



## Mate selection in the moth *Neoleucinodes elegantalis*: evidence for a supernormal chemical stimulus in sexual attraction

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We studied mate selection mechanisms in the tomato fruit borer moth *Neoleucinodes elegantalis*, and we found that males and females tended to mate monogamously. Males chose a female according to the blend of her sex pheromone, and they preferred heavier females for mating. Females that produced the preferred blend of the sex pheromone were heavier and had larger wings. Heavier males were more likely to initiate flight sooner and to be the first to copulate with the female. These results suggest that females compete for faster-responding males by producing an attractive blend of sex pheromone, and that males compete for females that synthesize the more attractive sex pheromone blend by responding to the calling female faster. We propose that the pheromone blend preferred by males constitutes a signal reflecting the genetic and physiological quality of the female that is difficult to achieve for biosynthetic reasons. We found that a synthetic 'supernormal' pheromone blend was more effective in attracting males than was the pheromone produced by calling females, thus providing a useful means of mass trapping of males (e.g. to control infestations in tomato plantations). The results support theoretical predictions that mate selection behaviour in both sexes regulates most sexual encounters, even those modulated by sex pheromones.

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Sex pheromones are important for species and sex recognition in a variety of organisms, and especially in insects. Some of these pheromones have an additional function as aphrodisiacs, and may contribute to sexual selection. Sex pheromones in insects have been widely studied and are increasingly being used for insect pest management (e.g. Foster & Harris 1997; Howse et al. 1998). The generally accepted view is that females are the more valuable sex because they manufacture the more rare and expensive gametes (eggs), whereas males can mate multiple times and produce numerous sperm, each inexpensively (Bateman 1948). One of many ramifications of this view is that males should seek out females without losing time

in mate selection, whereas females should be choosier. It is commonly assumed that the mating systems of moths using long-range sex pheromones follow this pattern (Thornhill & Alcock 1983). For example, arctiid moths show a highly sophisticated system of mate selection, whereby females select larger males following genetically determined mate preferences (LaMunyon & Eisner 1994; Iyengar & Eisner 1999; Iyengar et al. 2001, 2002) and males are assumed to follow the first pheromone plume that they encounter.

Although individual spermatozoa are relatively inexpensive to produce, sperm ejaculates are not. Therefore, seeking, finding and copulating with a female could be costly activities for males. Even if only a few spermatozoa are used to fertilize the eggs of a female, males must produce enough ejaculate to trigger fertilization, which could be at an important cost (e.g. Tang-Martinez 2000; Schaus & Sakaluk 2001; Weddel et al. 2002; Jaffe 2004; Tang-Martinez & Ryder 2005). Simultaneous selection by both sexes of the

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opposite sex is a very successful evolutionary strategy (Jaffe 1999) and thus it is likely to be found among extant species (e.g. Gowaty & Hubbell 2005). Theoretical studies suggest that evolution also has favoured the emergence of mate selection devices for males (Jaffe 2002), and thus, that Bate-man's (1948) principle that reproductive success of males is more variable than that of females does not hold in many cases (Tang-Martinez 2000; Tang-Martinez & Ryder 2005). Theoretical studies further suggest that sex without mate selection (Jaffe 2000) and/or gamete selection (Jaffe 2004) is evolutionarily unstable, and that signals such as sex pheromones probably have a function in mate selection.

For sex pheromones to work in mate selection, the chemical signal should provide information about the quality and/or genetic relatedness of the potential mate. Assessment of genetic quality (i.e. 'good genes') can be achieved by considering the complexity of the biochemical intermediaries required to synthesize pure chemical substances or precise blends of compounds to form a sex pheromone: any defect in the synthetic metabolism will affect the chemical purity or the precise pheromone blend. Specifically, when considering the difficulty in synthesizing precise blends of pure isomers, only females with little or no genetic and phenotypic defects may do so.

Sophisticated courtships involving chemicals are known to occur in moths (e.g. Krasnoff & Roelofs 1991), and thus, mate selection based on pheromones probably occurs in many other species. Many moth species share common compounds as sex pheromone components, and species specificity is achieved by precise blends of these components. Evidence that chemically pure or precise blends of moth sex pheromones attract the most mates support this view (Zagatti et al. 1991; Cork et al. 1992; Millar et al. 1992; Gries et al. 1993; Ho et al. 1996). According to Löfstedt (1990), the nature of the demonstrated variability within and between populations is critical for the evolution of moth pheromone communication systems and for pheromones to function in mate finding. Although individual variation in either the concentration or the ratio of sex pheromone components has been examined in some moth species (Miller & Roelofs 1980; Löfstedt et al. 1985; Barrer et al. 1987; Witzgall & Frérot 1989), to our knowledge, no experimental study has examined these factors in the context of mate selection.

We examined the role of sex pheromones in mate selection with the tomato fruit borer *Neoleucinodes elegantalis* (Lepidoptera: Crambidae). The biology and behaviour of this insect have been studied to a certain extent in the laboratory and in the field (Marcano 1991; Salas et al. 1991). Eggs hatch a few days after the females lay them on the tomato fruit. The larvae then perforate and enter the fruit. After some weeks feeding inside the tomato, larvae pupate in the soil. Adult moths emerge at night and live for only 1–4 days. Equal numbers of adult females and males emerge from pupae collected from the field. Both sexes are active only at night, and mating activity is restricted to 1–3 h per night. The sex pheromone of this insect has been isolated, identified and tested in the field (Cabrera et al. 2001; Badji et al. 2003), with traps designed by Mirás et al. (1997), and consists of a blend of at least five compounds. One compound,

(*E*)-11-hexadecenol, attracts males even when presented alone. The isomer (*Z*)-11-hexadecenol may act as a repellent, because it does not attract males by itself and it reduces catches when presented together with (*E*)-11-hexadecenol. Small amounts of (*Z*3,*Z*6,*Z*9)-tricosatriene, increase the attractiveness of (*E*)-11-hexadecenol, but larger amounts inhibit attraction. The tricosatriene has no attractive effect when presented alone.

We explored the role of the sex pheromone of *N. elegantalis* in mate selection and we looked for evidence of mate choice among females and males to test the theoretical prediction that mate selection mechanisms, directly or indirectly involving the sex pheromone, exist for both sexes.

## METHODS

### Laboratory Assays

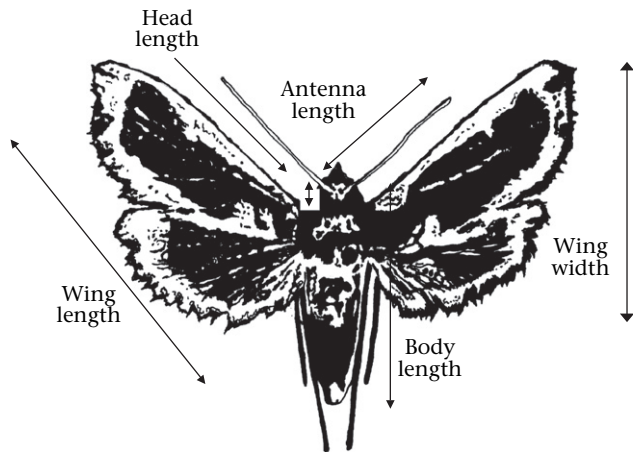
Insects were obtained from infested tomato fruits collected in commercial crops located in Pao de Zárata, Estado Aragua, Venezuela. The fruits were kept during several days in plastic containers in the laboratory until the larvae emerged from the fruit to pupate. To avoid mating, individuals were sexed as pupae and kept in separate cages (one pupae per cage) until emergence. Insects in the laboratory were maintained at 25°C, 70–80% relative humidity and a reversed 12:12 h photoperiod. Adults were fed with a 10% sucrose solution. We used 24-h-old virgin insects for bioassays to guarantee a more uniform motivation for mating.

Each experiment was replicated at least 10 times (preferably 20 times), using fresh 24-h-old virgin adults, except in experiments testing for multiple mating on consecutive days, where adults were older and were not virgin. Bioassays were performed at the hour of highest sexual activity (5–7 h into the scotophase), under darkness, in tulle cages (30 × 30 × 24 cm) that were exposed to a faint current of air. We observed each pair or group for a maximum of 0.5 h, under indirect red light from a 14-W lamp, to record mating. Adults were fed only sugar water and water. No food was presented during the experiments. After each bioassay, individuals were weighed and measured.

For experiments, we used only healthy adults (i.e. without any visible morphological defect). After experiments, we measured the morphological features of each adult, including body mass, body length, length and width of right and left forewings, head length and antennae length (Fig. 1).

### Female Choice in the Laboratory

We assessed female selection of males using only healthy, active females (i.e. with no visible morphological or behavioural defects) that showed the characteristic 'calling behaviour' at the beginning of scotophase: the female partially extended her wings, lifted her abdomen and extruded her ovipositor, exposing her sexual gland. At least 1 h after initiation of the scotophase, we placed each female in a separate cage. Ten minutes later, we released



**Figure 1.** Morphological features measured in *N. elegantalis* (drawing modified from Capps 1948).

five males into each female's cage. We observed females for rejection or acceptance of courting males. We classified the behaviour of males as (1) not stimulated, (2) flying or (3) copulating. Most of the adults lived for only 1 day, but females that survived longer than 1 day were retested each day (range 2–3 days) with fresh males in a similar way.

### Male Choice in the Laboratory

To assess male selection of females, we randomly selected five females that had emerged the day before the experiment and placed them together in cage. Once at least two of the females were 'calling', we waited 10 min, then released a male into the cage. We observed the behaviour of males and females, as described above, and we classified female behaviour as (1) not calling, (2) calling and (3) copulating. Surviving males were retested on day 2 and 3 in a similar way using fresh females.

### Relation between Female Morphology and the Chemistry of the Sex Pheromone

Chemical analyses of individual abdominal tips from 28 virgin females were performed by dissecting the tips at the hour when calling behaviour peaked (5–7 h into the scotophase). To obtain extracts from tips, each tip was placed in 15  $\mu$ l of an internal standard solution (1 ng/ $\mu$ l of *n*-heneicosane in *n*-hexane) for 30 min. Extracts were then sealed into glass microcapillary tubes and stored at  $-15^{\circ}\text{C}$  until analysis. Extracts were subjected to gas chromatographic (GC-FID) analysis, using a Hewlett Packard 5890 Series II gas chromatograph with DB-5 or Carbowax capillary-fused silica columns (30 m  $\times$  0.25 mm ID, Quadrex). For the DB-5 column, the temperature of the GC-oven was set at  $50^{\circ}\text{C}$  for 2 min, then increased, at  $8^{\circ}\text{C}/\text{min}$ , to  $280^{\circ}\text{C}$ . For the Carbowax column, temperature started at  $60^{\circ}\text{C}$  for 2 min, then increased, at  $15^{\circ}\text{C}/\text{min}$ , to  $140^{\circ}\text{C}$ , and finally increased, at  $2^{\circ}\text{C}/\text{min}$ , to  $200^{\circ}\text{C}$ . We used the Carbowax capillary column to analyse the two hexadecenol isomers *E* and *Z*, because this column achieved a better

chromatographic resolution than that obtained with the DB-23, as assessed from injections of the corresponding standards. The detection limit was 20  $\mu$ g. We identified each component of the sex pheromone, (*E*)-11 hexadecenol (*E*11-16:OH), (*Z*)-11 hexadecenol (*Z*11-16:OH), (*Z*)3, (*Z*)6, (*Z*)9-tricosatriene (*Z*3, *Z*6, *Z*9-23:Hy), (*E*)-11 hexadecenol (*E*11-16:Ald) and (*E*)-11 hexadecenyl acetate (*E*11-16:OAc) (Cabrera et al. 2001), by comparing their retention times with those from authentic standards. We purchased two of the components ((*E*)-11 hexadecenol, *E*11-16:OH: Pherobank, Wageningen, The Netherlands; (*Z*)-11 hexadecenol, *Z*11-16:OH: Aldrich Co., Milwaukee, Wisconsin, U.S.A.) and synthesized the other three components, as described in Cabrera et al. (2001).

### Male Choice in the Field

We conducted field tests exploring male preferences for female sex pheromone, as described in Mirás et al. (1997) and Cabrera et al. (2001). Briefly, we placed pheromone-baited traps in commercial tomato plantations and counted the number of male moths captured every 3 days. We tested the following baits: (1) two virgin females placed in a small cage; (2) a reconstructed synthetic chemical blend, using the same proportions as those found in the sex pheromone gland of virgin females (Table 4; Cabrera et al. 2001); (3) the main component of the pheromone: (*E*)-11 hexadecenol; (4) *E*11-16OH + 1% (*Z*)-11 hexadecenol; (5) *E*11-16OH + 2.5% (*Z*3, *Z*6, *Z*9)-tricosatriene; (6) *E*11-16OH + 5% tricosatriene; (7) *E*11-16OH + 10% tricosatriene. The pheromone mixes in these traps contained 1 mg of the main component. We tested more than 100 baited traps (range 9–12 traps per bait type). We also set out blank traps, but no males were caught in these traps, so we omitted them from analyses.

### Statistics

Statistical analysis was performed using the commercial package STATISTICA. We used nonparametric Mann–Whitney tests to compare outcomes of experimental tests in the laboratory and to compare the number of males captured in baited traps of each type in the field. We used a chi-square test to estimate the odds of obtaining a given outcome in the behavioural tests.

## RESULTS

### General Observations

*Neoleucinodes elegantalis* showed a slight sex polymorphism: females were heavier and larger than males (Tables 1, 2). The male/female ratio of moths that emerged from their cocoons in the laboratory was 0.95 ( $N = 4241$ ). Calling females were observed in the laboratory only during the night (2230–0130 hours), which coincided with maximum captures of males in the field.

Of the 20 virgin couples tested, none copulated on the night that they hatched, but 52% copulated 24 h after

**Table 1.** Correlation between the likelihood of copulation and morphological variables of *N. elegantalis* males (Spearman test,  $N = 30$ )

Variable	Mean±SD	<i>R</i>	<i>t</i>	<i>P</i>
Mass	12.2±2.6	0.396	2.281	<b>0.0304</b>
Body length	9.8±0.8	0.257	1.405	0.171
Head length	0.5±0.1	0.042	0.225	0.824
Antenna length	7.7±0.8	0.012	0.064	0.950
Wing length	8.1±0.7	0.525	3.261	<b>0.0029</b>
Wing width	3.2±0.4	0.452	2.681	<b>0.0122</b>

Mass (g); length and width (mm). Values in bold were statistically significant ( $P < 0.05$ ).

hatching, 30% copulated 48 h after hatching and 18% copulated 72 h after hatching. Of the 20 males that were each offered five females simultaneously, 80% copulated with only one female; the remaining males did not copulate. Similarly, all 20 females that were each offered five males also copulated with only one male. Of the moths that copulated 24 h after hatching and that survived for over 48 h, only 16% of males and 21% of females mated again on the second night. None of the insects tested mated on the third night. That is, only about 8% (20% of 40%) of the insects copulated twice when given the opportunity.

### Female Choice in the Laboratory

Results from laboratory bioassays showed that 82% of the females copulated with the first courting male. The remaining females rejected the first courting male but copulated with the second courting male. Males that copulated were heavier and had larger and wider wings than males that did not copulate (Table 1). Even after applying a Bonferroni correction for component tests (Rice 1988), wing length was significantly correlated with copulation success ( $\alpha < 0.05$ ). Behavioural experiments in the laboratory with groups of five males and one female showed that the bigger males (assessed through wing length) were more likely to be the first ones to become activated, and to fly to and copulate with the available female (linear correlation coefficient:  $R = 0.396$ ,  $t = 2.281$ ,  $P = 0.03$ ; Table 1).

**Table 2.** Correlation between the likelihood of copulation and morphological variables of *N. elegantalis* females (Spearman test,  $N = 24$ )

Variable	Mean±SD	<i>R</i>	<i>t</i>	<i>P</i>
Mass	20.2±3.9	0.476	2.541	<b>0.0186</b>
Body length	11.1±0.7	0.178	0.849	0.405
Head length	0.8±0.1	0.013	0.060	0.953
Antenna length	9.4±0.6	0.405	1.984	0.061
Wing length	11.3±0.9	0.301	1.481	0.153
Wing width	4.6±0.4	0.358	1.797	0.086

Mass (g); length and width (mm). Values in bold were statistically significant ( $P < 0.05$ ).

### Male Choice in the Laboratory

Of the males tested in the laboratory, 90% courted and subsequently copulated with one of the calling females. Only 1 of 18 active males was seen courting a noncalling female and was rejected. This male then copulated with a calling female.

Behavioural laboratory experiments with five females and one male showed that calling females that were not chosen by males for copulation were more likely to have a lower body mass than females that were chosen by males for copulation (linear correlation coefficient:  $R = 0.476$ ,  $t = 2.541$ ,  $P = 0.0186$ ; Table 2). That is, for calling females, there was a positive correlation between female body mass and the probability of being chosen for copula by males (Table 2).

### Sex Pheromone Variation

We assessed individual variation of sex pheromone components in the glandular extract of 28 *N. elegantalis* females (Table 3). The maximum and minimum amounts of the main component showed a 40-fold difference (range 3–124 ng), and those of the isomeric *Z* compound showed a 224-fold difference (range 20 pg to 4.5 ng). The major component of the pheromone, *E*11-16:OH, showed the lowest coefficient of variation ( $CV = 96$ ), and its isomer, *Z*11-16:OH, showed the highest coefficient of variation ( $CV = 177$ ).

Comparison of glandular extracts of virgin females showed that heavier and larger-winged females had significantly more of the main component of the sex pheromone (*E*11-16:OH) and less of the isomeric *Z* compound, which inhibits sexual attraction (Table 4). The ratio of *E*11-16:OH to *Z*3,*Z*6,*Z*9-23:Hy was negatively correlated with both female body mass and body length (Table 4).

### Male Choice in the Field

Traps baited with virgin females captured roughly the same number of males as did traps baited with 1 mg of the five-compound-mix reconstructing the pheromone secretion in the female gland (Fig. 2), suggesting that the natural and synthetic pheromones did not differ in their attractive potential. Traps baited with the pure major component of the pheromone, (*E*)-11 hexadecenol, captured many more males than did traps baited with either the natural pheromone or the synthetic pheromone. The addition of any

**Table 3.** Amounts (ng/moth) of the sex pheromone components of *N. elegantalis* females ( $N = 28$ )

	Range (ng)	Mean (ng)	SD	CV (%)
<i>E</i> 11-16:OH	2.99–124.11	40.88	39.18	96
<i>Z</i> 11-16:OH	0.02–4.47	2.05	3.62	177
<i>Z</i> 3, <i>Z</i> 6, <i>Z</i> 9-23:Hy	0.15–17.43	2.82	3.21	114
<i>E</i> 11-16:Ald	0.03–2.34	0.54	0.59	109
<i>E</i> 11-16:OAc	0.03–5.44	0.91	1.27	140
<i>Z</i> : <i>E</i> ratio	0.02–34.77	5.02	9.26	184

**Table 4.** Correlations between wing length and the amount and ratio of sex pheromone components of *N. elegantalis* (Spearman test,  $N = 28$ )

Variable	Total amount					Ratio to E11-16:OH			
	E11-16:OH	Z11-16:OH	Z3,Z6,Z9-23:Hy	E11-16:Ald	E11-16:OAc	Z11-16:OH	Z3,Z6,Z9-23:Hy	E11-16:Ald	E11-16:OAc
Mass	<b>0.44</b>	-0.20	-0.32	-0.20	<b>0.40</b>	-0.34	<b>-0.53</b>	-0.35	-0.01
Body length	0.36	-0.13	-0.23	-0.11	0.20	0.17	<b>-0.39</b>	-0.32	-0.13
RWL	<b>0.52</b>	<b>-0.44</b>	0.04	0.14	0.22	-0.33	-0.34	-0.18	-0.18
LWL	<b>0.58</b>	<b>-0.40</b>	-0.02	0.08	0.26	-0.35	<b>-0.40</b>	-0.22	-0.21
$\bar{X}$ WL	<b>0.55</b>	<b>-0.43</b>	0.01	0.11	0.24	-0.34	-0.37	-0.20	-0.19
RWL-LWL	-0.13	-0.31	0.32	0.34	-0.15	-0.08	0.20	0.10	0.08
RWW	0.33	-0.34	-0.08	0.04	0.24	-0.32	-0.31	-0.09	-0.03
LWW	0.34	<b>-0.43</b>	-0.12	-0.05	0.19	-0.32	-0.32	-0.20	-0.13
$\bar{X}$ WW	0.33	-0.35	-0.10	0.00	0.22	-0.29	-0.30	-0.12	-0.06
RWW-LWW	-0.00	0.09	0.14	0.22	0.12	-0.21	-0.06	0.18	0.20

RWL = right wing length; LWL = left wing length;  $\bar{X}$ WL = mean wing length; RWW = right wing width; LWW = left wing width;  $\bar{X}$ WW = mean wing width. Values in bold were statistically significant ( $P < 0.05$ ).

amount of the Z isomer of the principal component of the pheromone reduced the catches of males in the field. The mix that captured the most males in the field was (E)-11 hexadecenol + 5% tricosatriene. Small deviation from that mix significantly reduced catches. The optimal two-compound mix captured many more males than did the natural or the synthetic pheromone.

The chemical composition of the pheromone was much more important for attracting males than was the pheromone concentration. Although the amount of pheromone inside the gland varied by two orders of magnitude among females, the synthetic baits, with over 500 times more pheromone than the maximum found in female glands, captured approximately the same number of males as did traps baited with two virgin females when the mix of compounds and their relative concentrations were similar

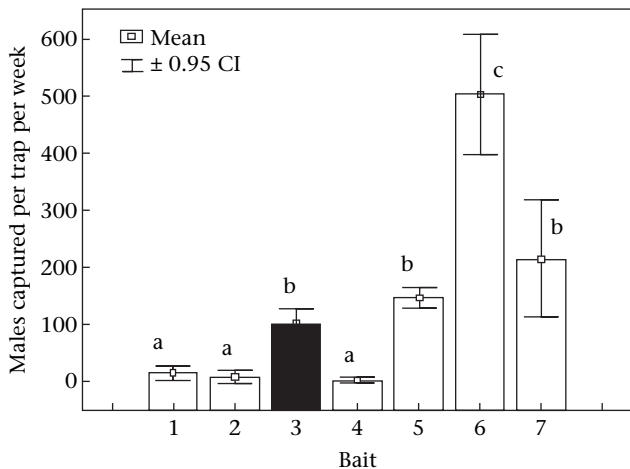
to those found in the glands of virgin females. In contrast, small variations in the relative chemical composition of the synthetic pheromone strongly affected the number of males that were attracted. Precise relative proportions of Z3,Z6,Z9-23:Hy to E11-16:OH, with a total absence of Z11-16:OH, attracted large numbers of males. This highly attractive blend was not synthesized by any of the female moths examined, and it attracted approximately 60 times more males than the average blend produced by virgin *N. elegantalis* females.

**DISCUSSION**

Our results suggest that *N. elegantalis* is monogamous, given its restricted time window for achieving copulations. These insects normally live for only 1 day, and each sex is active for only a few hours on a single night. Even when given the opportunity, individuals normally copulated only once. In addition, the sex ratio was close to 1 and slightly skewed towards females. Our results also suggest that males are attracted to females that produce a preferred pheromone blend, and that this preferred blend is produced mainly by larger females. The biggest males responded to the pheromone more quickly and had the highest mating success in the laboratory. Although mating success of males and females in the field is not known, it is probably lower than that in the laboratory.

Interestingly, the chemical composition of the pheromone was much more important than the pheromone concentration for attracting males, and the synthetic bait was far more effective in attracting males than was the mix found in female glands. This result suggests that females may indirectly select males that respond to sub-optimal mixes of compounds (Wiley & Poston 1966), or that male preferences cannot be matched by females.

Other compatible explanations for the wide range of variation in sex pheromone production are that (1) female moths that produce very weak signals select 'superior' males with better searching abilities (Greenfield 1981) and (2) sexually excited males may attempt to mate indiscriminately with any female that they encounter, especially when background levels are high, as occurs in the



**Figure 2.** Number of *N. elegantalis* males captured per trap per week, given as a percentage of the number captured using E11-16OH as bait (control: ■), when traps were baited with (1) two virgin *N. elegantalis* females, (2) a reconstructed synthetic chemical blend of *N. elegantalis* female pheromone, (3) E11-16OH, (4) E11-16OH + 1% Z11-16:OH, (5) E11-16OH + 2,5% tricosatriene, (6) E11-16OH + 5% tricosatriene and (7) E11-16OH + 10% tricosatriene. Bars with different letters were significantly different (Mann-Whitney U test:  $P < 0.05$ ).

crowded conditions in the laboratory (Barrer et al. 1987). Alternatively, Svensson et al. (1997) suggested that variation in pheromone production could result from different female strategies: a low-release strategy pays when population densities are high, by favouring the most sensitive males, and a high-release strategy pays when population densities are low (i.e. when finding any mate is more important than finding a high-quality sensitive male). None of these explanations allows for males to select females; they all refer only to sexual selection by females. Many other factors not studied here might, however, influence male choice of females (Parker & Tang-Martinez 2005).

Our finding that a synthetic sex pheromone blend was much more attractive to males than was the blend produced by females, and thus, that it behaved as a 'supernormal' stimulus, as originally described by Tinbergen & Perdeck (1950), suggests another plausible explanation for this phenomena: that male preferences in *N. elegantalis* are very demanding and possibly very difficult, or even impossible, to satisfy by the biochemical synthetic armory of females. This demanding male preference is compatible with a strong selection for 'good genes'. Mate selection mechanisms involving pheromones that focus on good genes are predicted to be common in nature (Jaffe 2002). Similar explanations could apply to the highly efficient trapping of Lepidoptera and other insects when synthetic pheromones are used as bait (Ando et al. 1995; Whittle et al. 1995; Zhu et al. 1995). The 'supernormal' synthetic pheromone that we used is very efficient for mass trapping of male *N. elegantalis* in the field, significantly reducing crop damage (Mirás et al. 1997; Sánchez & Pallares 2005), and it is used commercially in Colombia, Brazil, Ecuador and Venezuela to control infestations of this pest in tomato plantations.

Receptive females in our study were not very selective; they accepted the first male that courted them. Similar findings have been reported in the moths *Lymantria dispar* (Charlton & Cardé 1990) and *Operophtera brumata* (Van Dongen et al. 1998). Svensson (1996) suggested that the quick localization of a female should be the most efficient tactic for mating success of males. In such a system, the males with the highest probability of mating should be those that are the fastest to respond to the pheromone and the fastest to fly towards the calling female. That is, selection of males is indirect (Wiley & Poston 1966). As expected from theory, in our experiments, the first male to find the female was the fastest one to respond to the pheromone plume and he was also the largest. This result suggests that larger and more agile males have a higher probability of fertilizing the female and are thus more likely to be fitter. This explanation is compatible with the idea that females produce a suboptimally attractive pheromone blend that indirectly selects for males that are superior at perceiving the pheromone. Such a system would imply a divergent evolutionary race between the sexes and would imply ever-changing pheromone blends in the same population at different historical times. Data to test this hypothesis should be possible to obtain in the future.

Although there is considerable variability in pheromone concentration within populations of the same species,

variation of the Z:E ratio in *N. elegantalis* is particularly high (CV = 184%) compared with that of other moth species (*Grapholita molesta*: CV = 0.60%; Lacey & Sanders 1992; *Cryptophlebia leucotreta*: CV = 10%; Attygalle et al. 1986; *Argyrotaenia velutinana*: CV = 17%; Roelofs et al. 1975; *Yponomeuta padellus*: CV = 15%; Du et al. 1987). The larger variation in the case of *N. elegantalis* may reflect a greater dispersion of local populations, which is congruent with its wide geographical distribution in the Neotropics. Other moths with wide geographical distribution also show high variance in pheromone composition (e.g. European corn borer, *Ostrinia nubilalis*; turnip moth, *Agrotis segetum*; Oriental tobacco budworm, *Helicoverpa assulta*; Arn et al. 2000). Löfstedt et al. (1985) also found wide variation (CV = 127%) in the relative proportions of pheromone components of the turnip moth *A. segetum*, whose pheromone system does not consist of geometric isomers. Thus, different signals coded in the pheromone blend may be used for mate selection in different species. Other evolutionary forces promoting variability in the chemical composition of pheromones are certain to exist (e.g. asymmetric tracking; Roelofs et al. 2002).

Löfstedt (1990) suggested that the maximally attractive ratio between pheromone components should be the one that most closely approximates the natural average ratio produced by females. However, our results and those of Ono et al. (1990) do not support this view. Ono et al. (1990) found that *Argyrotaenia velutinana*, *Grapholita molesta* and *Trichoplusia ni* require a relatively precise blend of pheromones for male attraction and that variation in the ratio of pheromone components is minimal. Similarly, in other species, such as *Adoxophyes* sp. (Kou & Chou 1991) and *Pectinophora gossypiella*, males respond to a wider blend of ratios. Such differences could reflect coding for different strategies for signal selectivity in males and females (Ono et al. 1990).

Although much remains to be uncovered about the actual mate selection mechanisms used by *N. elegantalis*, the basic conditions allowing for mate selection to be modulated by sex pheromones (Jaffe 2002) are present, suggesting that similar systems might be found among other insects.

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