collaborators noted that *U. ornatrix* eggs were not eaten by predators such as lady beetles. We found (with James Resch, Karel Ubik, and Carl Harvis) these eggs to contain about 0.5 percent of pyrrolizidine alkaloid, to which their distastefulness could be attributed. (Eggs produced by parents raised on an alkaloid-free diet were eaten readily.) Not surprisingly, eggs laid by a normal female after mating with an alkaloid-free male were also chemically protected. However, it turned out that even eggs produced by an alkaloid-free female who *had mated with an alkaloid-containing male* were partially protected! A series of chemical analyses carried out on males, females, and eggs revealed that males can transmit approximately 15 percent of their total alkaloid content to their partners in a single mating, and that these females can put over half of the received alkaloid into their eggs. Both maternal and paternal investment in the chemical protection of offspring is thereby demonstrated. We have shown, in addition, that the size of a male's nuptial gift is in proportion to his alkaloid content, as is the amount of pheromone he produces. These findings provide some clues concerning the evolution of this type of chemical communication system, and also suggest that the "meaning" of some pheromones may be closely related to their chemical structure.

Top: Utetheisa ornatrix *on its* Crotalaria *food plant.*

Bottom: Crotalaria *seedpod with* Utetheisa ornatrix *larva and partially eaten seeds.*

Let us consider the situation of an *U. ornatrix* female who has attracted a number of competing males with her pulsed pheromone signal. What we observe is that she more readily accepts as a mate a male whose coremata are laden with hydroxydanaidal. We now realize that such a male can transmit in his spermatophore protective alkaloid, which the female can incorporate into her eggs. A male without hydroxydanaidal is most likely lacking in the alkaloid that serves as its biosynthetic precursor, and therefore he cannot contribute directly to the chemical protection of his offspring. Those females who can read a male's chemical message before mating are able to do a better job of insuring the future success of their own genes than can females who are blind to this signal; there should be clear selection for this ability.

We wondered whether an analogous argument would apply to the Florida queen butterfly and found that it did. In this case, males turned out to be capable of sequestering hundreds of micrograms of dietary monocrotaline. Much of this alkaloid is stored in the male reproductive tract. During mating, it is transmitted to the females, who incorporate it into their eggs with striking efficiency.

We can now suggest that hydroxydanaidal and danaidone provide females with chemical evidence of a male's ability to acquire defensive phytotoxins, and thus serve as valuable guides to male fitness. In a recent study, Silberglied reviewed the arguments for and against Darwin's conjecture that male butterfly coloration is an important factor in female choice, and added some of his own evidence indicating that females do not, in fact, appear to use visual cues for mate selec-

tion. Our own studies demonstrate that sexual selection can be based on a simple, organic chemical criterion.

It is tempting to speculate on how these sophisticated chemical communication systems may have evolved. In the example of the *U. ornatrix* male pheromone, the process can be imagined to have started with the insect's ancestors having "broken through" the chemical defenses of a pyrrolizidine alkaloid-producing plant, thereby gaining access to a new food source. Retention of some of the dietary alkaloid would then provide an additional benefit in the form of a chemical defense for the insects themselves. It would not be surprising if individuals bearing an especially heavy alkaloid load would excrete either alkaloid itself or an alkaloid metabolite. Since all animals have chemoreceptors that help them react appropriately to their environment, we can anticipate that members of the same species might be able to sense these products. As we have discussed, those females who could estimate the alkaloid content of their potential sexual partners would have a selective advantage, and so there would be a reward for the "tuning" of general chemoreceptors for this purpose.

Turning to the question of why a particular chemical structure has come to transmit a given message, we can see in the case of hydroxydanaidal and danaidone that these molecules provide direct, incontrovertible evidence that the bearer has acquired defensive alkaloid.

These studies raise many questions. What determines how much alkaloid is converted by an adult male into courtship pheromone versus how much is retained for defense? How widespread is the relation between chemical defense and sexual selection? Can this alkaloid-based chemical communication system be used to lie? How are pheromones detected? Can other pheromone structures be understood in terms of the messages they convey? What is the relationship between chemical structure and distastefulness or irritancy? With continued research on insects' activities as synthetic and analytical chemists, we can hope to gain new insight into many of the interactions that occur between organisms in nature. □

The alkaloid-derived pheromone hydroxydanaidal (figure 15) is disseminated during courtship by the coremata of the male Utetheisa ornatrix. *Photographs by Thomas Eisner.*