Unique sex chromosome mediated behavioral response specificity of hybrid male European corn borer moths

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Abstract. Unlike the narrow response windows exhibited by the parent races, hybrid male European corn borers resulting from crosses of the E and Z races respond to a wide range of sex pheromone blends. The F_1 response profile consists of some individuals that respond to both the Z pheromone and the 65:35 E/Z blend produced by F_1 females. Some F_1 males fail to respond to any blend and some do not respond as broadly as others. The hybrid male populations, however, are not tuned optimally to the pheromone blend produced by F_1 females and there is no coupling of F_1 blend production and response.

Key words. European corn borer; *Ostrinia nubilalis*; sex pheromone polymorphism; interracial hybrids; F_1 male-response profiles; flight tunnel.

The European corn borer (ECB), *Ostrinia nubilalis*, exhibits polymorphisms of important elements of its sex pheromone communication system. Within the species are populations, in both Europe and North America, that respond to and produce unique pheromone blends, which in turn affords considerable genetic isolation to members of these populations[1-5]. The within-race female-pheromone production and male-response profiles have been extensively studied for three races occurring in New York State, USA. These races, while morphologically indistinguishable, have distinct differences in voltinism (bivoltine vs univoltine) and in their sex pheromone system (E vs Z)[6-8] as well as some less pronounced differences in host plant range and susceptibility to insecticides[9-11]. These races are abbreviated BE, BZ, and UZ to denote their most important differences. In the Z races, females produce a blend of 3:97 (*E*)-11-tetradecenyl acetate/(*Z*)-11-tetradecenyl acetate (E11-14:OAc/Z11-14:OAc) as the sex pheromone, with peak male response occurring to this ratio. A second blend of 99:1E/Z11-14:OAc's is utilized as the sex pheromone by members of the E race[6,12-14].

A series of studies have elucidated the genetic bases of the sex pheromone communication system polymorphisms. The female-blend ratio is controlled by an autosomal factor that exhibits incomplete dominance in hybrid females resulting in a sex pheromone of approx. 65:35 E/Z11-14:OAc's[13-14]. The behavioral response of the males to appropriate pheromone stimuli in a flight tunnel has been shown conclusively to be under control of sex-linked genetic elements (males are the homogametic sex)[14,15]. The response profile of male ECB from the Z races is highly canalized with very few individuals responding to blends other than those close to the natural 3:97 E/Z ratio. E males, on the other hand, exhibit peak response to the natural blend of 99:1 E/Z, but with significant numbers also responding to the hybrid blend as well as 50:50 and 35:65 E/Z[14,15]. Behavioral responses of parent and hybrid males are summarized in figure 1.

Recently, there have been several studies undertaken to describe the interaction of Z and E races in sympatry[16,17]. A difficulty in describing these interactions stems from incomplete characterization of the hybrid male response. The initial study reported by this laboratory in which F_1 males were tested to various blends indicated that high numbers of males responded to 3, 35, 50, and 65% E blends with virtually none responding to 99% E[14]. In that study fewer individuals responded to 3% E than to 35, 50, or 65% E, with 50% E appearing

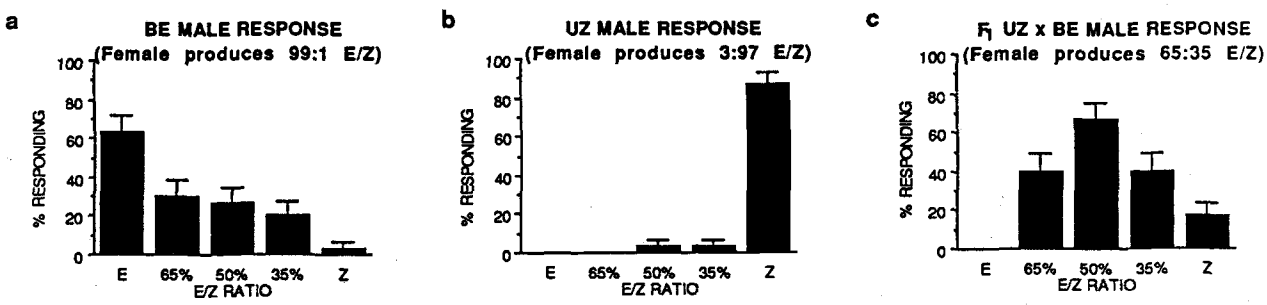

Figure 1. Male behavioral responses to blends to pheromone components for parent and hybrid European corn borer populations.

to elicit the maximum number of responses. A more recent study again demonstrated a wide range of responses in F_1 males, with between 17–67% responding to blends composed of from 65% to 3% E isomer and only 0–7% responding to the 99% E blend[15]. Peak response levels occurred to the 50:50 E/Z blend with 57% of the BE ♀ × UZ ♂ F_1 males and 67% of the UZ ♀ × BE ♂ F_1 males responding completely to this blend. It appeared that the 50:50 E/Z blend might be near the optimal blend for hybrid males.

The present study was undertaken to define precisely the response profile of E × Z hybrid males and to determine if there is a blend that will elicit peak response in hybrids and if any individual males respond broadly to both ends of the response profile. In addition, we wished to determine if the large numbers of non-responding males to one blend are actually tuned to and responsive to a different blend.

Materials and methods

Cultures of UZ and BE European corn borers were maintained using techniques and diet identical to those utilized in our earlier studies[6,7]. To generate the necessary F_1 males, individual pairs of moths (either UZ ♀ × BE ♂ or BE ♀ × UZ ♂) were placed in cardboard cages with a plastic-screened hole in the top. These mating cages were placed in a rearing room at 27 °C during the day and 18 °C at night with a 16:8 L:D photoperiod. Eggs were collected from waxed paper lining these cages. After hatching, larvae were reared at 30 °C with a 16:8 L:D photoperiod. Moths were sexed as pupae and stored separately by sex until eclosion. Adult male moths were kept isolated from females in a fluctuating temperature regime (27 – 18 °C) with a 16-h photoperiod until they were tested.

The pheromone blends were formulated from E11–14:OAC and Z11–14:OAc that were purchased from Dr S. Voerman at the Institute for Pesticide Research, The Netherlands. Both the E11–14:OAc and Z11–14:OAc were > 98.6% pure by GLC analysis and both contained < 0.05% of the opposite isomer. Blends were made in concentrations of 1 mg/ml using redistilled hexane as a solvent. Each blend composition was within 0.5% of the stated value confirmed by capillary GLC analysis. An appropriate amount of solvent was then applied to 5 × 9 mm rubber stoppers (Arthur H. Thomas Company, Catalog No. 8753-D22) so that the final amount of each blend was 30 µg. Preliminary studies indicated that the 30 µg dosage was the lowest concentration to elicit peak behavioral response levels in the flight tunnel.

Two different experiments were conducted using a previously described flight tunnel[7,8]. For both experiments the insects were tested 3–5 h into scotophase of their second day as adults. Temperature was controlled and ranged from 19–22 °C. The relative humidity was not controlled and ranged from 19–89%. The wind speed during all flights was approx. 0.4 m/s and the illumination was 5 lux of red light at the tunnel floor. The distance from the pheromone source to the insect release site was 1.2 m. In Experiment I, naive F_1 males from each cross were tested once to one of seven blends ranging from 62:38 E/Z to 38:62 E/Z (see table 1). After being placed on a release platform in a 3 cm × 6 cm screen cylinder, each insect was allowed 30 s to initiate flight. Insects that did not activate within that time period, but later demonstrated the ability to fly, were recorded as not responding (NR). For each responding insect, the final behavioral response was recorded using the following sequence of criteria: ACT = activation, rapid wing-beating and walking within the screen release cylinder; TF = taking flight; CAST = casting or looping flight near the release stand; UP = upwind, oriented flight in the pheromone plume; 15 cm = flight in the plume to within 15 cm of the pheromone source; TS = touching the source, landing on the pheromone source stand, and/or touching the rubber stopper; DIS = display, clasper extrusion with wings held vertically and abdomen waving slowly from side to side. The tests of the UZ ♀ × BE ♂ F_1 were conducted between 29 May 1990 and 28 June 1990 and for the BE ♀ × UZ ♂ F_1 were conducted between 5 March 1990 and 27 March 1990. On any given day at least three blends were tested in random order.

Experiment II, which was initiated to determine the breadth of individual F_1 male-response profiles, required testing males sequentially to a 65:35 E/Z blend, to the Z blend (3:97 E/Z), then again to the 65:35 E/Z blend. A second group of males from each F_1 were tested first to the Z blend, then to 65:35 E/Z, then again to the Z blend.

Final behavioral response of 2-day-old F_1 adult male *Ostrinia nubilalis* to various ratios of E11–14:OAc with Z11–14:OAc.

	UZ female × BE male F_1						
	62% E	58% E	54% E	50% E	46% E	42% E	38% E
NR	13	16	18	12	20	14	20
ACT	2	0	1	0	2	0	1
TF	8	10	13	11	8	15	12
CAST	1	3	2	0	1	2	1
UP	2	1	0	0	0	2	0
15 cm	5	2	2	4	3	2	0
TS	7	4	8	4	5	3	9
DIS	12	14	6	19	11	12	7
% TS + DIS	38a	36a	28a	46a	32a	30a	32a
	BE female × UZ male F_1						
	62% E	58% E	54% E	50% E	46% E	42% E	38% E
NR	19	18	23	19	17	25	21
ACT	3	1	2	1	1	2	2
TF	11	14	11	11	10	7	11
CAST	1	0	1	2	1	0	1
UP	2	1	0	0	2	0	0
15 cm	3	5	3	1	3	2	2
TS	2	2	4	5	5	2	5
DIS	9	9	6	11	11	12	8
% TS + DIS	22a	22a	20a	32a	32a	28a	26a

[a] Percentages in the same row followed by the same letter are not significantly different at the 5% level using the post hoc multiple comparisons procedure of simultaneous $\sqrt{\chi^2}$ to generate confidence intervals for simple contrasts.

For each insect there was approx. 30 min between trials. This protocol enabled the determination of individual response window dimensions with response criteria the same as for Experiment I. These sequences were devised after a variety of protocols had been tested. It was found that the flying of one hybrid male to all 4 treatments that elicit flight response (see fig. 1) gives poor results, both when tested in a single day and when tested over a two-day period. The tests reported here were completed between 31 October 1989 and 6 November 1989 for the BE ♀ × UZ ♂ F_1 and from 4 August 1989 and 7 August 1989 for the UZ ♀ × BE ♂ F_1.

Results

The results of Experiment I are summarized in table 1. For each blend in both F_1's, 50 naive males were tested, with the final behavioral response for each individual recorded. Individuals who touched the pheromone source and/or displayed were considered to have completed the behavioral sequence, so these two categories were combined for some analyses. In the F_1 produced by crossing UZ females with BE males, between 28 and 46% of the individuals tested completed the behavioral sequence (TS or DIS). These percentages were not significantly different at the 0.05 level when compared utilizing the simultaneous $\sqrt{\chi^2}$ procedure to generate confidence intervals for the simple contrasts [18]. Between 24 and 40% of the insects tested failed to respond (NR) with between 46 and 66% failing (NR, ACT, or TF) to exhibit oriented flight. Ryan's method of adjusted significance for proportions failed to demonstrate any significant differences ($p > 0.05$) in the response levels among the seven treatments at each step in the behavioral sequence [19].

The profiles for the reciprocal F_1 (BE females × UZ males) also showed remarkable consistency among the treatments. Between 22 and 32% of the individuals tested completed the behavioral sequence (TS or DIS), with between 34 and 50% failing to respond (NR) and between 56 and 72% failing to exhibit oriented flight (NR, ACT, or TF). Overall, the percent responding was somewhat lower for this second F_1 than for the previous one, most probably because the tunnel conditions were less optimal for the BE ♀ × UZ ♂ F_1 tested in March (RH ranged from 19–73%) than for the UZ ♀ × BE ♂ F_1 tested in June (RH ranged from 50–77%). Again Ryan's method of adjusted significance for proportions failed to demonstrate any significant differences ($p > 0.05$) in the response levels among the seven treatments at each step in the behavioral sequence. The percentages completing the behavior sequences were not significantly different among the treatments as determined by the simultaneous $\sqrt{\chi^2}$ procedure to generate confidence intervals for percentages.

In Experiment II each male was tested three times with approx. 30 min between trials. For each F_1, 30 males were tested to the 3% E, 65% E, 3% E sequence and 30 males were tested to the 65% E, 3% E, 65% E sequence of blends. In each test the insect either demonstrated oriented flight (UP, 15 cm, 5 cm, TS, or DIS) or failed to demonstrate oriented flight (NR, ACT, TF, or CAST). As a check on the protocol we tested Z population males in both test regimes and found that over 90% of them fully responded to the Z blend, and there was no response to the 65% E blend.

Figure 2 summarizes the results with the hybrid males. Figure 2a for the UZ ♀ × BE ♂ F_1 shows the results for

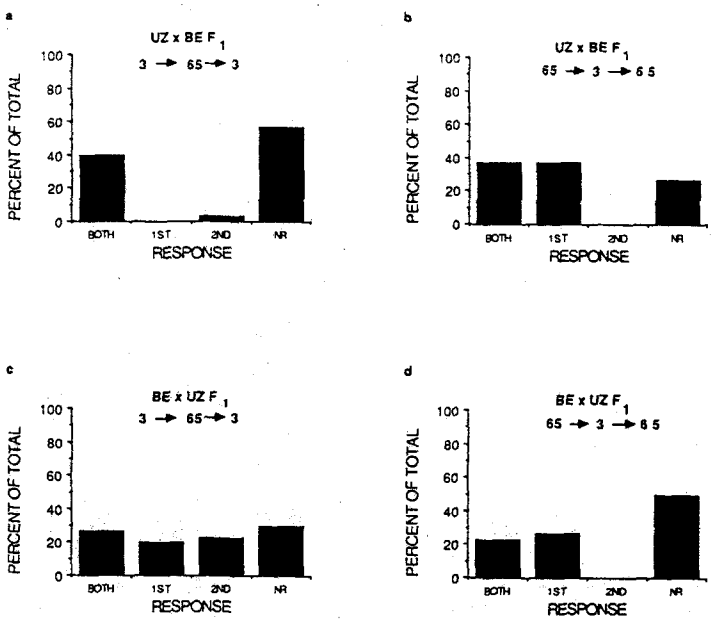

Figure 2. The percentages of hybrid males exhibiting oriented flight in sequential testing. Both = individuals responded to both 3% E and 65% E, 1st = individuals only responded to 1st blend tested, 2nd = individuals only responded to 2nd blend tested, NR = individuals failed to respond to either blend. Thirty insects were tested to each sequence in each F_1.

males flown first to 3% E then to 65% E. Twelve (40%) insects responded to both blends, none to only 3% E and one to only 65% E, whereas 17 (57%) failed to orient to either blend in any trial. Individuals from the same F_1 tested to 65% E first, then 3% E (fig. 2b), gave a somewhat different profile. Eleven (37%) insects responded to both blends, eleven (37%) to only 65% E and none to only 3% E, with eight (27%) failing to orient to either blend.

The reciprocal cross (BE ♀ × UZ ♂) F_1 tested to 3% E then 65% E is summarized in figure 2c. Eight insects (27%) responded to both blends, six (20%) only to 3% E, seven (23%) only to 65% E, and nine (30%) failed to respond to either blend. Individuals from the same F_1 tested to 65% E first then 3% E are summarized in figure 2d. Seven insects (23%) responded to both blends, eight responded only to 65% E, none responded only to 3% E, and 15 (50%) failed to respond to either blend. In both F_1's large numbers of the males tested demonstrated the ability to respond to both blends (between 23 and 40% of total) and large numbers of the males tested failed to respond to either blend (between 27 and 57% of total). Exposure first to 65% E seemed to reduce the number of insects responding to 3% E. Eleven (37%) in the UZ ♀ × BE ♂ F_1 and eight (27%) in the BE ♀ × UZ ♂ F_1 responded only to 65% E (figs 2b and d). No similar trend was exhibited in the complementary trials with 3% E tested first (Figs 2a and c). Disregarding order tested and F_1 origin, 33 insects responded to only one blend; of these, 27 responded to 65% E and only 6 responded to 3% E.

Discussion

Butlin and Ritchie view genetic coupling and coevolution of male and female components as alternative solutions to the problem of maintaining coordination between the sexes during evolutionary divergence of mate recognition systems[20]. They reviewed a number of studies and found no clear case of coupling as an explanation of the maintenance of coordination. The two clearest examples of failure to demonstrate genetic coupling were Zouros' work on sexual isolation in two sibling species of *Drosophila*[21] and the earlier work on ECB cited in the introduction of this report[14]. According to Löfstedt, 'Failure to demonstrate genes with pleiotrophic effects on critical sender and receiver traits, suggests that reciprocal selection on genetically independent sender and receiver loci is the more likely explanation for the generally observed coordination between pheromone production and response in moth populations.'[22]

However, Klun and Maini in their original paper describing the genetic basis of pheromone blend composition in ECB, reported that hybrid (E × Z) males in wing-fanning bioassays responded preferentially to the 65% E blend produced by hybrid females when compared to 3% E and 97% E. Somewhat later, Klun and Huettel interpreted results of a field study utilizing pheromone traps assuming that hybrid males respond only to the 65% E blend produced by their sisters and that the genetic bases of the response and production functions in the pheromone communication system of ECB are coupled or linked[16].

It was a primary goal of the studies reported here to elucidate the response capabilities of the hybrid males more fully and to determine if the response was in any way correlated with the female signal in hybrid populations. In Experiment I the seven blends tested, ranging from 62% E to 38% E, all generated approximately the same response profiles. Considerable numbers of individuals failed to exhibit any response and the numbers of individuals completing the behavioral sequence (TS and DIS) were not significantly different among the seven groups. Although the 50% blend had the highest number of full responders (46%) in the UZ ♀ × BE ♂ F_1 and tied for the highest number of full responders (32%) in the BE ♀ × UZ ♂ F_1, it is evident from table 1 that there is no clear optimal E/Z ratio for the hybrid males.

Furthermore, with Experiment II, we have for this system answered Löfstedt's question: 'Do the population response windows reflect variation between males in response to pheromone with each individual male being attracted to different pheromone compositions, or a wide response window of each individual male?'[22] We have demonstrated that many F_1 males have the ability to respond to a very broad range of pheromone blends including the natural Z blend and the natural hybrid blend. Others responded to a narrower range of blends and large numbers failed to respond to any blend tested. It is clear from the experiments reported here that Z ♀ × E ♂ or E ♀ × Z ♂ F_1 male populations have a broader range of response than do E or Z males parent populations. These hybrid males are not tuned to an optimal blend, such as that produced by hybrid females, and have most likely not experienced the stabilizing selection necessary for this optimum to develop. This indicates that there is no coordination between signal and receiver in the hybrid ECB populations, and that caution be exercised when assigning genotypes to individuals caught in pheromone traps in field studies.

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