Cross-attraction of Five Species of Stored-product Phycitinae (Lepidoptera: Pyralidae) in a Wind Tunnel

P. L. PHELAN AND T. C. BAKER

Department of Entomology, University of California,
Riverside, California 92521

Environ. Entomol. 15: 369–372 (1986)

ABSTRACT Sharing of the sex-pheromone communication channel by five species of phycitine moths was measured in a wind tunnel. Overall, male pheromonal response was characterized both by high levels of interspecific attraction and broad overlap in the circadian periods of male response, with attraction to nonconspecific females 75% of that to conspecifics, on average. *Cadra cautella* (Walker), *Plodia interpunctella* (Hübner), and *Anagasta kuehniella* (Zeller) males demonstrated high levels of upwind-flight response throughout an 8-h scotophase. *P. interpunctella* and *A. kuehniella* males responded to nonconspecific females at levels that were as high as to conspecifics. *Ephestia elutella* (Hübner) males showed the greatest level of response discrimination with the probability of flight to nonconspecific females only 41% of that to conspecific females, although relative importance of pheromonal differences and temporal effects could not be determined. Poor reproductive isolation by long-distance parameters points to a greater reliance on less efficient short-range mechanisms, such as courtship and male pheromones. Significance of these findings to current thought concerning evolution of reproductive isolating mechanisms is considered.

DESPITE THE APPARENT limited number of compounds that are utilized in the sex pheromone blends of most Lepidoptera and the sharing of many of these compounds, especially among closely related species, the chemical communication systems of most moths remain highly species-specific and act as important reproductive isolating mechanisms in many groups (Roelofs and Brown 1982). There are, however, exceptions to this general pattern, as interspecific attraction has been reported for *Plodia interpunctella* (Hübner) and *Cadra cautella* (Walker), both in laboratory studies (Ganyard and Brady 1971) and in the field (Ganyard and Brady 1972). This cross-attraction is at least in part due to (*Z,E*)-9,12-tetradecadienyl acetate (*Z,E*-9,12-14:Ac), a compound that has been reported as a major component of the female pheromone of a number of other phycitine moths (Vick et al. [1981] and references therein). These species, members of the so-called stored-product complex of phycitine moths, are largely cosmopolitan and are frequently found in an intense form of sympatry, coinhabiting grain warehouses and food stores (Levinson and Buchelos 1981). Temporal overlap in the periods of sexual activity of some species has also been reported, although Krasnoff et al. (1983) pointed to temporal differences of female calling behavior as a possible mechanism for reproductive isolation in other species of this group.

The present study investigates the species-partitioning of the sex-pheromone communication channel of five species of Phycitinae and measures the degree of isolation that can be attributable to female pheromonal differences and temporal differences in the sexual activity of these species. Analysis of male response to conspecific and nonconspecific females was carried out in a wind tunnel to partition the variables that can obscure this measure.

Materials and Methods

Five phycitine species were utilized in this study: *Ephestia elutella* (Hübner), *P. interpunctella*, *Anagasta kuehniella* (Zeller), *Cadra figulilella* (Gregson), and *C. cautella*. Colonies of each species were maintained separately on artificial media after the procedures described by Strong et al. (1968). Individuals were separated by sex at the pupal stage, and adults of all species were maintained on concurrent 16:8 (L:D) photoperiods with access to a 15% sucrose solution. *E. elutella* was obtained from a laboratory colony at the University of California, Riverside; the remaining species were in culture <1 year, having been established from wild populations collected in the Riverside area. Voucher specimens for each species were prepared and are available for examination.

Females were tested for their ability to elicit upwind flight of males in a wind tunnel during their respective periods of peak calling (Krasnoff et al. [1983] and references therein). For *C. cautella*, this was 0.5 h after the initiation of scotophase; for *C. figulilella*, 4 h into the 8-h scotophase; and for *A. kuehniella*, during the last hour of scotophase. *P. interpunctella* and *E. elutella* display broad windows of calling behavior, with >50% of the females calling during the second to the eighth hour of a 10-h scotophase (Krasnoff et al. 1983). These two species were tested beginning either with the second or the sixth hour of scoto-

phase, the order of which was alternated between replicates. Before the initiation of scotophase, 2- to 3-day old females of each species were transferred (two per cage) to cylindrical screen cages (4 cm diam by 4 cm long). Males were placed in the tunnel room 1 h before scotophase.

At the time described above for each species, a cage containing two females was placed on a sheet-metal platform elevated 15 cm above the tunnel floor, 50 cm from the upwind end of a flight tunnel (3.4 m long) described by Kuenen and Baker (1982). The wind velocity was maintained at 0.42 m/s, with a light level of 0.4 lx and a temperature of 22 ± 3°C. A different metal platform was used for females of each species to avoid possible pheromone contamination. To ensure that at least one of the females was calling, four or five conspecific males were released from downtunnel to test for response. If no response was observed, the females were replaced with another pair of the same species. This occurred infrequently and the response of these indicator males, whether positive or negative, was not included in the analysis. Once a positive response was obtained, five males of each species were individually released downwind of the females by placing them within the pheromone plume and allowing them to take flight from a small screen cone in which they were held. Testing of males of all species for the females of each species required ca. 1 h and the species order of males was randomized for each female, using six replicates in a randomized complete-block design. Male responses were statistically compared using Ryan's (1960) multiple test for proportions.

Measurement of interspecific pheromone response by *C. figulilella* males was not possible, as attempts to elicit even intraspecific upwind flight by these males were unsuccessful. When presented with conspecific females, *C. figulilella* males responded with wingfanning and by taking flight, but rarely exhibited oriented uptunnel displacement to the females. This could not be attributed to a lack of calling by the females because males of other species responded well to the same females. Despite many attempts to alter environmental conditions such as temperature, light level, and humidity, a consistent response could not be evoked. Use of two additional strains of this species, including the offspring from a feral population, was equally unsuccessful.

Results and Discussion

Response of males to nonconspecific females was remarkably high for most of the interspecific combinations tested (Table 1). For the males of four species, the level of response to nonconspecific females was, on average, 75% of that to conspecific females. These males flew 3 m upwind, landed on the cage containing the females, and walked rapidly around the cage while wingfanning. A high level of interspecific response is undoubtedly due to the multi-species sharing of at least one sex pheromone component, Z,*E*-9,12-14:Ac. This single compound has been used in traps to simultaneously monitor populations of several species of stored-product Phycitinae in warehouses (Levinson and Buchelos 1981). Within the no-choice conditions of this study, males of both *P. interpunctella* and *A. kuehniella* demonstrated a response to all nonconspecific females that was almost identical to that to conspecific females. The high level of attraction of *P. interpunctella* males to females of *C. cautella* is consistent with the work of Ganyard and Brady (1971), who found that when *Plodia* males were released in a room containing either *P. interpunctella* or *C. cautella* females, they were attracted in equal numbers to the two species. It should also be noted, however, that when females of both species were present, males were much more likely to locate conspecific females than *C. cautella* females, suggesting the presence of secondary pheromone components in either one or both species.

Sower et al. (1974) have identified (Z,*E*)-9,12-tetradecadienyl alcohol (Z,*E*-9,12-14:OH) as a secondary component of the *Plodia* female sex pheromone. This compound was also found to reduce the response of *C. cautella* males when combined with either conspecific pheromone extracts or synthetic Z,*E*-9,12-14:Ac. This explains the significantly reduced response of these males to *Plodia* females (40 versus 80% to conspecific females) in the present wind-tunnel studies. Furthermore, *E. elutella*, another species to which *C. cautella* males responded poorly (20%), also utilizes the alcohol as a pheromonal component (Krasnoff et al. 1984); response of *C. cautella* males to *C. figulilella* and *Anagasta* females (80 and 75%, respectively), from which Z,*E*-9,12-14:OH has not been identified, was

Table 1. Percentage of males of four phycitine species flying 3 m uptunnel and landing on a cage containing one of five species of females

♀♀	♂♂ (%)[ab]				$\bar{x}$ attraction of nonconspecifics/conspecifics
	C. cautella	*E. elutella*	*P. interpunctella*	*A. kuehniella*	
C. cautella	80a	11c	74a	74a	0.66
E. elutella	20b	60a	69a	71a	0.89
C. figulilella	80a	24bc	80a	67a	—
P. interpunctella	40b	44ab	77a	76a	0.69
A. kuehniella	75a	20bc	76a	76a	0.75
$\bar{x}$ response to nonconspecifics/conspecifics	0.67	0.41	0.97	0.95	

[a] Means for six replicates of five males per species

[b] Values within a column followed by the same letter are not significantly different ($P > 0.05$; Ryan's [1960] multiple test for proportions).

comparable with their response to conspecific females.

E. elutella males appeared to exhibit the most discriminating response. Their probability of flying upwind to any of the nonconspecific females was lower than the probability of flying to their own females; on average, interspecific response was 41% of that to conspecific females. Interestingly, their greatest interspecific response was to *Plodia* females. Again, this is probably due to the sharing of Z,*E*-9,12-14:OH as well as Z,*E*-9,12-14:Ac as pheromone components in these species.

In addition to high levels of overlap in pheromone-modulated response, it also appears that temporal overlap is greater than previously supposed. Krasnoff et al. (1983) considered the possibility that some reproductive isolation might be provided by temporal differences in sexual activity periods between *C. cautella, C. figulilella,* and *A. kuehniella,* based on previous studies of female calling periodicities in these species. Although the durations of male response periods typically approximate the circadian periods of female calling in moths (Roelofs and Cardé 1974, 103), the results of the present study indicate that males of many stored-product Phycitinae have much broader intervals of responsiveness than would be suggested by the calling windows of their females. Males of *P. interpunctella* and *A. kuehniella* responded equally well throughout the 8-h scotophase and *C. cautella* males showed high levels of response at the beginning, middle, and end of the scotophase. The reduced response of *C. cautella* males to *E. elutella* and *P. interpunctella* females cannot be attributed to temporal effects and is likely due to differences in pheromonal chemistry. The breadth of the period of *E. elutella* male response could not be ascertained by this study. Males responded equally well to conspecific females, whether tested at 2 or 6 h into scotophase; however, response to conspecific females was not tested before or after this 5-h period and may have been lower at these times. Therefore, although pheromonal differences probably are the cause of poor response to females of *C. cautella* and *A. kuehniella,* temporal effects cannot be ruled out in this case.

Chemical identifications of female sex pheromones for over a hundred species of moths have uncovered a relatively limited number of different compounds; these are primarily even-numbered 10- to 18-carbon straight-chain acetates, alcohols, and aldehydes, although exceptional compounds have also been elucidated (Cardé and Baker 1984). Despite a seemingly limited "alphabet" of chemicals, transmission of the sex pheromonal message is, typically, highly species-specific. Even when sympatric species share one or more pheromone components, interspecific cross-attraction is usually very low (Cardé et al. 1977, Greenfield and Karandinos 1979). This specificity may be brought about through a number of mechanisms: 1) use of additional pheromone components, 2) utilization of unique and precise ratios of the shared components in conjunction with a comparably precise male response, and 3) temporal differences in diel periodicities of sexual activity. Species-specific chemical communication in the Tortricidae is of paramount importance to reproductive isolation and well exemplifies the utilization of all these mechanisms (Roelofs and Brown 1982). For example, nine tortricine species may be found within a single apple orchard, all of which share pheromone components with at least one other species and eight of which overlap in their periods of sexual activity. Five species in this group have two components in common, (Z)- and (*E*)-11-tetradecenyl acetate. *Archips semiferana* (Walker) and *Choristoneura rosaceana* (Harris) maintain isolation through precise differential ratios of these two components. The pheromone systems of *A. argyrospila* (Walker) and *A. mortuanus* Kearfott are distinguished from those of other species by the presence of two additional components, (Z)-9-tetradecenyl acetate and dodecyl acetate, and are differentiated from each other by unique blend ratios of the four compounds. The fifth species of this complex, *Argyrotaenia velutinana* (Walker), is reproductively isolated by a combination of temporal differences and differences in blend ratio.

The results of this study suggest that, in contrast to the mating systems of the Tortricidae and most other moth groups, the long-range sexual communication channel of the stored-product Phycitinae is poorly partitioned. The utilization of secondary components in the sex pheromone, such as in *P. interpunctella* and *E. elutella,* reduces the probability of cross-attraction between some species pairs; however, this isolation is far from complete for any of the species in this group. This poor isolation along such long-distance parameters as diel periodicity, habitat, and female sex pheromone points to a greater reliance on less efficient, short-range factors. Grant et al. (1975) found high levels of reproductive isolation between *C. cautella* and *P. interpunctella* to be effected in the courtship arena due to differences in courtship behavior and courtship pheromones. Likewise, in the courtships of *E. elutella* and *C. figulilella,* females of both species display strong discrimination against nonconspecific males, making interspecific matings improbable (unpublished data). These rejections by females appear to be due to species differences in male pheromones.

Evolutionary theory predicts a relatively low level of energy/time expenditure for reproductive isolating mechanisms that have arisen through natural selection, whether such mechanisms were initially selected in response to interspecific mating mistakes (Dobzhansky 1940) or simply to increase the efficiency of specific-mate recognition (Paterson 1978, 1982). Isolation at the courtship stage occurs late in the mating process of moths, requiring considerable time wastage, especially for the male. This apparent inconsistency necessitates the

consideration of other models for the evolution of isolating mechanisms. The possible role of sexual selection in the evolution of reproductive isolation has been given greater attention recently (Thornhill and Alcock 1983, West-Eberhard 1983). A sexual selection explanation is more consistent with the evolution of the phycitine mating system, in that sexual selection would not necessarily favor an isolating mechanism with greater energy efficiency. In fact, such a selection process may occur in the absence of pressure from interspecific mating mistakes, with the reproductive isolation role arising only as an incidental by-product. The evolution of isolating mechanisms has remained an area of unresolved debate for the past 40 years. In the Lepidoptera, we presently entertain only a shallow understanding of the relationship between reproductive isolation and the evolution of chemical communication. The role of sexual selection in chemically mediated reproductive isolation in this group will be considered in a future work.

Acknowledgment

We thank R. S. Vetter for rearing the insects.

References Cited

Cardé, R. T., and T. C. Baker. 1984. Sexual communication with pheromones, pp. 355–383. *In* W. J. Bell and R. T. Cardé [eds.], Chemical ecology of insects. Chapman & Hall, New York.

Cardé, R. T., A. M. Cardé, A. S. Hill, and W. L. Roelofs. 1977. Sex pheromone specificity as a reproductive isolating mechanism among the sibling species *Archips argyrospilus* and *A. mortuanus* and other sympatric tortricine moths (Lepidoptera: Tortricidae). J. Chem. Ecol. 3: 71–84.

Dobzhansky, T. 1940. Speciation as a stage in evolutionary divergence. Am. Nat. 74: 312–321.

Ganyard, M. C., and U. E. Brady. 1971. Inhibition of attraction and cross-attraction by interspecific sex pheromone communication in Lepidoptera. Nature (London) 234: 415–416.

1972. Interspecific attraction in Lepidoptera in the field. Ann. Entomol. Soc. Am. 65: 1279–1282.

Grant, G. G., E. B. Smithwick, and U. E. Brady. 1975. Courtship behavior of phycitid moths. II. Behavioral and pheromonal isolation of *Plodia interpunctella* and *Cadra cautella* in the laboratory. Can. J. Zool. 53: 827–832.

Greenfield, M. D., and M. G. Karandinos. 1979. Resource partioning of the sex communication channel in clearwing moths (Lepidoptera: Sesiidae) of Wisconsin. Ecol. Monogr. 49: 403–426.

Krasnoff, S. B., K. W. Vick, and J. A. Coffelt. 1984. (*Z,E*)-9,12-Tetradecadien-1-ol: a component of the sex pheromone of *Ephestia elutella* (Hübner) (Lepidoptera: Pyralidae). Environ. Entomol. 13: 765–767.

Krasnoff, S. B., K. W. Vick, and R. W. Mankin. 1983. Female calling behavior in *Ephestia elutella* and *E. figulilella* (Lepidoptera: Pyralidae). Fla. Entomol. 66: 249–254.

Kuenen, L. P. S., and T. C. Baker. 1982. Optomotor regulation of ground velocity in moths during flight to sex pheromone at different heights. Physiol. Entomol. 7: 193–202.

Levinson, H. Z., and C. T. Buchelos. 1981. Surveillance of storage moth species (Pyralidae, Gelechiidae) in a flour mill by adhesive traps with notes on the pheromone-mediated flight behaviour of male moths. Z. Angew. Entomol. 92: 233–251.

Paterson, H. E. 1978. More evidence against speciation by reinforcement. S. Afr. J. Sci. 74: 369–371.

1982. Perspective on speciation by reinforcement. S. Afr. J. Sci. 78: 53–57.

Roelofs, W. L., and R. L. Brown. 1982. Pheromones and evolutionary relationships of Tortricidae. Annu. Rev. Ecol. Syst. 13: 395–422.

Roelofs, W. L., and R. T. Cardé. 1974. Sex pheromones in the reproductive isolation of lepidopterous species, pp. 96–114. *In* M. C. Birch [ed.], Pheromones. North Holland, Amsterdam.

Ryan, T. A. 1960. Significance tests for multiple comparisons of proportions, variances, and other statistics. Psychol. Bull. 57: 318–328.

Sower, L. L., K. W. Vick, and J. H. Tumlinson. 1974. (*Z,E*)-9,12-Tetradecadien-1-ol: a chemical released by female *Plodia interpunctella* that inhibits the sex pheromone response of male *Cadra cautella*. Environ. Entomol. 3: 120.

Strong, R. G., J. Partida, and D. N. Warner. 1968. Rearing stored-product insects for laboratory studies: six species of moths. J. Econ. Entomol. 61: 1237–1249.

Thornhill, R., and J. Alcock. 1983. The evolution of insect mating systems. Harvard University, Cambridge, Mass.

Vick, K. W., J. A. Coffelt, and R. W. Mankin. 1981. Recent developments in the use of pheromones to monitor *Plodia interpunctella* and *Ephestia cautella*, pp. 19–28. *In* E. R. Mitchell [ed.], Management of insect pests with semiochemicals. Plenum, New York.

West-Eberhard, M. J. 1983. Sexual selection, social competition, and speciation. Q. Rev. Biol. 58: 155–183.

Received for publication 17 October 1985; accepted 24 December 1985.