Sex pheromone production and perception in European corn borer moths is determined by both autosomal and sex-linked genes

(*Ostrinia nubilalis*/behavioral response/antennal receptors/pheromone gland/genetic analysis)

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ABSTRACT   Inheritance patterns for sex pheromone production in females, pheromone detection on male antennal olfactory receptor cells, and male pheromone behavioral responses were studied in pheromonally distinct populations of European corn borers from New York State. Gas chromatographic analyses of pheromone glands, single sensillum recordings, and flight tunnel behavioral analyses were carried out on progeny from reciprocal crosses, as well as on progeny from subsequent F_2 and maternal and paternal backcrosses. The data show that the production of the female pheromone blend primarily is controlled by a single autosomal factor, that pheromone-responding olfactory cells are controlled by another autosomal factor, and that behavioral response to pheromone is controlled by a sex-linked gene. F_1 males were found to possess olfactory receptor cells that give spike amplitudes to the two pheromone isomers that are intermediate to those of the high and low amplitude cells of the parent populations. Fifty-five percent of the F_1 males tested responded fully to pheromone sources ranging from the hybrid (*E*)-11-tetradecenyl acetate/(*Z*)-11-tetradecenyl acetate (E/Z) molar blend of 65:35 to the E/Z molar blend of 3:97 for the Z morph parents, but very few responded to the E/Z molar blend of 99:1 for the E morph parents. Data on the inheritance patterns support speculation that the Z morph is the ancestral and that the E morph is the derived European corn borer population.

Pheromonally distinct populations of European corn borer moth (*Ostrinia nubilalis*) exhibit strong behavioral isolation in the field but have enough genetic compatibility to produce viable and fertile hybrids. These populations thus offer an excellent opportunity for a genetic analysis of the mate recognition system, including the sex pheromone production in females, and its detection and behavioral response in males. The European corn borer moth, which was introduced into North America on at least two and probably three separate occasions during the early 20th century (1), has been characterized as belonging to three distinct races in New York State based on voltinism and sex pheromone blend differences (2). Generally, the bivoltine populations exhibit moth flight periods in June and August, whereas univoltine moth flight periods occur in July (3). Two specific pheromone blends distinguish a Z morph [characterized by the production and recognition of a 3:97 molar mixture of (*E*)-11-tetradecenyl acetate/(*Z*)-11-tetradecenyl acetate (E/Z)] (4, 5), and an E morph (99:1 E/Z molar mixture) (5, 6). Populations found in New York State include bivoltine Z, univoltine Z, and bivoltine E races.

Hybridization is readily achieved in the laboratory with the two pheromone morphs (7, 8), and analysis of hybrid females

from reciprocal crosses has shown that they produce the intermediate E/Z molar pheromone blend of 65:35 (9). Analysis of wild female moths from the United States (2, 9) and Europe (9, 10) has indicated that a low level of natural hybridization occurs in areas of sympatry. Klun and Maini (9) have shown that female pheromone production in the European corn borer is determined by two alleles at a single autosomal locus. In the present study, reciprocal crosses were carried out with the three European corn borer races from New York State to investigate the inheritance pattern for pheromone component ratios in female pheromone glands, for pheromone-responding olfactory cells on male antennae, and for male flight responses to different pheromone component ratios. Analyses were conducted on all types of F_1, F_2, and maternal and paternal backcross progenies. The most surprising result of this study was that the data show sex-linked inheritance patterns for male behavioral responses to pheromone but autosomal patterns for male pheromone-responding olfactory cells.

MATERIALS AND METHODS

Insect Cultures and Crosses. Laboratory cultures of the three European corn borer races were established from larvae, pupae, and adults collected from corn stubble in several areas of New York where a particular race was known to be predominant (5). Cultures were maintained on a wheat germ diet (2), and female moths were analyzed by capillary GLC periodically to verify the purity of the pheromone strains. Crosses were made by placing one female and one male moth in a 1-liter cardboard ice cream carton with a screened lid and a wax paper lining for egg laying. Families were reared separately and analyses were made of individuals from 10 families per cross or generation.

Analysis of Pheromone Component Ratios. Individual female pheromone glands were removed and extracted in 10 μl of Skelly B for GLC analysis using a 30-m Supelcowax 10 column (bonded carbowax PEG 20 M, Supelco, Bellefonte, CA), 0.32 mm i.d., using temperature programming from 80°C at 10°C/min (5). Quantitation of the components was carried out by adding 5 ng of standard tridecanyl acetate to each tip extract. The pheromone component ratio and amount were determined for 5–10 individual females from each of the 10 families produced per generation or cross.

Analysis of Pheromone-Responding Olfactory Cells. Single sensillum recordings were made with an antennal-tip recording technique (11, 12). The base of an excised male antenna was inserted into a capillary filled with Beadle–Ephrussi

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Abbreviations: E/Z, (*E*)-11-tetradecenyl acetate/(*Z*)-11-tetradecenyl acetate.
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Ringer's solution (0.1 M NaCl, 5 mM KCl, 2 mM CaCl$_2$), which also made contact with a grounded Ag/AgCl wire. The tip of a sensory hair, chosen randomly from the proximal half of the antenna, was amputated with two glass knives, and a glass capillary Ag/AgCl recording electrode, which also was filled with Ringer's solution, was gently slipped over the cut surface to establish contact with the neurons projected up inside the sensillum from the olfactory nerve cells situated below the base of the sensillum. The antenna was continuously flushed by a purified airstream with a velocity of 0.5 m/s. Odor stimuli were injected into the airstream with a plastic disposable syringe, which contained a piece of filter paper loaded with 100 μg of (Z)-11-tetradecenyl acetate or (E)-11-tetradecenyl acetate. A total of 8–20 recordings were made for each cross or generation in this study. The electrophysiological responses from most males were recorded on tape and analyzed quantitatively. The amplitude of the first five spikes after stimulation was measured, and the mean value was used for the analysis.

Male Behavioral Responses to Pheromone Component Mixtures. The flight tunnel, chemicals, and assay protocol have been described (5). In the present study, the flight response of male moths to 99:1, 65:35, 50:50, 35:65, and 3:97 E/Z molar ratios was determined. Pheromones (30 μg) were loaded on a rubber septum. Males were placed in the flight tunnel room before scotophase for conditioning and flew 3–5 hr into scotophase. The final behavioral response was recorded for each male using the following sequence of criteria: not responding, activation, taking flight, orientation flight in the pheromone plume, distance flown in the 1.8-m tunnel, landing and touching source, and clasper display. Available males each day were distributed among the five treatment groups until a total of 4 males from each of the 10 families were flown to each treatment, giving a total of 40 males that flew to each treatment per cross or per generation.

RESULTS

Data from all comparable univoltine Z and bivoltine Z crosses were very similar, and so were combined and treated as a single Z-morph population, regardless of differences in voltinism.

Female Pheromone Production. The results (Fig. 1) from GLC analyses of individual female pheromone glands confirmed the earlier findings of Klun and Maini (9) that pheromone production is controlled primarily by a single genetic factor with heterozygote females producing more E isomer than Z isomer. The pattern of pheromone blends found in progeny from the F_1 and F_2 generations, as well as those from the backcrosses, fit the inheritance pattern expected for a single autosomal gene with two alleles.

Male Electrophysiological Response to Pheromone. Electrophysiological recordings from single olfactory sensilla on male antennae showed that the reaction patterns of the Z-morph and the E-morph males were easily distinguishable (Fig. 1). The Z-male recordings exhibited a high-amplitude spike response from an olfactory cell responding to (Z)-11-tetradecenyl acetate and a low-amplitude spike response from an olfactory cell (≈35% of the summed amplitude of the Z-isomer and E-isomer spikes) responding to the E isomer. With the E males, however, the Z-spike amplitude was only ≈35% of the summed amplitude of the two cells, and the E isomer stimulated the olfactory cell with the high-amplitude spike response. F_1 males were characterized as intermediate by possessing two olfactory cells that gave similar spike amplitudes to the E and Z compounds. Analyses (Fig. 1) of males from the various generations and crosses in this study showed that the distribution of males belonging to the E, Z, and intermediate phenotypes were as expected under an

inheritance scheme determined by a single autosomal gene with two alleles.

Male Behavioral Response Inheritance Patterns. More than 90% of the tested Z males gave full-flight responses and touched the Z-morph source, whereas the males did not even activate to the E-morph source. None of the other mixtures evoked full responses from >7% of the males (Fig. 1). The E males exhibited a broader response range, with 90% exhibiting full-flight response to the E source, 27% to the source of the 65:35 E/Z blend, 10% to the 50:50 E/Z blend, 7% to the 35:65 E/Z molar blend, and none to the Z source. The F_1-generation males from all crosses exhibited similar response profiles to the five sources. Only 4% of the tested F_1 males flew to the E source, but each of the other four sources attracted ≈55% of the males (averages taken from all crosses). Using these response values as the intermediate phenotypic response, it is possible to interpret the (E × Z)F_2 response profile as a combination of half intermediate and half Z-type males, and the (Z × E)F_2 response profile as a combination of half intermediate and half E-type males. The (E × Z) paternal backcross response profile is the same as that of Z-parent males, and the (Z × E) paternal backcross males respond the same as the E-parent males. The (E × Z) maternal backcross response profile is similar to the one calculated for half intermediate and half E-type males, whereas the (Z × E) maternal backcross response profile is similar to the expected profile of half intermediate and half Z-type males.

DISCUSSION

The inheritance patterns for male pheromone-responding olfactory cells are very similar to those obtained for female pheromone production (Fig. 1) and are consistent with models in which one autosomal factor primarily determines female production and another is involved in control of the male antennal olfactory cells detecting the pheromone. The male behavioral response patterns found, however, are different from those expected for autosomal inheritance. The unusual behavioral response pattern observed for F_1 males to the five pheromone sources in the flight tunnel was obtained for all reciprocal crosses, which shows that male pheromone response is not determined by a locus on either the X or the Y chromosome of an XX-female/XY-male sex determination system or by a cytoplasmically inherited factor. The reciprocal crosses in these cases would produce F_1 males with different response profiles.

Using the observed F_1 response profile and that of the E- and the Z-parent populations, it is possible to calculate the expected response patterns for control by various numbers of autosomal genes and for control by sex-linked genes. Calculations for the latter involve the sex determination system that predominates in Lepidoptera in which males are homogametic (ZZ) and females heterogametic (ZW) (Fig. 2). Parental strains are considered to be homozygous for the Z or E alleles, and the F_1 generation will be heterozygous, irrespective of how the crosses are made and of the inheritance scheme. Although the expected male behavior from the maternal backcrosses is identical under both autosomal and sex-linked inheritance, it differs between the two inheritance schemes for the F_2 generations and the paternal backcrosses. Under autosomal inheritance, the F_2 males derived from the reciprocal F_1 families are expected to show identical response patterns. Under sex-linked inheritance, however, the (E × Z)F_2 generation should consist of half Z-type males and half intermediate males (Fig. 2), whereas the (Z × E)F_2 generation produces half E-type males and half intermediate males. Furthermore, simple autosomal inheritance should produce half intermediate males in both paternal backcross-

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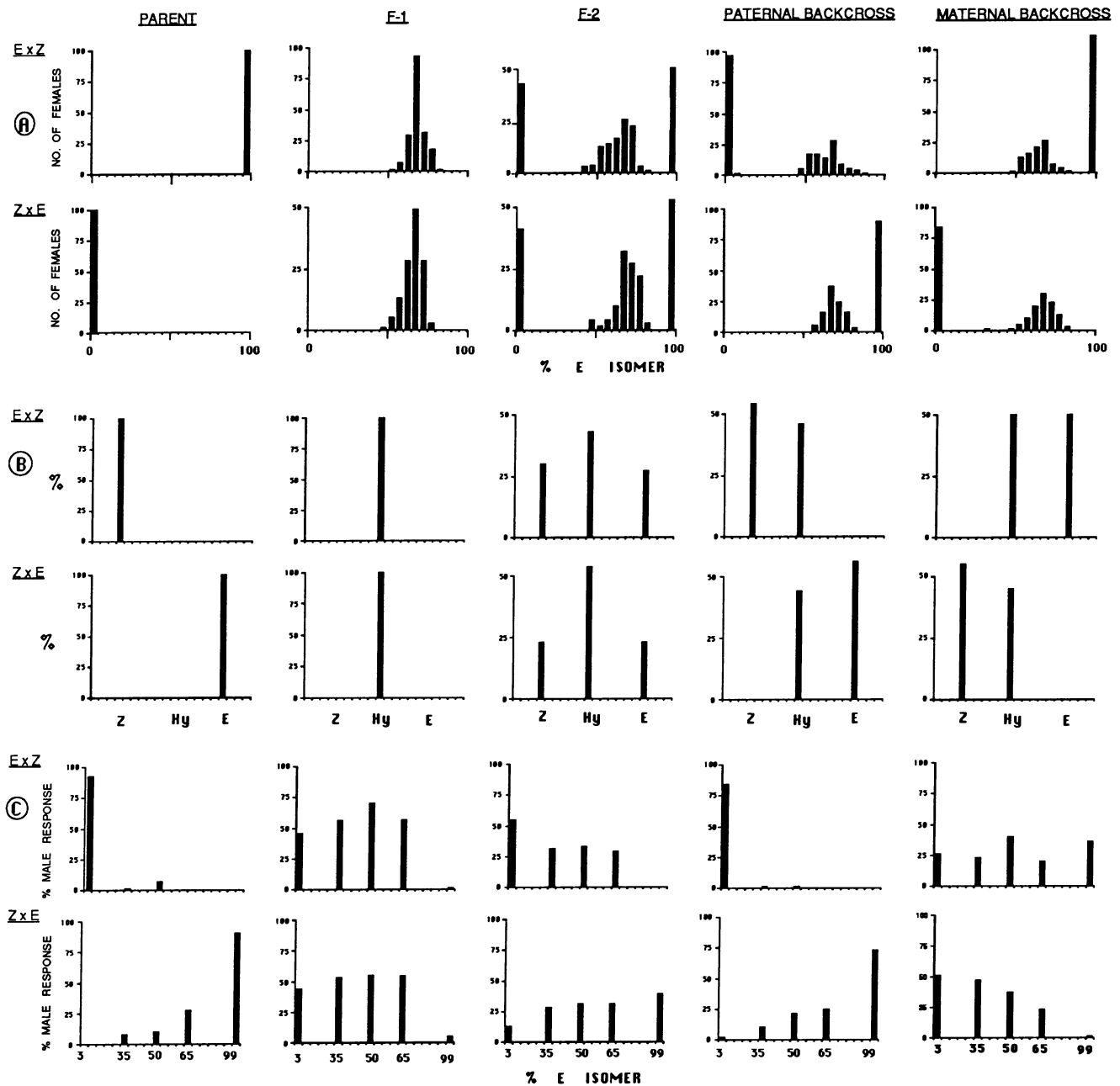*Proc. Natl. Acad. Sci. USA 84 (1987)*   7587

FIG. 1. Data summaries for parental European corn borer cultures, reciprocal F_1 progenies, F_2 progenies, and two backcross progenies for female pheromone production (*A*); male antennal olfactory cell responses (*B*); and male behavioral responses in the flight tunnel (*C*) (completed flights including touching the source and/or hair pencil display). Hy, hybrid.

es, whereas under sex-linked inheritance, the expected male behavior is as in the paternal parent strain.

The observed male response profiles (Fig. 1) for the F_2 generations and the backcrosses in every case are very similar to those calculated for allelic differences at a locus on the Z chromosome. For instance, the addition of half of the phenotypic response profiles of intermediate and Z-type males produces the observed response profile for the (E × Z)F_2 generation. Similarly, addition of half intermediate and half E-type responses produces the observed profile for the (Z × E)F_2 generation. Response profiles similar to that of a parental type in both paternal backcrosses are additional strong evidence that pheromone behavioral responses are under sex-linked inheritance. The good fit between the simple sex chromosome model and the data suggests that there is minimum interaction of the major factor on the Z chromosome and modifying factors elsewhere in the genome. It is not

known, however, if the major factor on the Z chromosome is a single gene or whether it consists of a set of closely linked genes.

The above results are consistent with models in which there are three genetic factors involved in the sex pheromone communication system of the European corn borer. It is suggested that one autosomal factor determines female pheromone production, whereas at least two factors are involved in male response, one carried by an autosome and the other by the Z chromosome. The male autosomal factor, which appears to control the ability of the male to detect the pheromone components, could be linked to the female autosomal factor for determining production of these components. However, it is interesting to find that the flight response to the components is controlled by a different genetic factor. Recombinations then may generate some unusual males. For example, the (E × Z)F_2 generation could
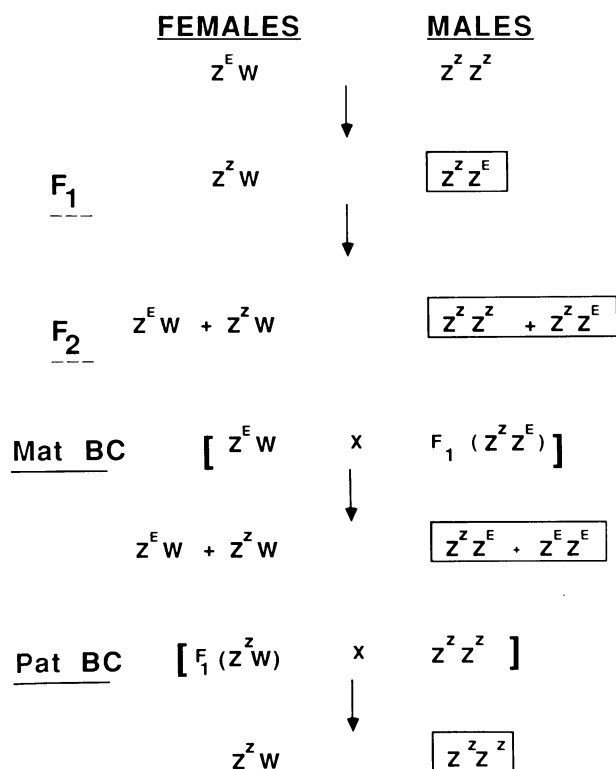
FIG. 2. Expected genotypes for male behavioral response genes in various generations from an (E × Z) cross assuming sex linkage. (The large Z and W represent the sex chromosomes, whereas the small z and E represent alleles for the pheromone type.) Mat, maternal; Pat, paternal.

include some males that possess antennae of parental E type but respond behaviorally as intermediate or Z-type males with no response to the E source. Also, the (E × Z) paternal backcross must include males that possess an intermediate antennae but respond only to the Z source. The genetic control of this moth system appears to differ from that found in a similar study of two pheromonally distinct populations of *Ips pini* bark beetles (13). In that study hybrid receptor cells were not found, but rather the parent populations and hybrids had identical kinds of receptor cells that were found in different proportions. So far they have not found genetic control of pheromone production or response to be sex linked.

Although the major pheromone component stimulates the receptor cells with the largest spike amplitude in the parental strains, all males have sensory cells sensitive to both pheromone compounds. Central nervous system input from these cells appears to be differentially interpreted to give the various phenotypic behavioral responses. Regulatory genes on the Z chromosome could be involved by controlling factors, such as levels of octopamine and cAMP, that modulate central nervous system behavioral response thresholds for the pheromone components (14). Species-specific regulatory genes on the Z chromosome have been proposed to explain the large influence of the Z chromosome over most of the phenotypic characters that distinguish two species of sulfur butterflies, including visual and olfactory signals of the courtship communication system (15).

Control of pheromone production by a single gene would be a remarkable feat if the entire biosynthetic pathway is considered. However, in the present case, the various phenotypic pheromone blends can be produced by effecting a specific change in the final reduction sequence to the acetate components. The pheromone components, (*E*)- and (*Z*)-11-tetradecenoyl acetate, have been shown in other species (16)

to be derived from acetate by way of the fatty acid cycle to palmitic acid. Palmitic acid is shortened to a 14-carbon intermediate acyl compound through β oxidation, and then $\Delta 11$ desaturation occurs to give the (*E*)- and (*Z*)-11-tetradecenoyl precursors, which are finally reduced to the unsaturated acetate pheromone compounds. In the redbanded leafroller moth, the intermediate 14-carbon unsaturated acids are present in an E/Z molar ratio of 60:40, but the resulting acetates are produced in a very tightly controlled 9:91 molar ratio. Attempts to shift the pheromone blend to ratios beyond the natural range by artificial selection through 24 generations were unsuccessful (17). In the European corn borer the last reduction sequence also appears to be the critical stage for determination of the final pheromone blend, since both E- and Z-parent strains, as well as F_1 males, have intermediate unsaturated 14-carbon acyl compounds with similar E/Z ratios of $\approx 70:30$ (L. Sreng and W.R., unpublished data). With similar unsaturated precursor acids in the different pheromone morphs, the autosomal gene evidenced in this study must encode for a factor effecting specificity in the final reduction sequence.

The allelic effects are strong at all three loci for which the pheromone morphs of the European corn borer have been found to differ. What evolutionary causes and forces led the species to split into two pheromonally distinct types are unknown, but at one time there could have existed a population with a genetic polymorphism at both the emission locus and the response locus. The present behavioral response data from the flight tunnel indicates that a male, which is heterozygous for its sex-linked response locus, would have great difficulties in finding an unfertilized mate in a population dominated by E females, even if some females in the population emitted an intermediary pheromone blend. However, the same male would have a much greater mating success if it were present in a population dominated by Z females. Thus, from this point of view, it can be speculated that the European corn borer originally used a Z-dominated pheromone blend for its mate recognition system and that the E-pheromone morph evolved out of the Z morph by a few simple genetic substitutions, some of which could have occurred simultaneously.

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