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23. Supported by NSF grant BNS84-00875 to I.B.L. We thank J. Lisman, E. Marder, G. Carrow, and R. Kramer for comments.

10 March 1987; accepted 17 June 1987

Pheromone Components and Active Spaces: What Do Moths Smell and Where Do They Smell It?

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The pheromone-mediated flight behavior of male Oriental fruit moths was observed in the field to test the hypothesis that male activation far downwind of a female is initiated by the major, or most abundant, component of the pheromone blend. Males responded at significantly greater distances to the three-component pheromone blend produced by females than to the major component alone or to either binary mixture containing the major component and one minor component. These results support the alternative hypothesis that the active space of a multicomponent pheromone is a function of male perception of the female-released blend of components, rather than of the major component alone, and that so-called minor components have a greater impact on male behavior farther downwind of a female than previously thought.

ONE OF THE MAJOR QUESTIONS IN the study of lepidopteran multicomponent sex pheromones concerns the role of individual chemicals in influencing the dimensions of the active space, defined here as the area downwind of a calling female over which males are able to detect and respond to the pheromone (*1, 2*). Two conflicting hypotheses have been presented to explain this problem. The first, or component, hypothesis states that the maximum distance at which a male orients to the odor plume and initiates upwind flight is a function of the male's ability to detect the major, or most abundant, component in the blend (*3–7*). Accordingly, minor components do not participate in long-range attraction; rather, they initiate short-range approach, landing, and courtship behaviors. The second, or blend, hypothesis states that the female-released blend of components acts as a unit to effect optimal sensitivity in males over the entire response range (*8, 9*). Even though minor components may be present in very small amounts (usually <10% of the major component), these components are necessary for optimal levels of activation and upwind flight.

To test these two hypotheses, we recently conducted a series of flight tunnel tests with three moth species, the Oriental fruit moth (OFM) [*Grapholita molesta* (Busck)], the red-banded leaf roller (RBLR) [*Argyrotaenia velutinana* (Walker)], and the cabbage looper (CL) [*Trichoplusia ni* (Hübner)] (*10*). Our results showed (i) that over the dose series used the female-released blends significantly enhanced the number of males activating and completing upwind flights to the source over that observed with the major component alone, and (ii) that male response to lower doses of the major component (at which no upwind flights were recorded) was significantly enhanced by addition of the complement of minor components (*11*). From these results we hypothesized that downwind of a female, represented in the flight tunnel by lower doses of pheromone, males are most responsive to the complete blend of components and that the active space of the pheromone is a function of male perception of the female-released blend and not simply the major component. Here we present additional evidence for the blend hypothesis from studies with the OFM in which the active space of the pheromone was measured under field conditions. We suggest that this hypothesis represents an important general principle in insect chemical communication systems and is most consistent with the existing paradigm stating that multicomponent pheromones in the Lepidoptera function as species-specific mating signals.

The behavior of male OFM to synthetic pheromone [(*Z*)-8-dodecenyl acetate (Z8-12:OAc), with 6% (*E*)-8-dodecenyl acetate (E8-12:OAc) and 3% (*Z*)-8-dodecenol (Z8-12:OH) (*12*)] was observed in a large open field (*13*). The pheromone plume was localized by a parallel stream of bubbles released at a point 1 m from the pheromone source (*14*). In the first test, males were exposed to the major component, Z8-12:OAc, alone and to the three-component mixture at three doses (1, 10, and 100 μg) and over two temperature ranges (19° to 21°C and 25° to 28°C) representing the extremes that occur during the activity period of this insect. Males exhibited vigorous wing-fanning and walking responses in significantly greater numbers and at greater distances at all three doses of the three-component blend than to the major component (Fig. 1) (*15, 16*). The distance at which males responded was approximately doubled at the higher temperature range. In the second test males were

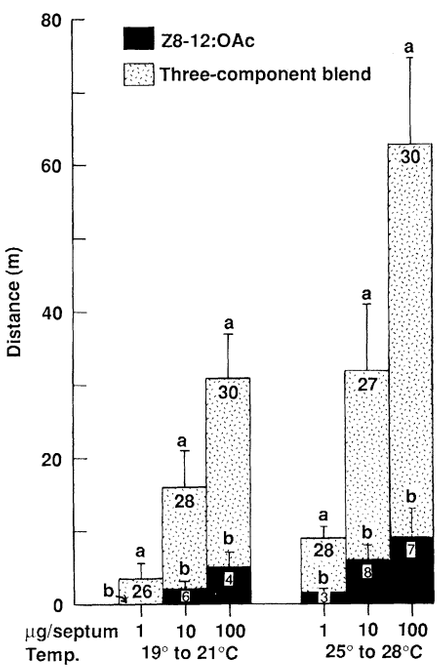

Fig. 1. Distance downwind (in meters) of a pheromone source that prompted male OFM wing-fanning and walking responses. Three doses of the major component (Z8-12:OAc) or the three-component blend (Z8-12:OAc with 6% E8-12:OAc and 3% Z8-12:OH) were used over two temperature ranges. Response values are means ± SD, $n = 30$, for each treatment. Values inside each bar are the number of responders. Comparisons of mean response values were made between the pair of treatments at each dose within each temperature range by analysis of variance. Means within each pair with different letters indicate a significant difference ($P < 0.05$).

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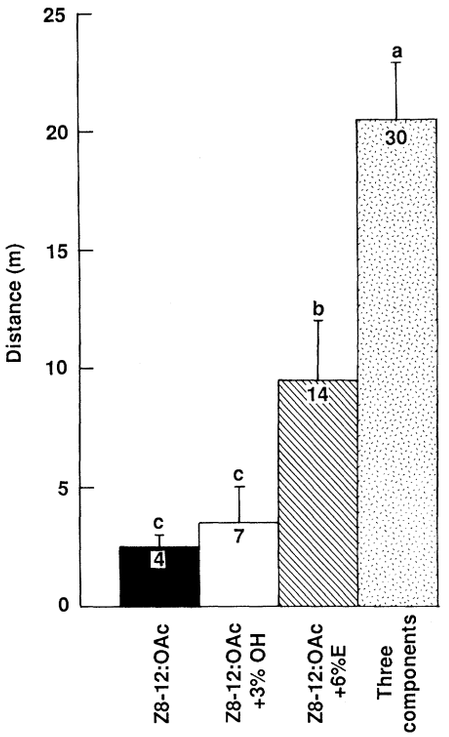

Fig. 2. Distance downwind (in meters) of four pheromone treatments (Z8-12:OAc, Z8-12:OAc + 3% Z8-12:OH, Z8-12:OAc + 6% E8-12:OAc, and the three-component blend) that prompted male OFM wing-fanning and walking responses. Temperature was 19° to 21°C; dose was 10 μg. Values are means ± SD, $n = 30$, for each treatment. Values inside each bar are the number of responders. Means having no letters in common are significantly different according to analysis of variance and Student-Newman-Keuls test for separation of means ($P < 0.05$).

exposed to the major component alone, the three-component blend, and the two binary blends composed of Z8-12:OAc and one of the minor components. Again, males responded in significantly greater numbers and at greater distances to the 10-μg dose of the female-released blend than to either of the two-component mixtures or to Z8-12:OAc alone (Fig. 2). In the third test we observed male flight behavior in response to Z8-12:OAc, the Z8/E8-12:OAc mixture, and the three-component blend (*17*). With Z8-12:OAc alone only 5 of 30 males took flight, and these exhibited random flight away from the cage. With the Z/E mixture 15 males took flight, with 12 of these exhibiting stationary flight in the odor plume before flying away. With the three-component blend, all of the insects took flight, with 24 orienting in the plume and 21 of those initiating upwind flight over a distance of at least 2 m (*18*).

The results of these tests support the blend hypothesis by showing that males were more sensitive to the complete blend of components at distances far downwind of the female than they were to the major component alone. The results also demonstrate that minor components can have a significantly greater effect on male behavior downwind of a female than has previously been believed, and that they do not function simply as releasers of short-range courtship behaviors (*19*).

The conclusion expressed here for OFM, and also in supporting flight-tunnel studies with CL and RBLR, represents a reversal of earlier results with these species that supported the component hypothesis (*3, 4, 20*). Our recent flight-tunnel and field studies were conducted as a result of reinvestigations of the sex pheromone of each species, which showed that the female-produced blends were not the same as those previously identified and used in behavioral studies (*12, 21*). The earlier studies utilized partial blends or blends containing ratios of components different from those released by females, but blends that had proven to be very attractive in field-screening trials. It is clear that although these partial blends can elicit certain behaviors from male insects, these responses are inferior, both qualitatively and quantitatively, to those exhibited with the female-produced blends.

Our conclusion concerning the active space of the pheromone is also in better agreement with a major paradigm which states that multicomponent pheromones function as species-specific mating signals (*2, 22*). With respect to interactions involving the chemical signal, males in a population face two major problems in locating a mate. The first is intraspecific competition from other males. Unmated females represent a potentially limiting resource, and therefore the sensitivity of a male to the pheromone could be an important factor in his ability to respond quickly to conspecific females. Second, the signal serves as a barrier to cross-attraction between closely related species that utilize some chemical components in common (*2*). A male must be able to recognize not only the conspecific pheromone blend but also variants of that signal that might resemble the pheromone of a closely related species. Thus a male who is able to assess the qualitative and quantitative nature of the signal at the farthest point possible downwind could have an advantage in locating mates. Future studies at the evolutionary, behavioral, and neurophysiological levels should focus on the problems related to perception of blend quality and quantity, rather than on the less heuristic search for functions of individual chemicals.

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11. The proportions of components in the RBLR pheromone are Z11-14:OAc (100), E11-14:OAc (0.9), 14:OAc (0.5), 11-12:OAc (0.3), Z9-12:OAc (0.1), E9-12:OAc (0.2), and 12:OAc (0.6); the proportions in the CL pheromone are Z7-12:OAc (100), Z5-12:OAc (0.9), 11-12:OAc (0.3), 12:OAc (0.8), Z7-14:OAc (0.2), and Z9-14:OAc (0.1) (*21*); for the OFM see text.
12. A. M. Cardé, T. C. Baker, R. T. Cardé, *J. Chem. Ecol.* **5**, 423 (1979). The chemicals used in the present study were the same as those used in (*10*).
13. Tests were conducted in an open 5-acre (2-hectare) plowed field within the experiment station grounds. The procedure was modified from that of T. C. Baker and W. L. Roelofs [*Environ. Entomol.* **10**, 211 (1981)]. Males were tested over a 2-hour period immediately prior to scotophase (16:8 light-dark photoperiod), when peak response to pheromone occurs. By offsetting the photocycles, we were able to test two groups each day, one from 9 to 11 a.m. and the other from 2 to 4 p.m. Tests were conducted from June through mid-July 1986. Males 4 to 5 days old were isolated individually in screen cylinder cages (15 by 10 cm) and held upwind of the release site. The Z8-12:OAc isomer was shown by capillary gas liquid chromatography (GLC) to be >99.9% pure. The mixture of Z8- and E8-12:OAc (6% E) and that of Z8-12:OAc and Z8-12:OH (3% OH) were checked by capillary GLC, as was the three-component blend. The single component and the mixtures were dissolved in hexane and applied to rubber septa (A. H. Thomas Company). The stated doses represent the amount of Z8-12:OAc on the rubber septum, with the minor components added to this amount to achieve the desired proportions. The septa were placed individually in glass vials and held at −10°C when not in use. In the field, the septa were placed open end up on a pin at the end of a 1.5-m metal pole.
14. Bubbles were generated by hand from a commercial children's bubble mix and could easily be seen for up to 100 m. The observer, with two cages held at eye level, walked upwind along a parallel path 0.5 to 1 m from the bubbles. Tests were conducted at wind speeds of <2 m/sec. Under conditions of excessive wind speed or erratic shifts in wind direction the observer would stop and either move perpendicular to the original line of sight to the source until contact was made with the stream of bubbles or wait until the wind shifted back to the original position. When a male moth exhibited vigorous wing-fanning and walking, a numbered colored flag was dropped. Response distances were recorded after a maximum of ten males had been tested to each treatment. During each test period all of the treatments for a particular experiment were tested, and each treatment was tested on at least 3 days. For analysis only those males exhibiting a response within 0.5 m of the source were included. The temperature and wind speed were recorded frequently during the tests to maintain constant experimental conditions.
15. The release rate of the 10-μg source (1.2 ng/hour) closely approximates that of a female OFM (3.2 ng/hour) [T. C. Baker, R. T. Cardé, J. R. Miller, *J. Chem. Ecol.* **6**, 749 (1980)].
16. The mean distances shown in Fig. 1 compare favorably with those reported for the three-component blend by Baker and Roelofs (*13*).
17. The observer approached the source from downwind, using, as in the other experiments, the bubbles as a guide for plume location. Once the males initiated wing-fanning, the observer stopped and opened the cage, allowing the insect to take flight from the release cage. Male flight behavior in the area of the odor plume was then observed.

18. Observations over distances greater than 2 m were difficult because of the rapid flight speed, small size, and dark coloration of the insects.
19. Most recently championed by Bradshaw, Baker, and Lisk (*7*) and P. E. Howse, J. C. Lisk, and J. W. S. Bradshaw [in *Mechanisms in Insect Olfaction*, T. L. Payne, M. C. Birch, C. E. J. Kennedy, Eds. (Oxford Univ. Press, Oxford, 1986), pp. 157–162].
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23. We thank K. Poole for aid in rearing the insects, G. Rule for preparing the synthetic chemicals and blends, and R. Mcmillan-Sticht and J. Ogrodnick for preparing the figures. This study was supported by NSF grant BNS-8518855.

2 March 1987; accepted 2 June 1987

Evidence for Reduced Recombination on the Nondisjoined Chromosomes 21 in Down Syndrome

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Trisomy 21 usually results from nondisjunction during meiosis I. In order to determine whether nondisjunction results from failure of normal chromosome pairing or premature unpairing, recombination frequencies were estimated between DNA polymorphic markers on the long arm of chromosome 21 in families containing one individual with trisomy 21. The recombination frequencies on chromosomes 21 that had undergone nondisjunction were then compared to those on chromosomes 21 that had disjoined normally. The data indicate that recombination is reduced between DNA markers on nondisjoined chromosomes 21. These results are consistent with the hypothesis that reduced chiasma formation predisposes to nondisjunction, resulting in trisomy 21 in humans.

TRISOMY 21 RESULTS FROM CHROMOSOME nondisjunction, which occurs most frequently in maternal meiosis I (*1*). Studies in other species, most notably *Drosophila*, have demonstrated reduced recombination frequencies on chromosomes undergoing nondisjunction during meiosis (*2*). We have estimated the frequency of recombination between loci identified by DNA polymorphisms on human chromosomes 21 that have undergone nondisjunction. These frequencies were compared with the estimated frequencies of recombination between the same DNA loci (i) on chromosomes 21 from normal control families and (ii) on chromosomes 21 that disjoin normally in the families containing one individual with trisomy 21. These comparisons demonstrate that recombination is significantly reduced on chromosomes 21 that have undergone nondisjunction.

Nondisjunction of chromosomes occurs commonly during meiosis, and the resultant aneuploidies cause significant human morbidity and mortality. Trisomies occur in approximately 4% of clinically recognized pregnancies and trisomy 21 occurs in approximately 0.1% of live births and 0.5% of all conceptions (*3*). Trisomy 21 (Down syndrome) is the commonest known genetic cause of mental retardation (*3*). Nondisjunction of chromosome 21 is strongly influenced by maternal age (*3, 4*).

The following hypotheses have been proposed (*5*) to account for the abnormal segregation of chromosome 21 leading to Down syndrome in humans: nondisjunction results from (i) asynapsis (failure of normal pairing of the homologous chromosomes at meiosis I) or (ii) desynapsis (premature unpairing of the homologous chromosomes after normal pairing). Theoretically, asynapsis during meiosis will lead to absence of recombination, whereas desynapsis will lead to normal recombination on the homologous chromosomes undergoing nondisjunction. In this study, we tested these hypotheses by comparing linkage maps of chromosomes 21 that had disjoined normally and chromosomes 21 that had undergone nondisjunction.

A linkage map of human chromosome 21 was constructed with DNA polymorphisms adjacent to several single-copy DNA fragments derived from human chromosome 21. The recombination values obtained between pairs of DNA markers are shown in Table 1.

The DNA markers D21S1 and D21S11 are closely linked; no recombinants were observed in over 135 meioses (*6, 7*). These two markers were thus treated as a single locus in subsequent analyses. Similarly, the DNA markers D21S3 and D21S23 are closely linked; one recombinant has been observed in 23 meioses [this study and (*7*)]. The estimated recombination value is $\hat{\theta} = 0.04$ (LOD score $\hat{Z} = 5.14$). Thus, D21S3 and D21S23 were also treated as one locus in subsequent analyses.

Multilocus linkage analysis by the computer program package Linkage reveals that the most probable location of CW21pc is proximal to D21S13 (Table 2). The locus CW21pc thus appears to be close to the centromere, as D21S13 maps proximal to a breakpoint in band q21 (*8*). The linkage map of chromosome 21q in control families is illustrated in Fig. 1.

The linkage map of chromosomes 21 from normal families was compared with the linkage map of human chromosomes 21 that have nondisjoined. Nondisjoined chromosomes were identified in informative fam-

Table 1. Values for recombination between polymorphic DNA markers on chromosome 21 presented with LOD scores. Tests of linkage in the control families were performed with the maximum likelihood LOD score method of Morton (*23*) and the computer program Liped (*24*). For each LOD table, the maximum likelihood estimate $\hat{\theta}$ and the maximum LOD score $\hat{Z}$ were computed with the interpolation formulas of Rao *et al.* (*25*). The 95% confidence limits on the recombination value were computed by the method of Buetow *et al.* (*26*).

Locus pair	$\hat{\theta}$	$\hat{Z}$	95% confidence limits
CW21pc-D21S13	0.14	2.05	0.01–0.27
CW21pc-D21S1/D21S11	0.17	3.87	0.09–0.25
D21S13-D21S1/D21S11	0.17	2.51	0.07–0.27
D21S1/D21S11-SOD1	0.08	4.96	0.00–0.19

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