# No Linkage Between Genes Controlling Female Pheromone Production and Male Pheromone Response in the European Corn Borer, *Ostrinia nubilalis* Hübner (Lepidoptera; Pyralidae)

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ABSTRACT

The E and Z pheromonal strains of the European corn borer, *Ostrinia nubilalis*, are characterized by female production of and male preference for opposite blends of (*E*)-11-and (*Z*)-11-tetradecenyl acetate. It is known that the pheromone production is controlled by an autosomal gene and that the males' behavior is determined by a sex-linked gene. A third gene, autosomally inherited, has been shown to determine the organization of the male pheromone receptors. In the present study the linkage relationship between the autosomal genes controlling sex pheromone production and male olfactory sensilla was investigated. A recombination experiment showed unequivocally that the genes determining the variation in pheromone production and male pheromone receptors are not closely linked and are most likely inherited independently.

COMMUNICATION implies that sender and receiver traits are matched in some way, *i.e.*, the sender should produce a signal that is recognized by the receiver. The evolution of species specific communication systems, commonly involved in mate-finding and reproductive isolation, thus requires parallel changes on both sides of the system. Since many closely related species are isolated strictly by differences in behavior (mediated by chemical, acoustical or visual signals) an understanding of the genetic basis for premating reproductive isolation may also provide unique insight into the speciation process (BUSH 1986).

Different hypotheses have been formulated about how corresponding changes of signal production and response take place (see for instance DOHERTY and HOY 1985) but the direct experimental tests of these are few, if any. "The genetic coupling hypothesis" originates from ALEXANDER's (1962) suggestion that acoustic signal production and recognition may have neural elements in common, and implies that these are specified by a common set of genes. In the case of pheromone communication, similar proteins that are coded for by the same genes, could be involved in the specific female pheromone production and in male detection of the pheromone (HANSSON, LÖFSTEDT and ROELOFS 1987). In contrast, "the coevolution hypothesis" states that coordination of sender and receiver mechanisms results from similar selection pressures acting on genetically independent sender and receiver mechanisms.

The European corn borer is an ideal experimental insect for the genetic analysis of sex pheromone evolution. Two pheromone strains of the European corn borer *Ostrinia nubilalis* (Lepidoptera: Pyralidae) (Hübner), the so-called E and Z strains, occur in Europe and North America (KOCHANSKY *et al.* 1975; KLUN and cooperators 1975; ANGLADE *et al.* 1984; BARBATTINI *et al.* 1984; PEÑA *et al.* 1988). The strains exhibit behavioral isolation in the field, but hybrid individuals can be produced in the laboratory and are found in areas where the strains occur in sympatry (KLUN and HUETTEL 1988). Females of the E-strain produce a pheromone containing (*E*)-11-tetradecenyl acetate (E11–14:OAc) with 1–3% of (*Z*)-11-tetradecenyl acetate (Z11–14:OAc), whereas Z-strain females produce approximately the opposite blend. Males of the two strains are selectively attracted to the respective blends (KLUN *et al.* 1973; KOCHANSKY *et al.* 1975; KLUN and cooperators 1975). The two pheromone components are detected by different specialized receptors in the olfactory sensilla on the male antenna. In each sensillum Z-strain males have a receptor cell characterized by a large spike amplitude that responds to the Z isomer and a cell firing with a small spike amplitude tuned to the E isomer. In E-strain males the situation is the reverse; a large spike amplitude cell is tuned to E11- and a small spike amplitude cell to Z11–14:OAc (HANSSON, LÖFSTEDT and ROELOFS 1987).

The difference between the two strains has been

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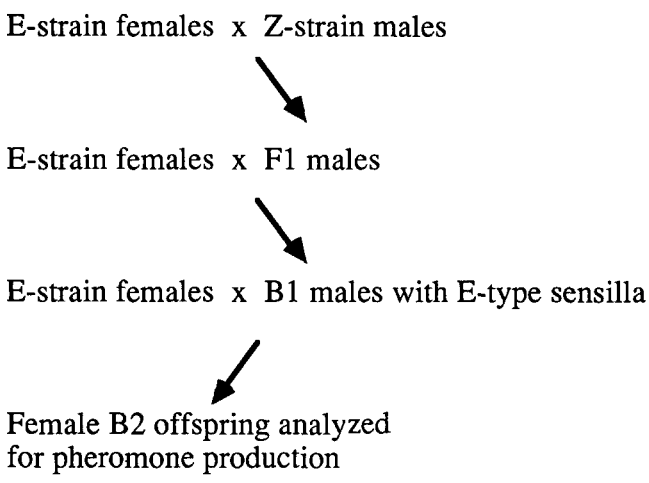


FIGURE 1.— Outline of the crosses on which the experiment is based. With complete linkage between the genes specifying female pheromone production and male sensillum type, all tested females should produce the same pheromone blend as the E-strain females do.

shown to depend on allelic differences for, at least, three different genes (ROELOFS *et al.* 1987). One of the genes, determining the behavior of males in flight tunnel experiments, is carried on the Z chromosome. (Males of the order Lepidoptera are homogametic, ZZ, and females are heterogametic, ZW.) The other two genes, one determining the pheromone blend produced by females and the other the males' sensillum type, are autosomally inherited. There are two alleles known for each gene and the heterozygotes show an intermediary phenotype (for further details on the genetic analysis, see ROELOFS *et al.* 1987).

The purpose of the present investigation was to study whether the autosomal genes are closely linked. Close linkage between genes controlling female pheromone production and male response would help in building up linkage disequilibrium between alleles specifying new types at these loci, and would thereby strongly affect the evolutionary process which leads a population to substitute one pheromone blend for another.

## MATERIALS AND METHODS

**Insects:** Laboratory cultures of the E and Z strains of the European corn borer were established from larvae, pupae and adults collected from corn stubble in several areas of New York state where a particular race was known to be predominant (ECKENRODE, ROBBINS and ANDALORO 1983). The E culture used in the present study was established from bivoltine and the Z culture from univoltine insects, but rearing was carried out under conditions that allowed continuous generations to be produced in the laboratory. The insects used in the present experiment were drawn from the same cultures as utilized in our earlier studies (ROELOFS *et al.* 1987; HANSSON, LÖFSTEDT and ROELOFS 1987). Matings were obtained by placing one female and one male moth in a 1-liter cardboard carton with a screened lid and a wax paper lining for egg laying. The larvae were maintained on a wheat germ diet (ROELOFS *et al.* 1985).

TABLE 1

**Expected genotypes of animals in the experiment**

| Insect | | Genotype at locus specifying: Female pheromone production | Genotype at locus specifying: Male sensillum type |
|---|---|---|---|
| E-strain female | | $S_ES_E$ | $R_ER_E$ |
| Z-strain male | | $S_ZS_Z$ | $R_ZR_Z$ |
| $F_1$ male | | $S_ZS_E$ | $R_ZR_E$ |
| $B_1$ male with E-type receptors[a] | $r$ | $S_ZS_E$ | $R_ER_E$ |
| | $1-r$ | $S_ES_E$ | $R_ER_E$ |

[a] $r$ is the frequency of recombination in $F_1$ males between the locus for female pheromone production and the locus for male sensillum type.

**Crosses:** The crosses on which the experiment was based are outlined in Figure 1. $F_1$ hybrids were obtained by crossing E-strain females with Z-strain males and the $F_1$ males were backcrossed to females from the E strain to produce the $B_1$ generation. The $B_1$ males were tested for their electrophysiological response by recordings from one of their antennae. Twenty males having the E-type sensilla were then selected and mated with females from the E strain. At least ten daughters from each family were then investigated for their genotype at the production locus by analysis of their pheromone blend production.

Table 1 gives the expected genotypes of the insects in the experiment. The following notation is used for the different alleles: $S_X$ stands for an allele at the locus determining female pheromone production (the "sender locus"), $R_X$ stands for an allele at the locus determining male sensillum type (the "receiver locus"), and $X$ denotes the type of the allele by showing the strain in which it normally occurs.

**Electrophysiological recordings:** Recordings from single olfactory sensilla were made with the tip recording technique on excised male antennae (HANSSON, LÖFSTEDT and ROELOFS 1987; and references therein). The antenna was continuously flushed by a purified airstream with a velocity of 0.5 m/s. Odor stimuli (>98.5% pure with respect to geometric and positional isomers) were injected into the airstream with a disposable plastic syringe, which contained a piece of filter paper loaded with 10 μg of E11- or Z11–14:OAc. The action potentials generated were monitored on the screen of an oscilloscope and the male olfactory type was determined from the relation between the action potentials of the cells tuned to E11- and Z11–14:OAc respectively (HANSSON, LÖFSTEDT and ROELOFS 1987).

**Gas chromatographic analysis of pheromone composition:** Extracts of female pheromone glands were prepared in 8 μl of hexane with 4 ng of (*Z*)-8-tridecenyl acetate added as an internal standard. The extracts were analysed on a Hewlett Packard 5880 gas chromatograph equipped with a 30 m x 0.25 mm id DB-wax fused silica capillary column (J. & W. Scientific, Folsom, California 95630). The column temperature was maintained at 80° for 2 min after injection and then raised to 230° by 10°/min; the injector temperature was 225°; hydrogen was used as carrier gas, supplied at 40 cm/sec linear velocity. The pheromone component ratio for each female was obtained by comparing the heights of the E11- and Z11–14:OAc peaks.

TABLE 2

**Phenotypes among daughters in investigated families**

| Family No. | *N* | Frequency of phenotypes | | | Average (range) of E/Z ratio in intermediary females |
|---|---|---|---|---|---|
| | | Unknown[a] | E-type | Intermediary | |
| 4 | 11 | 2 | 3 | 6 | 75.0 (69.2–80.4) |
| 5 | 13 | 0 | 5 | 8 | 83.0 (79.4–85.8) |
| 6 | 10 | 1 | 4 | 5 | 70.4 (67.0–73.8) |
| 9 | 17 | 0 | 13 | 4 | 74.1 (71.0–77.2) |
| 10 | 22 | 7 | 11 | 4 | 87.1 (85.5–89.0) |
| 17 | 10 | 1 | 9 | 0 | — — |

[a] Pheromone titre below limit of quantification.

## RESULTS

Six of the 20 attempted matings between E-strain females and $B_1$ males with E-type sensilla produced eggs that developed into adult insects. The mating success rate was lower than that of previous backcrosses, and was probably negatively affected by the removal of one antenna from the males. Among the offspring produced, 83 females were analyzed for their pheromone composition. Eleven of these did not contain enough pheromone to allow a reliable quantification (Table 2). The remaining 72 females were assigned to one of two phenotypes; either they produced an intermediary E/Z blend, or they produced a pheromone with more than 99% E (Table 2).

The experiment was so constructed that $B_1$ males with E-type sensilla would have the $S_ZS_E$ genotype at a frequency determined by the recombination rate between this locus and the locus specifying the males' electrophysiological response (Table 1). As seen from Table 2, five out of the six investigated families contained daughters producing an intermediary pheromone blend. They must, thus, have inherited the $S_Z$ allele from their father. The chance that the father also in the sixth family was a heterozygote is small (the probability that nine offspring inherit the same allele from their father is $0.5^8 = 0.004$). The ratio of E-type and intermediary females in the segregating families (36:27) conforms well to the expected 1:1 segregation ratio ($\chi^2 = 1.286$, d.f.$= 1$, $P > 0.05$).

It was noted during the experiment that the pheromone composition of the females with the $S_ZS_E$ genotype varied between families. For example, the mean of the E/Z ratio among intermediary females was 70.4 in family number 6, but 87.1 for family 10. The variation between families was highly significant when tested by analysis of variance ($F = 24.5$, $P < 0.0001$).

## DISCUSSION

The experiment clearly demonstrates that there is no close linkage between the autosomal genes determining the female pheromone production and the organization of olfactory receptors in *O. nubilalis*. The design of the experiment was such that a single intermediary female among those tested would have falsified the hypothesis of complete linkage. In fact five of the six families turned out to be segregating for the $S_Z$ allele, making independent segregation ($r = 0.5$) between the genes the most likely interpretation of the data. The probability of getting at least five segregating families out of six tested is $r^6 + 6r^5(1 - r)$, where $r$ is the recombination fraction between the two genes. This value becomes smaller than 0.05 for $r < 0.42$, so the recombination fraction is unlikely to be less than 0.4.

Our conclusion that there is no linkage between genetic factors for female pheromone production and male pheromone sensilla is based on the assumption that the Z-strain and E-strain parents were homozygous for both production and receptor alleles. However, only homozygosity of the E-strain females for the E-pheromone production allele is really critical for the experimental outcome. The absence of contamination for the production alleles in the respective cultures was previously established (ROELOFS *et al.* 1987) and all samples taken from subsequent generations have confirmed the purity of the cultures.

The E/Z ratio produced by the intermediary $B_2$ females, being heterozygous for the major genetic factor controlling female production, varied between families and was high compared to earlier reports (KLUN and MAINI 1979; ROELOFS *et al.* 1987). We take these observations to indicate the presence in the material of independently segregating modifier genes affecting the exact ratio produced by $S_ZS_E$ heterozygous females (also observed by T. J. GLOVER and W. L. ROELOFS, unpublished results). In no case was the ratio produced by a female with a quantifiable amount of pheromone such that it was uncertain whether she should be classified as producing an intermediary or an E-type blend.

It has now been shown that the pheromone variation in *O. nubilalis* is controlled primarily by three independently inherited factors, the two autosomal genes discussed in this paper and the sex-linked male behavioral response gene. The European corn borer is the first species where the degree of genetic linkage between sender and responder characteristics in a sex pheromone communication system has been directly investigated. The result is interesting, since it could have been expected that unless the genes determining sender and receiver properties of the communication system were closely linked, the evolution of a new pheromonal strain would be very difficult (see *e.g.*, O'DONALD (1962) for comments on the role that linkage may play in the evolution of sexual communication systems). In Lepidoptera genetic analysis of interspecific hybrids between the sulfur butterflies *Colias eurytheme* and *Colias philodice* have provided

evidence for the existence of Z-chromosomal supergenes which exert a very large influence on most of the traits which distinguish these species, such as wing pigmentation, wing coloration pattern, ultraviolet reflectance pattern, and male pheromone production, as well as female response to the visual and chemical cues (GRULA and TAYLOR 1980). The idea of linkage between factors controlling female production and male response has also found some empirical support in earlier studies on moths where pheromone component ratios may be under polygenic control. Thus, in a laboratory selection experiment, male redbanded leafroller moths (*Argyrotaenia velutinana*) responding to a high percent E isomer in Z11–14:OAc seemed to carry genes enabling their daughters to produce a high percent E pheromone (ROELOFS *et al.* 1986). Furthermore, COLLINS and CARDÉ (1989) found that females producing a high ratio between the pheromone components in the pink bollworm *Pectinophora gossypiella* increased male wing fanning response to "high blends" in the same line. However, the lack of replicates in these moth studies make them inconclusive, although it is, of course, perfectly possible that linkage exists in these species but not in the European corn borer. More precise genetical studies, of the kind performed in *O. nubilalis*, are needed in additional species before any general conclusions can be drawn about the genetic organization of species-specific communication systems.

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