Genetic Variation in the Strongly Canalized Sex Pheromone Communication System of the European Corn Borer, *Ostrinia nubilalis* Hübner (Lepidoptera; Pyralidae)

Junwei Zhu,* Christer Löfstedt* and Bengt O. Bengtsson†

*Department of Ecology and †Department of Genetics, Lund University, S-223 62 Lund, Sweden

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ABSTRACT

The major difference in pheromone production between the so-called E and Z strains of the European corn borer *Ostrinia nubilalis* is controlled by two alleles at a single autosomal locus. E-strain females produce an (E)-11-tetradeccenyl acetate pheromone with 1–5% of the Z isomer, whereas Z-strain females produce the opposite blend. In laboratory-reared insects we found that F₁ females produced, on average, a 71:29 E/Z ratio, but the distribution was clearly bimodal. The variability in pheromone blend produced by heterozygous females could be explained by the existence of two different alleles in the Z strain which in combination with the E-strain allele for the major production locus cause the production of a component mixture either high or low in the E isomer. In addition, evidence was found for an independently inherited factor, existing in the E strain, with a dominant effect on the amount of E isomer produced by females homozygous for Z alleles at the major production locus. Thus, the low variability normally found in the pheromone mixture produced by *O. nubilalis* and other moth females may, by canalization, hide a considerable amount of underlying genetic variation.

MOTh sex pheromone communication systems appear to be strongly canalized. Intrapopulation and even interpopulation variation in sex pheromone production and response is generally very low (LÖFSTEDT 1990) and attempts to produce behaviorally significant shifts in sex pheromone production by mass selection in the laboratory have so far failed (ROELOFS et al. 1986; COLLINS and CARDE 1989). This may be attributed to the important role that the sex pheromones play in mate finding and reproductive isolation, which implies that the pheromone communication system must be under strong stabilizing selection.

Several studies indicate that differences in sex pheromones between closely related taxa are controlled by a small number of Mendelian genes (for reviews, see LÖFSTEDT 1990, 1991). Under these conditions a mutation at a major locus controlling pheromone production or response may produce a mutant that does not "communicate" with the wild type of the opposite sex in the population. With little or no preexisting heritable variation in the pheromone communication system, it is difficult to envisage how such a mutation can be efficiently reproduced, as it is unlikely that simultaneous and parallel mutations occur in both the production and response systems (LÖFSTEDT et al. 1989, 1993). Therefore, for pheromone evolution to be understandable, more must be known about the nature of genetic variation in the pheromone communication system (BUTLIN 1995). In particular, it is important to learn if the canalization of the pheromone production and response functions hides an underlying genetic variation that may become apparent under certain circumstances.

*O. nubilalis* has become a model species for the study of pheromone genetics. Two pheromone strains, the so-called E and Z strains, occur in Europe and North America (KLUN et al. 1975; KOCHANSKY et al. 1975; ANGLADE et al. 1984; BARRATTINI et al. 1985; PÉÑA et al. 1988). They are behaviorally isolated in the field, but hybrid individuals can be produced in the laboratory and are found in nature in areas where the strains occur in sympatry (KLUN and HUETTEL 1988). Three major genes have been found to determine the pheromone differences between the two strains. One locus controls male behavioral response (ROELOFS et al. 1987), whereas the male electrophysiological response is determined by a second locus (HANSSON et al. 1987). The difference in female pheromone production is controlled by a third locus (KLUN and MAINI 1979; ROELOFS et al. 1987).

Females of the E strain produce a well-defined pheromone mainly containing (E)-11-tetradeccenyl acetate (E11-14:OAc) but with 1–5% of Z11-14:OAc, whereas Z strain females produce the opposite blend. The difference in pheromone production between the two strains is controlled by two alleles at a single autosomal locus, with heterozygotes producing an intermediary blend (KLUN and MAINI 1979). This locus has been suggested to determine the specificity of the reductase enzyme
responsible for production of alcohols from fatty acid precursors in the pheromone biosynthesis (ROELOFS et al. 1987).

In the course of two recent studies involving strain crosses (ROELOFS et al. 1987; LÖFSTEDT et al. 1989), we found evidence in female corn borers for additional variation in the precise blend of pheromone components. This additional variation could not directly be explained under the basic one locus, two allele model. The results, thus, raised the possibility that there are genetic modifiers which have an influence on pheromone production in *O. nubilalis*. If proven to exist, such factors would be of great importance for our conceptual models of how pheromone communication systems evolve. A study was therefore initiated to make a more detailed investigation of the genetic architecture of the ratio of pheromone components produced by individual *O. nubilalis* females. During the course of the study, it became possible for us to benefit from a newly developed nondestructive technique for determining the pheromone ratio produced by an individual female.

In presenting the results obtained, we wish to show that while the standard model used to interpret the differences in pheromone blends between *O. nubilalis* strains still stands unchanged, the exact pheromone blend of an *O. nubilalis* female is determined by more than two complementary alleles at a single locus. From our data we conclude that at least two extra factors may play a role. Due to the variability in the system and the difficulty to obtain large offspring numbers, it is, however, easier to establish the existence of modifier genes than to determine their exact modes of action.

**MATERIALS AND METHODS**

Insects: Laboratory cultures of the bivoltine E and Z strains of the European corn borer were obtained from New York State Agricultural Experiment Station, Geneva, NY. The cultures had been established from larvae, pupae, and adults collected from corn stubble in areas of New York state where a particular race was known to be predominant (ECKENRODE et al. 1983). The E culture originated from a field collection in 1988, whereas the Z culture was established in 1989. Derived cultures were started at the Department of Ecology, Lund University, from approximately 25 males and 25 females of each strain and these cultures were reared approximately six generations before being used in the present study.

Matings were obtained by placing males and females in a 200-ml plastic cup, covered by a nylon net and with a wax paper lining for egg laying. The larvae were maintained in our laboratory on a diet consisting of white beans, 470 g (soaked in water overnight and homogenized); yeast, 71 g; methyl 4-hydroxybenzoate, 4.4 g; ascorbic acid, 7 g; sorbic acid, 2.2 g; chloretacryline hydrochloride, 0.13 g; sodium benzoate, 2 g; propionic acid, 2 ml; distilled water, 0.45 liter and agar, 28.2 g.

**CROSSES:** The crosses performed are outlined in Figure 1. F₁ hybrids were obtained by reciprocal mass-matings of males and females of the two strains. Backcrosses were produced by mating F₁ males with females from the E and Z cultures respectively. Duplicate matings of individual males were obtained after recovery of mated males, one or two nights after mating, and subsequent remating with a new female of the same strain. Altogether more than 40 families were successfully initiated, and from 27 of these families female offspring were analyzed for pheromone production.

Selection of an individual female, on the basis of her produced pheromone blend, was made possible by a nondestructive analysis of the pheromone secreted before mating. The surface of the pheromone gland was scrubbed with a piece of filter paper soaked in redistilled hexane. The filter paper was then extracted using the same procedure as described for glands below. The accuracy of the method was confirmed by analysis of filter paper samples and corresponding gland extracts from 20 females producing between 0 and 98% E isomer. There was a perfect correlation between the ratios found in filter paper extracts and gland extracts ($r^2 = 0.998$).

Using the described filter paper technique, assumed heterozygote females with relatively high E (80–90% E isomer) and low E values (55–65% E isomer) were selected from the offspring to backcross matings. The two types of selected females were mated individually with Z or E males. Out of 22 attempted matings 10 resulted in female progeny that were analyzed for pheromone production.

**Gas chromatographic analysis of pheromone composition:** Individual female pheromone glands were excised during female calling time and extracted in 6 μl of hexane, with 1 ng of (Z)-8-tridecetyl acetate added as an internal standard. The extracts were analyzed on a Hewlett-Packard 5880 gas chromatograph equipped with a 30 m × 0.25 mm ID DB-wax fused silica capillary column (J & W Scientific, Folsom, CA). The column temperature was maintained at 80° for 2 min after injection and then raised to 290° by 15°/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. The pheromone component ratios were determined for at least 20 female offspring from each family in the backcrosses and conditional crosses.

**RESULTS**

**Pheromone variation:** *E* and *Z* strain females: Pheromone analysis of females from the parental strains confirmed that the E strain females produced an approximate 99:1 mixture of the E11- and Z11-14:OAc, whereas the Z strain females produced the opposite blend. In both strains the variation in pheromone composition was very low (Figure 2).

**F₁ females:** *F₁* females were produced by crossing the *E* and *Z* strains in both directions. The *F₁* females...
showed a range of pheromone blends from 59% E to 86% E (Figure 2). An apparent bimodality was observed in the frequency distribution, with one peak around 65% E and the other around 85% E (Figure 2).

No statistically significant difference was found between the offsprings' distributions of pheromone blends with respect to the direction in which the cross was performed (a chi-square heterogeneity test based on a comparison of the distributions after a split at 75% E gave a value of 3.55 with one degree of freedom). The data from reciprocal crosses will therefore be considered together throughout the article.

The difference that is possibly seen between the distributions of reciprocal F1 females can therefore be interpreted in two ways. Either the difference was due to chance, which implies that it would have disappeared if larger samples were studied. Or the difference was real but too weak to be detected statistically with the sample sizes used; the likely cause of the effect would then be some sex-linked factor. This possible effect was not further pursued.

Backcross females: Males from the F1 crosses were used as fathers in backcrosses to the E and the Z strains, with some of them being mated to more than one female. Female offspring from 27 such families were analyzed for their pheromone production. Large differences in pheromone composition were observed within and between the families (described in Table 1 and summa-
TABLE 1

Pheromone production (% EL1-14:OAc) among female offspring heterozygous at the major production locus in 27 backcross families

<table>
<thead>
<tr>
<th>No.</th>
<th>Family name*</th>
<th>n</th>
<th>Mean ± SD</th>
<th>Min</th>
<th>Max</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>E × (E × Z): A</td>
<td>13</td>
<td>65.97 ± 5.23</td>
<td>61.30</td>
<td>82.32</td>
</tr>
<tr>
<td>2</td>
<td>E × (E × Z): A2</td>
<td>8</td>
<td>64.96 ± 1.89</td>
<td>62.30</td>
<td>66.59</td>
</tr>
<tr>
<td>3</td>
<td>E × (E × Z): C</td>
<td>12</td>
<td>59.79 ± 1.80</td>
<td>56.07</td>
<td>63.14</td>
</tr>
<tr>
<td>4</td>
<td>E × (E × Z): C2</td>
<td>15</td>
<td>60.67 ± 4.90</td>
<td>47.78</td>
<td>68.60</td>
</tr>
<tr>
<td>5</td>
<td>E × (E × Z): B2</td>
<td>8</td>
<td>60.12 ± 1.88</td>
<td>57.69</td>
<td>62.70</td>
</tr>
<tr>
<td>6</td>
<td>E × (E × Z): D2</td>
<td>12</td>
<td>81.00 ± 6.50</td>
<td>62.00</td>
<td>87.00</td>
</tr>
<tr>
<td>7</td>
<td>E × (E × Z): Y</td>
<td>8</td>
<td>62.00 ± 1.90</td>
<td>59.00</td>
<td>65.00</td>
</tr>
<tr>
<td>8</td>
<td>E × (Z × E): A</td>
<td>5</td>
<td>63.86 ± 3.55</td>
<td>60.71</td>
<td>69.51</td>
</tr>
<tr>
<td>9</td>
<td>E × (Z × E): A2</td>
<td>11</td>
<td>65.22 ± 8.35</td>
<td>58.91</td>
<td>89.65</td>
</tr>
<tr>
<td>10</td>
<td>E × (Z × E): B</td>
<td>8</td>
<td>82.09 ± 1.11</td>
<td>80.43</td>
<td>83.30</td>
</tr>
<tr>
<td>11</td>
<td>E × (Z × E): E</td>
<td>8</td>
<td>83.32 ± 7.34</td>
<td>69.95</td>
<td>89.15</td>
</tr>
<tr>
<td>12</td>
<td>E × (Z × E): I</td>
<td>12</td>
<td>82.94 ± 1.38</td>
<td>81.06</td>
<td>85.28</td>
</tr>
<tr>
<td>13</td>
<td>E × (Z × E): D2</td>
<td>10</td>
<td>89.96 ± 5.83</td>
<td>70.71</td>
<td>85.95</td>
</tr>
<tr>
<td>14</td>
<td>E × (Z × E): X</td>
<td>9</td>
<td>85.00 ± 2.00</td>
<td>83.00</td>
<td>88.00</td>
</tr>
<tr>
<td>15</td>
<td>Z × (E × Z): A</td>
<td>10</td>
<td>67.00 ± 3.50</td>
<td>64.00</td>
<td>76.00</td>
</tr>
<tr>
<td>16</td>
<td>Z × (E × Z): B</td>
<td>10</td>
<td>78.00 ± 4.90</td>
<td>73.00</td>
<td>84.00</td>
</tr>
<tr>
<td>17</td>
<td>Z × (E × Z): C</td>
<td>7</td>
<td>70.43 ± 6.17</td>
<td>62.42</td>
<td>77.90</td>
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<tr>
<td>18</td>
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<td>60.81 ± 4.42</td>
<td>57.74</td>
<td>71.56</td>
</tr>
<tr>
<td>19</td>
<td>Z × (E × Z): D2</td>
<td>10</td>
<td>77.93 ± 4.89</td>
<td>70.56</td>
<td>84.01</td>
</tr>
<tr>
<td>20</td>
<td>Z × (E × Z): II</td>
<td>7</td>
<td>67.57 ± 2.00</td>
<td>64.20</td>
<td>70.21</td>
</tr>
<tr>
<td>21</td>
<td>Z × (E × Z): A</td>
<td>11</td>
<td>75.00 ± 7.90</td>
<td>67.00</td>
<td>85.00</td>
</tr>
<tr>
<td>22</td>
<td>Z × (E × Z): A2</td>
<td>9</td>
<td>85.12 ± 2.05</td>
<td>82.61</td>
<td>88.28</td>
</tr>
<tr>
<td>23</td>
<td>Z × (E × Z): D2</td>
<td>11</td>
<td>68.18 ± 5.84</td>
<td>61.15</td>
<td>77.93</td>
</tr>
<tr>
<td>24</td>
<td>Z × (E × Z): E</td>
<td>10</td>
<td>78.80 ± 7.02</td>
<td>71.44</td>
<td>89.59</td>
</tr>
<tr>
<td>25</td>
<td>Z × (E × Z): I</td>
<td>7</td>
<td>72.06 ± 4.92</td>
<td>64.16</td>
<td>78.61</td>
</tr>
<tr>
<td>26</td>
<td>Z × (E × Z): III</td>
<td>4</td>
<td>66.12 ± 4.70</td>
<td>61.56</td>
<td>72.12</td>
</tr>
<tr>
<td>27</td>
<td>Z × (E × Z): III2</td>
<td>6</td>
<td>87.06 ± 1.31</td>
<td>85.44</td>
<td>88.83</td>
</tr>
</tbody>
</table>

*The system for naming crosses is such that E × (E × Z): A and E × (E × Z): A2 denotes backcrosses of the same F1 male, produced by an E × Z cross, to two different E-strain females. Males were used for repeated crosses of the same type only.

rized in Figure 2). A clear bimodality among heterozygous females was found in the backcross to E, while this effect was not detectable in the backcross to Z.

Variation: The results are consistent with the established notion that there is a single genetic locus with two major types of alleles which determines the females' pheromone production (UUN and MAIN 1979; ROELOFS et al. 1987). The segregation ratios were not perfectly 1:1 in all the backcross families (data and analysis not shown), but the deviations may well have been due to viability effects being involved in these interracial crosses. In no case did we obtain a female whose pheromone production contradicted the basic model, for example a daughter from a backcross with the Z strain that predominantly produced the E component. In the rest of the analysis, the basic one locus model will therefore be taken to be correct. We will also regard the possible difference between reciprocal crosses to be either absent or so small that the effect can be ignored with respect to the following questions.

From Figure 2 can be seen that the variability in pheromone ratios observed in our crosses was so large that it sometimes was difficult to know which genotype to ascribe to a particular female. It could be argued that the experimentally produced females, being derived from interstrain crosses, were less well canalized and that they therefore showed greater variation in their pheromone production than females with a less mixed genetic background (compare with studies on morphological asymmetries in interracial crosses; e.g., see the review by ZAKHAROV 1991). However, another effect of the crosses may have been to unveil otherwise hidden genetic variation in the parental strains. The variability among the females showed, furthermore, some clear patterns that could be given specific genetic interpretations, rather than being ascribed solely to general effects. To analyse this possibility, two separate issues were considered. The first was the bimodality shown by females heterozygous for the major locus, and the second was the variance shown by homozygote females in backcrosses to the Z strain. By looking for Mendelian factors with major effects in the material, a problem arises in
that the families become the informative units while being limited in numbers. The results of such an analysis are, however, much easier to grasp than if the analysis was performed in a strictly biometrical fashion.

**Two types of heterozygotes: F1 generation:** In the F1 generation a considerable variation was found among the females heterozygous for the major locus. The distribution of E produced by individual females showed two clear peaks around 65% E and 85% E (Figure 2).

**Backcross experiment:** In the analysis of the backcross data, we regarded all females with a pheromone blend between 40% E and 90% E as heterozygous for the major locus. Under this assumption, the mean and the standard deviation of the heterozygous females in the individual families could be calculated (Table 1). Both values fluctuated from family to family, but a pattern became visible when the standard deviations were plotted against the means (Figure 3).

From this graph it appears that the 10 families with relatively uniform assumed heterozygotes (standard deviation ≤2.05) fell into two classes with respect to their family means. The females in five families produced a pheromone with a low proportion of the E isomer, ranging from 60% to 68% E, while the daughters from the other five families produced pheromones with high proportions of E, ranging from 82% to 87% E. These pheromone values produced by females from uniform families correspond well to the distribution peaks seen in the F1 females.

In the remaining 17 families with more variable putative heterozygotes (standard deviation ≥3.50) no family means were lower or higher than recorded among the less variable families, but intermediary family means were here also observed. Such intermediary families were, however, only found after backcrossing to the Z strain. In the backcrosses to the E strain, all the family means fell in the same distinct ranges as before.

These results indicate the presence of three kinds of backcross families: (1) those whose heterozygote daughters all have a comparatively low proportion of E; (2) those whose heterozygote daughters all have a high proportion of E; and (3) those who produce daughters of both types; this last type occurred only after backcrossing to the Z strain. A genetic model consistent with these results is described in the discussion.

In addition to this variation between families, there may also be a difference in the amount of variability around the genetically predisposed pheromone blend. The lack of any bimodality when heterozygote females from backcrosses to the Z strain are considered in toto (Figure 2), we believe to be due to such an increased variability which blurs the underlying bimodal distribution. Whether the increase in variability is due to experimental or genetic effects is impossible to determine from the present experiment. The expected bimodality is, however, clearly seen in the Z backcross families reported on in the next experiment.

**Conditional crosses:** To test the suggested genetic model we made use of a new nondestructive technique for selecting individual females with respect to their pheromone production. Crosses were performed by selecting females with pheromones comparatively high (>83%) or low (<65%) in the E component among the daughters to the backcross generation. These selected females were then mated individually with E or Z strain males (Figure 1).

In the crosses with E strain males, the average pheromone production among the heterozygous progeny never deviated far (more than 5.5 percent units in %E) from that of their mother, and the offspring distributions appeared unimodal (Table 2 and Figure 4). However, in the crosses with the Z strain males, strong differences could be found between the pheromones produced by the mothers and by their daughters (Table 2 and Figure 4). Two families were particularly informative in this respect: In family number 9, a 60% E mother produced totally eight heterozygous daughters, all with 80–90% E, and in family number 7 a 57% E mother produced totally 10 heterozygous daughters with 82–93% E. The distributions of daughters in the other families (nos. 6, 8 and 10) produced by crosses with Z strain males showed clear indications of the same bimodality as observed before (Figure 4).

Thus, in this set of experiments there was no simple correlation between the pheromone blend of heterozygous mothers and of their heterozygote daughters. Instead, offspring families were obtained which appeared as being either of a pure or a mixed type. This result fits, again, very well with the genetic model suggested,
based on a single modifier with a quantitative effect in heterozygotes.

**Variability in homozygotes:** Normally, most females could easily be classified as being either heterozygous or homozygous, based on their pheromone values. However, this was not the case with all the female offspring derived from the backcrosses to the E strain, shown in Figure 2. Among the offspring to these backcrosses, there were daughters that produced pheromone blends of 92.7 and 95.8% E, which made them difficult to classify. But the most unexpected observation was the occurrence of a large number of females producing between 5 and 25% E in the backcrosses to the E strain (Figure 2). (The single female with 37.7% E will not be further considered, since the relevance of this heterozygote is such, that the ratio of insects with no and one copy of the E allele among the backcross offspring. For this to be the case, the most likely interpretation is that the females with pheromones in the range 5 to 25% E were homozygous for alleles coming from the Z strain. Since females taken directly from the Z strain never show any such variation, there must have been some independent dominant factor, contributed from the E strain, that gave the females homozygous for production alleles coming from the Z strain their unusually high %E pheromone blend.

**Variance in titers:** Different pheromone component ratios can be produced in different ways. For instance, the high %E production among some assumed heterozygotes may have been due to an increased production of the E isomer, a low production of the Z isomer, or a combined effect of both. To analyze the mechanism behind the production of the different pheromone ratios, we determined the titers of the two pheromone components among the progeny in the 10 selected families. The females were classified into the five classes discussed above: Z homozygotes with high or low E values, assumed heterozygotes with high or low E values, and E homozygotes. The results of the estimates are given in Table 3.

The variance in the pheromone titers was very large, as was expected from other studies of individual variation in pheromone production (see Schluter and Birgersson 1989, and references therein). But the table also shows that there was no clear difference in the total amount of pheromones produced by females in the different classes. Females with a higher percentage of E11-14:OAc in their pheromone seem to produce a higher titer of this isomer, as well as a lower titer of the corresponding Z isomer, compared to females with a lower %E blend. An exception to this pattern was the mean amount of E11-14:OAc produced by E-type homozygotes compared with the mean value for heterozygotes with a high E-value, but the underlying distributions had such a broad variance that the estimated means were not statistically significantly different from each other. Thus, the changes in pheromone ratios produced by different females appear to be a combined effect of changes in both the E- and Z-isomer titers.

### DISCUSSION

Our analyses of offspring produced from crosses between different pheromone strains demonstrate a hitherto unknown variation in the pheromone communication system in *O. nubilalis*. As hybridization between the two pheromone strains occurs frequently under natural conditions (Klun and Maini 1979; Roelofs et al. 1985; Klun and Huettel 1988; Peña et al. 1988), the variation revealed by us cannot be considered as a laboratory artifact but must be regarded as a factor of potential importance for the evolutionary dynamics of the system.

The variation in pheromone ratios produced by females in the parental strains was very small. Z strain females all produced less than 5% E isomer and E strain females all produced more than 95% E isomer, in accordance with earlier results by Roelofs et al. (1987). The variation in the pheromone production became visible after crosses between the E and the Z strains, not only among females heterozygous for the major production locus but, in later generations, also among females carrying two Z-derived production alleles. In the backcross families we found females producing almost any pheromone blend (see Figure 2)—a remarkable result given the normally low variation in ratios between E and Z pheromone component isomers (Sreng et al. 1989).

This increased variability shows that the pheromone production system in *O. nubilalis* is such, that the ratio of components can, under certain circumstances, vary almost continuously between closely related females. From our data can also be deduced that at least some of this variability must have a direct genetic basis. To
Figure 4.—Frequency diagrams of %E11-14:OAc produced by daughters of 10 selected female *O. nubilalis*. The pheromone blend produced by the mother (%E) is given for each family.
identify all the heritable factors involved is impossible in this system, which, though excellent from the point of view of pheromone research, nevertheless carries some genetic and experimental limitations. However, among all the variance causing effects, we believe that two genetic factors with comparatively large effects can be recognized.

The first is a dominant effect contributed by animals from the E strain which affects the E/Z ratio produced by animals with two Z alleles. In the absence of this factor only a small amount of the E substance is produced, while in its presence the pheromone produced may contain up to 25% E. Very little can be said about this factor, which may itself have a complex genetic background.

More is known about the second factor which affects the variability among heterozygous females. It appears as if it can explain a large part of the variation found in the middle range of the pheromone spectrum, including the bimodality of heterozygotes found, for example, in the distribution of F1 females.

**Genetic interpretation of the variability among heterozygotes**: Our suggested extension of the basic model is built on the idea that in the Z strain there are two variant alleles for the major pheromone production locus. These alleles from the Z strain differ with respect to the pheromone blend produced by heterozygote females carrying one of them together with the corresponding (monomorphic) allele from the E strain. The heterozygotes can be written as ZE and ZE, where the females of the first type have a pheromone mean around 65% E and females of the second type a mean around 85% E. This simple extension of the basic model accounts for the bimodality of the F1 females, and can also explain the existence of three kinds of backcross families: those with all heterozygous daughters ZE, those with all heterozygous daughters ZE, and those with heterozygous daughters of both types. The last family type would occur after backcrossing to the Z strain and not to the E strain - in accordance with the results on family means described in Figure 3.

The suggested factor will account for some of the observed variability also through its associated sampling variance. Thus, in the backcrosses to the E strain (Table 1), the families derived from E × (E × Z) crosses behave as if six families segregated the "low" allele and one the "high" Z allele. In the next seven families, derived from E × (Z × E) crosses, the corresponding numbers are two and five (which is not significantly different from the earlier numbers; \( P = 0.103, \) Fisher's exact probability test, two-tailed). The difference in family means between the E × (E × Z) and the E × (Z × E) crosses can, thus, be understood solely by reference to the chance sampling of the Z allele, and does not need any separate explanation.

It is possible that the genetic variation in the Z strain does not exist at the major pheromone production locus itself but at another locus. However, in the backcrosses to the Z strain a wider range of genotypes would then occur than in the backcrosses to the E strain, leading presumably to a wider range of pheromone mixtures in the Z than in the E strain backcrosses. No such effect was seen (Figure 3), so we find this alternative less attractive. We cannot, however, exclude that the modifying locus, influencing the pheromone produced by the heterozygotes for the major locus, is independent but closely linked to the major locus.

In heterozygous offspring to the E backcross, the Z derived allele at the major production locus came from the selected mother (who only carried one such allele), while in the reciprocal backcross the daughters got their Z allele from their father (who carried two). Thus, the closer correspondence observed between mothers and daughters in the first than in the second case, gives strong evidence for the genetic variation at the major production locus existing in the Z strain rather than in the E strain. All in all, the results in the conditional crosses conform perfectly to the genetic interpretation.

---

**TABLE 3**

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>N</th>
<th>E11-14:OAc Mean ± SD</th>
<th>Z11-14:OAc Mean ± SD</th>
<th>Total Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Z homozygotes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>With low %E</td>
<td>33</td>
<td>0.18 ± 0.21</td>
<td>10.18 ± 7.60</td>
<td>10.47 ± 7.82</td>
</tr>
<tr>
<td>With high %E</td>
<td>12</td>
<td>0.81 ± 0.75</td>
<td>7.35 ± 6.04</td>
<td>8.06 ± 6.76</td>
</tr>
<tr>
<td>Heterozygotes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>With low %E</td>
<td>28</td>
<td>8.18 ± 8.07</td>
<td>4.36 ± 4.17</td>
<td>12.57 ± 12.58</td>
</tr>
<tr>
<td>With high %E</td>
<td>72</td>
<td>14.62 ± 21.15</td>
<td>2.97 ± 4.68</td>
<td>17.72 ± 25.89</td>
</tr>
<tr>
<td>E-homozygotes</td>
<td>35</td>
<td>9.61 ± 8.10</td>
<td>0.07 ± 0.19</td>
<td>9.68 ± 8.23</td>
</tr>
</tbody>
</table>

Values are means ± SD.

* Means in the same column followed by the same letter are not significantly different at the \( P < 0.01 \) level (ANOVA followed by pairwise comparisons of means by Fisher’s protected least significant difference test; \( \ln(x + 1) \) transformation of raw data).
of the heterozygotes' variability given above, again with the caveat that we cannot know whether the genetic variability in the Z strain exists at the major pheromone production locus or at a locus closely linked.

It is reasonable to assume that this polymorphism did not occur in the laboratory stock of Z type animals used in the experiments by Roelofs et al. (1987), as no bimodality was observed among heterozygotes in those experiments. From the bimodality among F1 females one can estimate the frequency of the Z, allele giving "low E" heterozygotes to be around 61%, giving its allelic alternative Z2 the frequency 39%. Again, these frequencies correspond well with the results obtained in the later experiments. The limited number of, for example, tested back-cross families must, however, be remembered before any strong weight is given to this argument.

Biosynthesis: The pheromone components of O. nubilalis are derived biosynthetically from tetradecanoic acid by Δ11 desaturation, producing (E)- and (Z)-11-tetradecenoate. These acyl moieties are subsequently reduced and acetylated to produce the pheromone component acetates (Wolf and Roelofs 1987). The shift in which isomer dominates the blend, controlled by alleles at the major locus, is most likely accounted for by the geometric specificity of a monomeric reductase as suggested before (Roelofs et al. 1987), but the precise ratio of the two isomers may very well be controlled by interaction between the desaturase and reductase systems operating in the pheromone gland. Part of the explanation for high %E females producing a larger amount of E11-14:OAc than low %E females, may be that they have a larger pool of E11-14:1acyl precursor available for reduction than the low E females have.

Pheromone evolution: The low variability found in the parental strains is certainly due to a canalization effect, but a general loss of variability among the laboratory-kept insects may also have played a role. Studies on field collected insects as well as laboratory cultures have sometimes shown significant variation among both E and Z strain insects (Roelofs et al. 1985; Peña et al. 1988). Putative heterozygotes collected from European populations and analysed by Peña et al. (1988) showed a fairly constant blend of 65% E, but two populations from New York State investigated by Roelofs et al. (1985), seem, in retrospect, to have contained heterozygous females producing both low and high %E values. Thus it appears that different local populations show different degrees of variability. We suggest that the significant variation in the E/Z ratio observed in the present study probably occurs under natural conditions but that it may have been overlooked in the past under the basic model of two alleles at one locus controlling the female pheromone production.

The mechanisms by which divergence occurs in sex communication systems, and the relevance this divergence may have for speciation, is a controversial area of evolutionary biology (e.g., see the discussions in Lambert et al. 1987; Löfstedt 1991, 1993; Phelan 1992; Linn and Roelofs 1995; and White et al. 1995). It has even been questioned whether pheromone systems may change due to selective processes; the lack of any significant heritable variation has been used as an argument in favor of this view (e.g., Lambert et al. 1987; White et al. 1995).

The results presented here show that the lack of genetic variation for the composition of pheromone blends may be only apparent. Crosses with slightly different conspecific members may help to reveal an otherwise hidden extensive pool of variation, which rapidly may respond to any selective pressure present. Thus, not only pheromone blends are genetically controlled, but also the variability of these blends, i.e., their degree of canalization.

The proposed extension of the basic model, assuming there to be more than one type of Z-alleles at the major production locus, furthermore shows a likely solution to the dilemma raised in the introduction - how can a new pheromone phenotype type determined by an allele at a "major locus" spread when it cannot communicate with anybody? If at a locus with potentially major effects the individual alleles differ only slightly from each other, then a selective process can be envisaged where a series of increasingly different alleles are successively favoured in different populations. As in O. nubilalis, the process may result in two kinds of populations with remarkably different animals, where the difference is determined by a simple allelic change. This large difference between these alleles should, however, be regarded as a derived trait, rather than reflecting an underlying molecular necessity.

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LITERATURE CITED


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