doi:10.1111/j.1558-5646.2011.01252.x

FITNESS COST OF PHEROMONE PRODUCTION IN SIGNALING FEMALE MOTHS

Ally R. Harari,[1,2,3] Tirtza Zahavi,[4,5] and Denis Thiéry[6,7]

[1]*Department of Entomology, the Volcani Center, Bet Dagan, 50250, Israel*

[2]*E-mail: aharari@agri.gov.il*

[3]*Chemical Ecology, Lund University, Lund, Sweden*

[4]*Extension Service, Ministry of Agriculture, Kiryat Shmona, 10200, Israel*

[5]*E-mail: tirtzaz@yahoo.com*

[6]*INRA, UMR 1065 Santé and Agroecologie du Vignoble (SAVE) BP 81, 33883 Villenave d'Ornon, France*

[7]*E-mail: thiery@bordeaux.inra.fr*

Received July 10, 2010
Accepted January 28, 2011

A secondary sexual character may act as an honest signal of the quality of the individual if the trait bears a cost and if its expression is phenotypically condition dependent. The cost of increasing the trait should be tolerable for individuals in good condition but not for those in a poor condition. The trait thus provides an honest signal of quality that enables the receiver to choose higher quality mates. Evidence for sex pheromones, which play a major role in shaping sexual evolution, inflicting a signaling cost is scarce. Here, we demonstrate that the amount of the major component of the pheromone in glands of *Lobesia botrana* (Lepidoptera) females at signaling time was significantly greater in large than in small females, that male moths preferred larger females as mates when responding to volatile signals, and small virgin females, but not large ones, exposed to conspecific pheromone, produced, when mated, significantly fewer eggs than nonexposed females. The latter indicates a condition-dependent cost of signaling. These results are in accordance with the predictions of condition-dependent honest signals. We therefore suggest that female signaling for males using sex pheromones bears a cost and thus calling may serve as honest advertisement for female quality.

KEY WORDS: **Behavior, fitness, selection—sexual.**

Sexual selection acts on secondary sexual traits when certain phenotypic characters lead to an increase in reproductive success of individuals competing for mates. It has been argued that individuals should use the expression of these traits as signals to evaluate the condition of their mates (Andersson 1994). Theoretical models have predicted (Zahavi 1977; Grafen 1990) and behavioral experiments have confirmed (Kotiaho 2000, 2001, 2002) that signals can be evolutionarily stable if they are costly to the signaler and are condition dependent, such that the cost is correlated with the signaler's quality. Most research has focused almost exclusively on visual (Kotiaho 2002; Walther and Clayton 2005; Velando et al. 2006) or acoustic signals (Burk 1988; Ryan 1988; Rivero et al. 2000) while chemical signals have been largely ignored (but see Rantala et al. 2003; Zala et al. 2004; Jaffe et al. 2007).

Sex pheromones have long been recognized as important signals in sexual selection (Wyatt 2003; Johansson and Jones 2007), and evidence demonstrating that chemical signals provide information on morphological and general conditions of the signaler has recently been accumulating (Insects: Moore et al. 1997; Worden et al. 2000; Rantala et al. 2003; Jaffe et al. 2007; Lizards: Olsson et al. 2003; Lo'pez et al. 2006; Mammals: Zala et al. 2004). The vast majority of studies of mate choice by means of pheromones concern female choice of mate (Bonduriansky 2001), while male choice has largely been neglected, even though males are sensitive to small changes in the ratio of the pheromone components (Collins and Cardé 1985). This bias partially emerges from the distinctive difference in male and female pheromone biosynthesis in the Lepidoptera. Males often use various

secondary plant components as precursors for their sex pheromones (e.g., pyrrolizidine alkaloids (Löfstedt et al. 1989; Eisner and Meinwald 1994; but see Fitzpatrick et al. 1985; Landolt and Heath 1990), and the pheromones honestly signal the amount of the alkaloids in the spermatophore that is later incorporated in the eggs as a means of protection against predators (Eisner and Meinwald 1994). Females, however, synthesize their species-specific sex pheromone de novo from available fatty acids (Jurenka 2004) that are not influenced much by the origin of the larval diet (Miller et al. 1976), and the typically small amount of the pheromone (ngs) (El-Sayed 2010) released by the females is traditionally considered as not costly to females (Cardé and Baker 1984; Kokko and Wong 2007). Thus, whereas male pheromones are accepted as a secondary sexual trait that advertises the male quality, female pheromones were typically taken as a mean for species- or sex-recognition (see Johansson and Jones 2007 for review) that do not affect the male choice of mate.

Male choice is predicted to be adaptive when mating is costly (Kokko and Monaghan 2001; Byrne and Rice 2006), when mating is restricted to a small number of females (Parker 1983; Owens and Thompson 1994; Johnstone et al. 1996), and when variance in female phenotypic condition is large (Harari et al. 1999; Kvarnemo and Simmons 1999). Cost of mating for males may arise from an energetically expensive courtship display (Segoli et al. 2006), the production of nutritive ejaculates (Thornhill 1980; Dewsbury 1982), intense intrasexual competition for mates (Bonduriansky and Brooks 1999; Fromhage and Schneider 2005), and limited sperm supply (Friedlander et al. 2005; Teng and Zhang 2009).

Female sex pheromones vary among individual females in quantity and in the ratio of their components (Collins and Cardé 1985; Witzgall and Frérot 1989; Löfstedt 1990; Jaffe et al. 2007). In addition, pheromones are subjected to rapid changes in quality and quantity (AliNiazee and Stafford 1971; Webster and Cardé 1982; Liu and Haynes 1994) and therefore may be regarded as phenotypically condition-dependent traits. There is very little evidence for honest advertisement by means of olfactory cues in relation to female mate choice and less still in relation to male choice (Bonduriansky 2001). Most known examples involve advertisement of male dominance (Moore et al. 1997) or male resistance to parasites (Worden et al. 2000). The scarcity of studies testing female pheromone as chemical advertisement may result from the small amount of pheromone produced and released by females, which led to the perception that the cost of pheromone production is low (Cardé and Baker 1984; Alberts 1992) and from the hypothesis that female moths that produce weak signals select for "superior" males with better searching abilities (Lloyd 1979; Greenfield 1981). These factors are coupled with the substantial difficulties in measuring the metabolic costs of pheromone production (but see Schlyter and Birgersson 1989).

In this study, we used the behavioral response of female moths to their species-specific pheromone to manipulate their calling behavior, and thus their pheromone production rate. Response of female antennae to conspecific female sex pheromone is well demonstrated for various lepidopteran families and species (Arctiidae: *Panaxia quadripunctaria*, Schneider et al. 1998; *Utetheisa ornatrix*, Grant and O'Connell 2000, Noctuidae: *Spodoptera littoralis*, Ljungberg et al. 1993).

Exposure to calling conspecific females and/or their synthetic sex pheromone has been reported to induce signaling in females (Tortricidae; *Choristoneura fumiferana*, Palaniswamy and Seabrook 1985; *Cydia fagiglandana* and *C. splendana*, Den Otter et al. 1996), delay signaling in females (Tortricidae; *Adoxophyes sp*. and *Homona magnanima*, Noguchi and Tamaki 1985), and attract (Tortricidae; *C. fagiglandana* and *C. splendana*; Otter et al. 1996) or repel females (Noctuidae; *Heliothis armigera* and *H. zea*, Saad and Scott 1981). However, the impact of the behavioral changes resulting from this exposure on the fitness of the receiver female has not been analyzed, and the general subject of the cost of female advertisement remains largely unexplored.

The moth *Lobesia botrana* (Lepidoptera: Tortricidae) can serve as a model organism for studying the role of sex pheromones as honest indicators of female quality (i.e., as chemical ornaments) for several reasons: (a) females release a sex pheromone (Roelofs et al. 1973; Witzgall et al. 2005) that attracts mate-seeking males at long range; (b) there is evidence that female calling behavior is affected by female size and age (Torres-Vila et al. 1997a); (c) female sex pheromone characteristics may be heritable (Torres-Vila et al. 1997b), and (d) males are expected to be choosy because they contribute a spermatophore to the sexual bond, rather than sperm alone. The volume of the spermatophore is reduced with each subsequent mating, increasing the risk of female remating (Dewsbury 1982; Torres-Vila et al. 1997a), and moreover, sperm are not renewed in the adult lifetime (Friedlander et al. 2005). All the above provide *L. botrana* males with the motivation for choosiness and females in various conditions to choose from. These, by no means, preclude the possibility that females also chose among mates in this species, which is not, however, in the scope of this research. The objective of this study, which used *L. botrana* as a model organism, was to quantify the fitness cost of pheromone production in signaling female moths.

Here, we demonstrate, for the first time, that female sex pheromones are costly and condition dependent, and, thus, may serve to signal the female quality. We also show that males prefer larger females based on their chemical advertisement.

Materials and Methods

GENERAL

Moths were obtained from the Entomology Department, Volcani Center, Israel. They were reared on an artificial diet (38-0600,

WARD'S, New York) at 25°C with a 17:7 L:D photoperiod. The colony was replenished with field-collected moths every year. The sexes were separated as pupae to prevent mating.

MEASURING THE AMOUNT OF PHEROMONE IN GLANDS OF LARGE AND SMALL FEMALES

Lobesia botrana females were taken from the Israeli culture and reared from pupae to adults in a climate chamber at Lund University, Sweden, under a 17:7 L:D photoperiod. The females were sorted visually by size into three groups: small, average, or large. Only small and large females were used (*t*-test on the weight of random samples of 30 small and 30 large female pupae: $N = 60$, $P < 0.001$). Adult females emerging from these pupae were kept individually in a 200 cc sealed plastic box. The pheromone glands of virgin 2-day-old females were dissected at the time of the peak calling period, 30 min after the onset of the scotophase. Excised glands were placed individually in 10 μL redistilled hexane, with 10 ng (*Z*)-8-tridecenyl acetate (Z8–13:OAc) as an internal standard, for 5 min. The pheromone compounds were identified by comparing their retention times with those of reference compounds; retention times were measured with a Hewlett-Packard HP 5890 series II GC (Waldbronn, Germany) equipped with a flame ionization detector and a capillary column (Innowax, 30 m length × 0.25 mm inner diameter). The oven temperature was programmed to 80°C for 2 min, then increased to 200°C at 10°C/min, held for 5 min, and then increased to 230°C at 20°C/min, holding for 10 min. Inlet temperature was 230°C and the detector temperature was 250°C; hydrogen carrier gas flow was 5 min in splitless injection mode. The amount of each component was calculated in relation to the amount of the internal standard in each injected sample.

COST OF SIGNALING

Effect of conspecific pheromone on calling behavior

Calling behavior of 1-day-old virgin females was observed at calling time, at the beginning of the scotophase. One female was placed in a sealed glass cup (300 mL) together with either (1) four 1-day-old females, each in a glass vial (30 mL) closed with a perforated cap, or (2) four empty glass vials (30 mL). In the first treatment, the tested female was exposed to conspecific pheromone emitted by five calling females (including her own) whereas in the second treatment, the female in focus experienced her own pheromone only. The calling behavior of each female was recorded by two independent observers every 10 min for 130 min starting from 10 min before the onset of the scotophase on the first night ($N = 58$), second night ($N = 58$), fourth night ($N = 58$), and fifth night ($N = 43$).

The calling behavior of female *L. botrana* is characterized by a typical posture in which the wings slightly open and the abdominal tip is pressed to the cup wall. Females often fly short distance between calling bouts. These short flying periods were included in summarizing the duration of calling behavior. In addition, as females that have been exposed to the pheromone of neighboring females may become agitated and spend more energy in attempts to escape competition (for males or future food resources), the duration of flying females was analyzed separately. Flying and calling behavior of females in the two treatments was compared using ANOVA with each specific female as a covariate (repeated measures was not possible because some females died during the week). The contrast procedure of SPSS was used to compare calling behavior of females in the two treatments each night.

Here, we hypothesize that females in a group elevate their pheromone-releasing activity to compete with neighboring females for males, whereas females that are alone optimize their calling activities in the absence of competition.

Effect of pheromone emission on egg laying

We tested the effect of continuous signaling of virgin females on the number of eggs oviposited after mating. Females were sorted visually as pupae into three sizes as described earlier, and only females from the small and large categories were used. One large or one small 1-day-old virgin female in a clear 30-mL covered vial was placed in a 2-l sealed container together with four females (one female per covered 30-mL clear vial) of the same size category. In the first treatment, each female was visually exposed to the other four females but received no chemical stimuli from her neighbors, for three consecutive nights. In the second treatment, the covers of all vials in the container were perforated to allow the transfer of volatile pheromone, such that all females received visual and chemical stimuli from the four other females for three consecutive nights. Females in both treatments received a male on the fourth evening. On the following morning, the males were removed and each female was transferred to an oviposition tube. In the control treatments, females, both large and small, were placed in a clear 30-mL vial with a cover, following the same treatments as above but control females received a male on the first day of calling; on the following morning, the males were removed, and the control females were transferred individually to an oviposition tube.

The eggs were counted every day in all treatments until the female died. Because Levene's test for homogeneity of variance revealed significant differences among treatments, the nonparametric Kruskal–Wallis test was used to compare numbers of oviposited eggs. Females that laid eggs only on the last 2 days before dying were excluded from the analysis. These females were assumed to be unmated because virgin females typically drop their eggs before dying.

Here, we hypothesize that if calling is costly ([a] above), females in a group will trade-off calling with fecundity. We also

hypothesize that this cost will be more noticeable in small females than in large females.

Effect of pheromone emission on survival

To test the effect of conspecific pheromone on the life span of signaling females, we assigned females to one of three treatments: (1) exposure only to their own pheromone, (2) exposure to pheromone emitted by four other calling conspecific females, and (3) exposure to pheromone emitted by nine other calling conspecific females. One-day-old virgin females were placed individually in clear 30-mL vials with a cover; 10 vials were placed in 2-l sealed containers. In the first treatment, each female in the container was visually exposed to other females but received no chemical stimuli from their neighbors ($N = 50$). In the second treatment, the cover of five vials were perforated, thus each of these five females focused upon was visually exposed to 10 females but received chemical stimuli from only five females (including her own) ($N = 50$). In the third treatment, the covers of all vials were perforated to allow the transfer of the volatile pheromone, such that each female received visual and chemical stimuli from 10 females sharing the sealed container ($N = 49$). Live females were counted every day, and each dead female in the three treatments was replaced with a new 1-day-old female such that the number of calling females was constant in each container during the experiment. The survival time of females in each treatment was compared using the Tarone–Ware survival test (the survival times of females that replaced dead females were not included in the analysis).

As a control for the effect of the pheromone emitted by virgin females, similar treatments were prepared with 1- to 2-day-old mated females, which typically do not call for males. Individual females were kept with a male in a sealed plastic box (200 cc) upon emergence. After mating, females were placed individually in a clear 30-mL vial with a cover; the vials were placed in a 2-l sealed container. In the first control, each mated female was placed with 10 virgin females, each in a sealed vial ($N = 31$). In the other two controls, each mated female was placed with either five ($N = 31$) or 10 ($N = 30$) virgin females, each in a vial with a perforated cover; in these latter two controls, the mated females were therefore exposed to the pheromone of either five or 10 females. The number of calling females in the container was kept constant by replacing dead females with 1-day-old virgin females. As before, survival time of the mated females in each treatment was compared using the Tarone–Ware survival test.

Here, as well, we hypothesize that if calling is costly (a above) females in a group will pay a survival cost and their life expectancy will be shorter than that of females that were kept alone.

MALE MATE PREFERENCE

One-day-old females from the large and small groups were used in this experiment. We placed three large females at one end of a transparent cage (100 × 60 × 60 cm) and three small females at its opposite end. Each female was enclosed in a perforated plastic cylinder (4 × 3 cm) that was wrapped with cotton mesh to prevent the males from seeing the females but to allow pheromone to flow from the cylinders. Each cylinder was placed in the cage after female calling behavior was observed. After a 2-min acclimation period, 20 males were released into the cage. For the following 45 min, we recorded every 30 sec the number of males hovering within 40 cm above and 15 cm to the side of the females in the cylinders at each end of the cage. Every 10 min, the cage was turned 180° to avoid directional bias. The experiment was repeated three times. A G-test was used to test for differences between the expected and observed results ($N = 3$, with 20 males in each repetition and a total of 396 choosing events).

Here, we hypothesize that if females' phenotypic condition (i.e., size) is revealed in their pheromone characteristics (2 above) males are expected to choose the better fit females (i.e., more fecund) as mates.

Results

THE AMOUNT OF PHEROMONE IN FEMALE PHEROMONE GLANDS

One pheromone component (E9–12Ac) was not detected in any of the small female glands ($N = 38$) but was detected in a few glands of large females (3 of 31). A second measured component (Z9–12Ac) was detected in more glands of large females (67.8%) than of small females (57.8%), whereas the main pheromone component (E7, Z9–12Ac) was detected in all females. The amount of the main component differed significantly between the two size groups (Large females = range: 0.12–6.18, mean ± SE 1.25 ± 0.32 ng; Small females = range: 0.10–2.30, mean ± SE 0.62 ± 0.10 ng. ANOVA: $f_{67,1} = 4.23$, $P = 0.04$).

COST OF CHEMICAL SIGNALING

Effect of conspecific pheromone on calling behavior

The effect of the nights on calling behavior (Area Under the Curve (AUC)) was significant ($f_{204,3} = 2.825$, $P = 0.005$), as was the interaction between different nights (first, second, fourth, and fifth) and the treatments (GLM: $f_{204,3} = 4.601$, $P = 0.004$). Thus, the effect of the treatment for each night was analyzed separately. The effect of an individual female as a covariate was not significant. The effect of pheromone of conspecific females on calling behavior changed gradually during successive nights. On the first night, females exposed to conspecific pheromone called significantly more than females that were exposed to their own pheromone only (ANOVA: Contrast, $t = 1.994$.

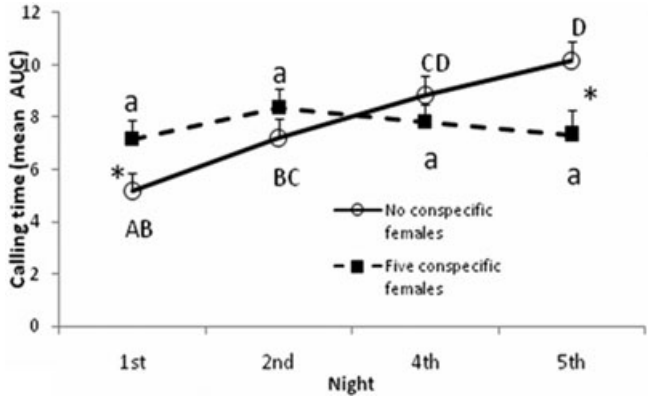

Figure 1. Calling time (mean area under curve of females calling on each night) of virgin females kept alone or kept in a group of five for five successive nights. Different letters indicate significant differences between nights of the same treatments; * = significant differences between the two treatments (Tukey HSD $\alpha = 0.05$). AUC = area under curve.

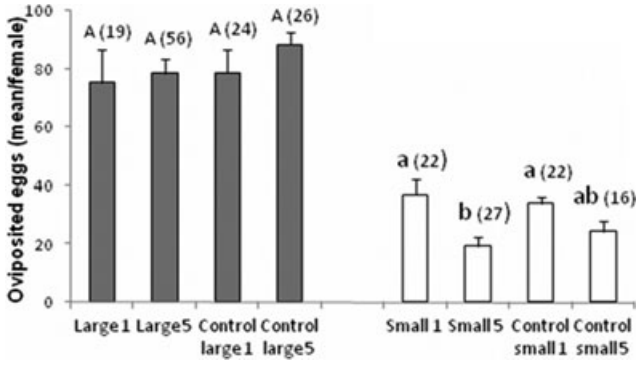

Figure 2. Number of eggs (mean/female) oviposited by (1) Large females that were kept alone and mated on the third night of signalling (Large 1); (2) Large females exposed to pheromone of five females and mated on the third night of calling (Large 5); (3) Large females that were kept alone and mated on the first night of calling (Control Large 1); (4) Large females that were exposed to pheromone of five calling females and received a male on the first night of calling (Control Large 5); (5) Small females that were kept alone and mated on the third night of signalling (Small 1); (6) Small females exposed to pheromone of five females and mated on the third night of calling (Small 5); (7) Small females that were kept alone and received a male on the first night of calling (Control Small 1); (8) Small females that were exposed to pheromone of five calling females and received a male on the first night of calling (Control small 5). Different letters indicate significant differences (Tukey HSD $\alpha = 0.05$).

df = 200, $P = 0.048$). On the second and fourth nights, no significant difference was detected ($t = 1.117$ and 0.931 respectively, df = 200, $P > 0.05$). On the fifth night however, females exposed to conspecific pheromone called significantly less than females that were exposed only to their own pheromone ($t = 2.400$. df = 200, $P = 0.017$) (Fig. 1), whereas females that were exposed to neighboring females' pheromone continued to call at a similar rate (Tukey's post-hoc test using model MSE of 15.067, 103 df, $P > 0.05$ for all comparisons). Females isolated from the pheromone of other calling females increased their calling time each night with significant differences between the first night and both the fourth and fifth nights (Tukey's post-hoc test using model MSE of 14.063 with 97 df, $P < 0.001$) (Fig. 1).

The effect of an individual female (as a covariate) on flying behavior of females in the two treatments was not significant ($P > 0.05$), and so was the effect of different nights (ANOVA of Area Under the Curve (AUC): $f_{204,3} = 1.625$, $P > 0.05$), and that of the treatments ($f_{206,1} = 1.625$, $P > 0.05$), but the interaction between different nights and the treatments was significant ($f_{204,3} = 5.134$, $P = 0.002$). Thus, the effect of the treatments was analyzed separately for each night. The results revealed that female flying behavior in the two treatments was not significantly different in either of the successive nights (ANOVA: Contrast, df = 200, $P > 0.05$).

Effect of conspecific pheromone on egg laying

Some females from all treatments did not lay eggs or eggs were laid one to two days before the females' death. These females were removed from the analysis.

The Kruskal–Wallis test for differences of independent means revealed a significant effect of the treatments (Chi-square statistics = 94.317, df = 7, $P < 0.001$). Because egg numbers are often correlated with female size (Honěk 1993), we examined the differences between mean numbers of eggs among treatments in a separate analysis for each size group. The Kruskal–Wallis test revealed no significant effect of the treatments among all large females (Chi-square statistics = 1.391, df = 3, $P > 0.05$), but a significant effect was observed for small females (Chi-square statistics = 16.183, df = 3, $P = 0.001$). Small females exposed to pheromone of five females (including their own) for three nights before mating laid significantly fewer eggs than small females exposed to either own pheromone for one or three nights before mating (Fig. 2).

Effect of pheromone emission on survival

Virgin females exposed only to their own pheromone survived longer (mean ± SD: 12.08 ± 2.10 days) than females that were exposed to pheromone of either five (9.52 ± 1.43 days) or 10 (9.68 ± 1.32 days) calling females throughout their lifetime (Tarone–Ware survival test: Chi-square = 46.07, df = 2, $P < 0.0001$ and following Tukey HSD, $\alpha = 0.05$) (Fig. 3). No significant difference in survival was found when mated females were kept with 10 virgin females in sealed vials (9.06 ± 1.48 days) or when mated females were exposed to pheromone of five conspecific virgin females (8.33 ± 1.63 days) or 10 virgin females (8.36 ± 0.87 days) in a container (Tarone–Ware survival test:

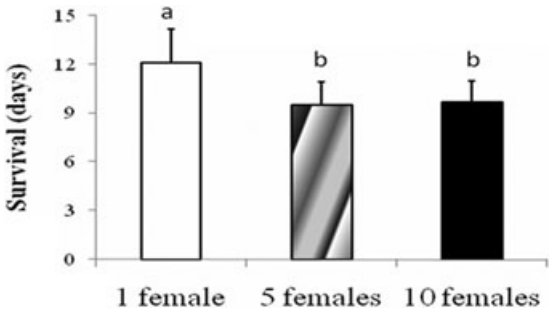

Figure 3. Survival (days, mean per female) of virgin females that were kept alone (exposed to their own pheromone only) and females that were exposed to either four or nine conspecific calling females on successive nights. Different letters indicate significant differences (Tukey HSD $\alpha = 0.05$).

Chi-square = 4.591, df = 2, $P > 0.05$ and, following Tukey HSD, $\alpha = 0.05$) (Fig. 4).

MALE MATE PREFERENCE

We tested the hypothesis that male moths are more attracted by volatile materials emitted by large females than by small females when given a choice of females of different sizes. The results indicated that caged adult *L. botrana* males presented with large and small calling females, concealed in opaque perforated cylinders, preferred to fly toward and stay close to the large ones (mean ± SE: 76.82 ± 4.40%, $N = 3$, with 20 males in each trial and total of 396 choosing events; G-test: G (homogeneity) = 2.64, df = 2, $P > 0.05$; G(p) = 112.96, df = 2, $P < 0.0001$). This suggests that males were capable of discerning large from small females by emitted volatile.

Discussion

In this study, we demonstrated that (a) more of the large females have detectable amounts of the three pheromone components, and large females have larger amounts of the main pheromone component in their glands than small females, (b) female moths advertising for males suffer a cost of survival and small advertising females were less fecund, and (c) based only on pheromone emission, males prefer larger females. All three pieces of evidence support the hypothesis that female sex pheromones serve as sexual signals that convey honest information regarding female quality to males.

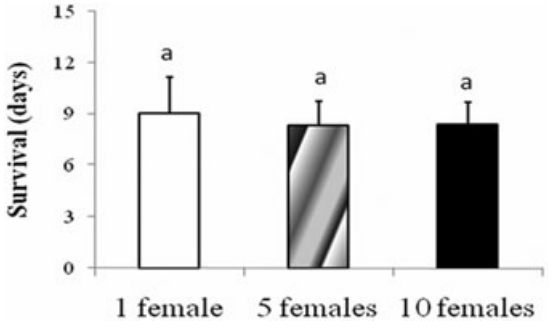

Figure 4. Survival (days, mean per female) of mated females that were kept alone and females that were exposed to either five or 10 conspecific virgin calling females on successive nights. Different letters indicate significant differences (Tukey HSD $\alpha = 0.05$).

We found that *L. botrana* females vary with respect to the pheromone amount and the ratio of its components (as two of the pheromone components were detected in more of the large females). We further found that more of the large females had a detectable level of the secondary components of the pheromone (E9-12Ac and Z9-12Ac), and that large females had a significantly greater amount of the main component of the pheromone (E7, Z9-12Ac).

Females responded to the pheromonal signal (calling) of conspecific females. Females that were exposed to the conspecific pheromone on their first night signaled at a significantly higher rate than females that were kept alone. However, on consecutive nights, these females gradually signaled less than lone females (Fig. 1). These differences were due to an increase in calling behavior among females that were kept alone. When not mated, single females calling at their own adaptive rate increased their calling bouts on successive nights. In contrast, females exposed to conspecific pheromone kept calling at a similar pace on successive nights, hampering their chances for attracting a male. We suggest that females that amplified their signaling rate in the first night could not further increase their calling due to fitness costs involved in the process. This was evident when virgin females that were exposed to signals of conspecifics survived for significantly fewer days than females that were kept alone (Fig. 3). The cost of signaling was also condition dependent because extensive signaling did not affect the number of eggs laid by large females but strongly affected the number of eggs laid by small females (Fig. 2). Further support for this explanation is provided by the observation that there was no effect of conspecific pheromone on survival time of mated females, which usually do not signal and thus did not expend energy on signaling (Fig. 4). The noticeable reduction in fitness of females in groups as compared to solitary females is not explained by agitation of females in the presence of larger amount of pheromone released by the females in a group, as no significant difference was detected between flying behavior of females in the two groups.

Female sex pheromones may thus qualify as chemical ornaments, honestly advertising the quality of calling females. In this study, male *L. botrana* flew across the cage but hovered for significantly more time above large signaling females than over small ones, indicating a preference for the pheromone emitted by the large females. Males may have based their preference on the detectable differences in quality and/or quantity of pheromone

emitted by females of the two size categories. Different amounts of pheromone and ratios of component produced by small and large females have been detected in a few moth species (*Neoleucinodes elegantalis*, Jaffe et al. 2007; *Agrotis segetum,* Harari et al. (unpubl. data) *Trichoplusia ni*, Kenneth Haynes, pers. comm.), and age-dependent pheromone release has been demonstrated in various studies (Miller and Roelofs 1980; Raina et al. 1986; Noldus and Potting 1990).

Males should be choosy if there is a cost involved in mating. Male moths do not overtly fight for females, although intrasexual "repellent pheromone" has been demonstrated in some cases (Hendricks and Shaver 1975; Hirai et al. 1978; Fitzpatrick and McNeil 1988; Otter et al. 1989). In moth species, sperm reserves are depleted after repeated copulations (Friedlander et al. 2005; Teng and Zhang 2009). In some species, the total quantity of accessory fluid that a male can produce is limited (Callahan and Cascio 1963), whereas in other species repeated mating reduces male survival (Amoako-Atta and Mills 1977; Kehat and Gordon 1977; Fitzpatrick and McNeil 1989). There is evidence that males prefer heavier females (*N. elegantalis*, Jaffe et al. 2007; *A. segetum,* Harari et al. (unpubl. data). Males may prefer large females because body size has been reported to affect female fecundity in many insect species (Andersson 1994; Harari et al. 2003), and large females oviposit larger eggs (Torres-Vila et al. 2002). That small females lay significantly fewer eggs than large females was also evident in control treatments of this study with *L. botrana* (females in control treatments, Fig. 2).

Although evidence has accumulated concerning the response of females to conspecific pheromone (Birch 1977; Light and Birch 1979; Palaniswamy and Seabrook 1985; Schneider et al. 1998; Grant and O'Connell 2000; Groot et al. 2005; Lim et al. 2007), adaptive explanations of this phenomenon have been suggested in only a few cases. Response of females to conspecific pheromone has been suggested to: (1) generate intrasexual competition among females under high female density (Lim and Greenfield 2007); (2) decrease intrasexual competition by spreading the beginning and ending of the calling window (Noguchi and Tamaki 1985; Gökçe et al. 2007; Yang et al. 2009); and (3) increase spacing on food plants (Otter et al. 1996).

Examples of the harmful effects of pheromone on conspecific females as demonstrated in this study have been reported previously in several studies. For instance, Dunkelblum and Kehat (1987) showed that females exposed to their own pheromone did not mate although males were at a touching distance. Palaniswamy and Seabrook (1985) demonstrated that virgin females were induced to call earlier and lay prematurely with more eggs in a clutch when exposed to conspecific female pheromone. Signaling behavior of *Adoxophyes* sp. and *H. magnanima* was delayed significantly when the females were exposed to a small amount of their own pheromone under outdoor and laboratory conditions (Noguchi and Tamaki 1985), and Ellis et al. (1980) reported a reduction in female signaling events when females were exposed to pheromone in laboratory trials. Reduced oviposition of moths when exposed to an excess of pheromone in the orchard was suggested by Weissling and Knight (1996).

Several studies have directly demonstrated increased signaling behavior by females when exposed to conspecific pheromone (Palaniswamy and Seabrook 1985; Lim and Greenfield 2007). Lim and Greenfield (2007) have termed this phenomenon "female pheromonal chorusing" and suggested that it represents a form of intrasexual competition for mates, which might have selected females to reach the signaling level of their neighbors, as was observed in mating systems using acoustic communication (Gerhardt and Huber 2002). Although Lim and Greenfield (2007) restricted this observation to cases when there was a strong female-biased operational sex ratio, this phenomenon may also apply to species in which males are choosy and female advertise for mates (Kokko and Monaghan 2001; Byrne and Rice 2006).

A possible mechanism by which females exposed to excessive conspecific pheromone suffer a fitness cost is that selective pressure to release more pheromone may take its toll on their survival and reproductive potential. Thus, females that repeatedly signal early and intensively may suffer higher mortality due to the energy costs of signaling and/or producing large amounts of pheromone during the calling period (Foster 2009). In contrast, lone females presumably adjust their investment in calling and pheromone production to the environmental situation; in the absence of competing females they minimize the investment in calling, whereas, in the absence of males, as they get older and still virgin, they increase their investment in calling to secure mating. These females, when virgin, increased their rate of signaling on successive nights, survived for longer periods, and enhanced their chances for attracting a mate. When finally mated, small lone females oviposited more eggs than small females that signaled intensely for males. When signaling for males is costly, females may increase their calling rate to assure mating and trade-off calling for future fecundity. There is evidence for such a trade-off when females invest more in resistance to insecticides than on the account of their future progeny. For example, in *H. virescens* fecundity of pyrethroid-resistant females was significantly lower (half) than that of susceptible females (Campanhola et al. 1991). That result is in agreement with our study demonstrating reduced fecundity of small females that increased calling. This suggests that advertising is costly for females and the cost is higher for small females that allocate energy for calling at the expenses of future fecundity.

An alternative explanation, although not mutually exclusive, for minimizing the investment in calling by lone females during the first night, is an indirect selection pressure for the most sensitive males as mates, as suggested by Lloyd (1979). In the absence

of these sensitive males, females increase their calling rate during successive nights to secure any mate.

In this study, no attempt was made to disentangle the costs of signaling (releasing pheromone) from the costs of pheromone production per se. Although cost of pheromone production for female moths has traditionally been considered to be low (Cardé and Baker 1984; Alberts 1992) there is indirect evidence that pheromone production is costly. For example, acquiring resistance to pesticides reduces pheromone production in females (Campanhola et al. 1991; Delisle and Vincent 2002), suggesting a trade-off between obtaining resistance and pheromone production, thus inferring a high cost due to the two metabolic activities. Recently the effect of low hemolymph trehalose concentration on pheromone production was demonstrated for both virgin and mated *H. virescens* females (Foster 2009; Foster and Johnson 2010). Feeding on sucrose-restored pheromone production, thus suggesting an energy cost of pheromone production in itself. Foster (2005) suggested that trehalose together with fatty acids serve as a metabolic reservoir for pheromone biosynthesis. Trehalose is used for various energy-consuming activities, such as flying and egg production (Foster and Johnson 2010) and probably calling. Accordingly, the increase in calling behavior and the release of pheromone may decrease the trehaslose concentration in the female haemolymph thereby lessening the trehaslose available for pheromone production and for other metabolic activities. This may explain the decrease in life span and egg production that followed the increase in calling behavior in this study.

Our results suggest that males prefer larger females that produce more pheromone. However, density dependence is an alternative explanation for the observed preference of males for large females (Lucas and Howard 1995). Svensson et al. (1997) suggested that a low-release strategy may be advantageous for females when population densities are high, giving priority to the most sensitive male (gaining an indirect fitness from his "sexy sons"), and a high-release strategy pays when population densities are low (increasing the chance of finding any mate). The results of our study are inconsistent with these explanations because all females in a group (high population density) called more intensively than lone females (low population density) in the first days of calling. Moreover, females changed their calling intensity in response to their calling experience rather than in response to their experienced population density. Furthermore, the results of this study and others (Webster and Cardé 1982; Schal et al. 1987; Ono et al. 1990; Babilis and Mazomenos 1992; Delisle and Royer 1994; Delisle and Simard 2003; Xiang et al. 2010) show that individual females change the amount of pheromone they release over their lifetime (a few days), during which a change in population density is not expected.

Lucas and Howard (1995) suggested that small females may cease signaling to reduce energy investments in a pheromone-saturated environment. In our study and others (Jaffe et al. 2007; Harari et al. unpubl. data), however, males preferred large signaling females over small ones, and would have probably preferred either of these over nonsignaling females, which they obviously cannot detect. Furthermore, there is evidence that delayed mating in *L. botrana* females reduces their reproductive fitness (Torres-Vila et al. 2002). Thus, the cost of deferred mating or not mating that is imposed on nonsignaling females far exceeds the energy outlay for the production of sex pheromone.

Several reports of pheromone-mediated mate choice suggest a positive correlation between male olfactory attractiveness and other male traits such as performance (Moore et al. 1995), physiological condition (Shelly and Dewire 1994), and parasite load (Moore et al. 1997). Honest advertisement by odor, although rare, has been demonstrated in male mice (Lemington 1983; Zala et al. 2004) and insects (Moore 1988) in relation to dominant males and spermatophore size. In females, honest advertisement by odor has been hypothesized in the goldfish, *Carassius auratus* (Sorenson and Stacey 1999), and demonstrated for the tomato fruit borer, *N. elegantalis* (Jaffe et al. 2007).

Pheromone production by females seems to fit the definition of a "revealing handicap," suggesting that the trait or its maintenance needs to bear a cost, which should be condition dependent to serve as an honest signal of quality (Nur and Hasson 1984; Iwasa et al. 1991).

Although evidence exists that male choice of females for mating is correlated with female body size and pheromone production (Phelan and Baker 1986; Jaffe et al. 2007), to our knowledge no previously published study has documented that a female moth pheromone can be a condition-dependent trait. We demonstrate that chemical signaling is costly for females although we did not determine whether the cost is in the production of the pheromone from its fatty acid precursor or in the expansion of energy during signaling. This fitness cost is expressed in decreased (a) signaling behavior, (b) fecundity, and (c) survival of the signaling female. In light of our results, we suggest that males can detect the size of a female according to pheromone-related signals. Male preference can result from differences in the amount of pheromone released by females or the ratio of its components (Collins and Cardé 1985; Jaffe et al. 2007), which may in turn reveal differences in female condition (Phelan 1997). Males that discriminate between small and large females at long range by means of female pheromone titre may benefit by choosing higher quality mates that will produce more or higher quality offspring. We therefore conclude that the female sex pheromone of *L. botrana* can serve as a "revealing handicap" (Phelan and Baker 1986) that honestly signals the female's general condition. We believe that this phenomenon is not unique to the species studied but is a general trait of species in which females release sex pheromone and males bear a cost of mating.

ACKNOWLEDGMENTS

We thank A. Zahavi, Y. Lubin, J. Brockmann, V. Soroker, and P. Weintraub for comments on early drafts. We thank Christer Löfstedt, Maria Strandh, Hong-Lei Wang, Jean-Marc Lassance, Marjorie Lienárd, Camilla Ryne, Olle Anderbrant, Jette Knudsen, Glenn Svensson, and Erling Jirle from of the chemical ecology group of Lund University, Sweden, and T. Keasar, A. Bouskila, M. Segoli, D. Gottlieb, N. Morag, and A. Sadeh from Ben Gurion University, Beer Sheva, Israel for their helpful comments on experimental design and interpretation of results.

LITERATURE CITED

Alberts, A. C. 1992. Constraints on the design of chemical communication systems in terrestrial vertebrates. Am. Nat. 139:S62–S89.

AliNiazee, M. T., and E. M. Stafford. 1971. Evidence of a sex pheromone in the omnivorous leaf roller, *Platynotas tultana* (Lepidoptera: Tortricidae): laboratory and field testing of male attraction to virgin females. Ann. Entomol. Soc. Am. 64:1330–1335.

Amoako-Atta, B., and R. B. Mills. 1977. Gamma radiation effects on mating frequency and delayed mating of male *Cadra cautella* (Walker) (Lepidoptera: Pyralidae). J. Stored Prod. Res. 13:139–143.

Andersson, M. 1994. Sexual selection. Princeton Univ. Press, Princeton.

Babilis, N. A., and B. E. Mazomenos. 1992. Pheromone production in *Sesamia nonagrioides*: diel periodicity and effect of age and mating. J. Insect Physiol. 38:561–564.

Birch, M. C. 1977. Responses of both sexes of *Trichoplusia ni* (Lepidoptera: Noctuidae) to virgin females and to synthetic pheromone. Ecol. Entomol. 2:99–104.

Bonduriansky, R., and R. J. Brooks. 1999. Why do male antler flies (*Protopiophila litigata*) fight? The role of male combat in the structure of mating aggregations on moose antlers. Ethol. Ecol. Evol. 1:287–301.

Bonduriansky, R. 2001. The evolution of male mate choice in insects: a synthesis of ideas and evidence. Biol. Rev. 76:305–339.

Burk, T. 1988. Acoustic signals arm races and the cost of honest signalling. Fla. Entomol. 71:400–409.

Byrne, P. G., and W. R. Rice. 2006. Evidence for adaptive male mate choice in the fruit fly *Drosophila melanogaster*. Proc. R. Soc. Lond. Ser. B. 273:917–922.

Callahan, P. S., and T. Cascio. 1963. Histology of the reproductive truck and transmission of sperm in the corn earworm, *Heliothis zea*. Ann. Entomol. Soc. Am. 56:535–555.

Campanhola, C., B. F. McCutchen, E. H. Baehrecke, and F. W. Plapp, Jr. 1991. Biological constraints associated with resistance to pyrethroids in the tobacco budworm (Lepidoptera: Noctuidae). J. Econ. Entomol. 84:1404–1411.

Cardé, R. T., and T. C. Baker. 1984. Sexual communication with pheromones. Pp. 355–383 *in* W. J. Bell and R. T. Cardé, eds. Chemical ecology of insects. Chapman and Hall, London.

Collins, R. D., and R. T. Cardé. 1985. Variation in heritability of aspects of pheromone production in the pink bollworm moth, *Pectinophora gossypiella* (Lepidoptera: Gelechiidae). Ann. Entomol. Soc. Am. 7:229–234.

Delisle, J., and L. Royer. 1994. Changes in the pheromone titer of obliquebanded leafroller, *Choristoneura rosaceana*, virgin females as a function of time of day, age, and temperature. J. Chem. Ecol. 20:45–69.

Delisle, J., and J. Simard. 2003. Age-related changes in the competency of the pheromone gland and the pheromonotropic activity of the brain of both virgin and mated females of two *Choristoneura* species. J. Insect Physiol. 49:91–97.

Delisle, J., and C. Vincent. 2002. Modified pheromone communication associated with insecticidal resistance in the obliquebanded leafroller, *Choristoneura rosaceana* (Lepidoptera: Tortricidae). Chemoecology 12:47–51.

Dewsbury, D. A. 1982. Ejaculate cost and mate choice. Am. Nat. 119:601–610.

Dunkelblum, E., and M. Kehat. 1987. Sex pheromone precursors in *Spodoptera littoralis* (Lepidoptera: Noctuidae). Insect Biochem. 17:877–881.

Eisner, T., and J. Meinwald. 1994. The chemistry of sexual selection. PANS 92:50–55.

El-Sayed, A. M. 2010. The pherobase: database of insect pheromones and semiochemicals. Available at http://www.pherobase.com (accessed December 10, 2010).

Ellis, P. E., L. C., Brimacombe, L. J. McVeigh, and A. Dignan. 1980. Laboratory experiments on the disruption of mating in the Egyptian cotton leafworm *Spodoptera littoralis* (Boisduval: Noctuidae) by excesses of female pheromone. Bull. Entomol. Res. 70:673–684.

Fitzpatrick, S. M., and J. N. McNeil. 1988. Male scent in lepidopteran communication—the role of male pheromone in mating behavior of *pseudaletia unipuncta* (HAW) (Lepidoptera: Noctuidae). Mem. Entomol. Soc. Can. 146:131–151.

———. 1989. Life time mating potential and reproductive success in males of the true armyworm, *Pseuduletia unipuncta* (HAW) (Lepidoptera, Noctuidae). Func. Ecol. 3:37–44.

Fitzpatrick, S. M., D. Miller, I. Weatherston, and J. N. McNeil. 1985. Determining pheromone content of hairpencils from individual virgin males of *Pseudaletia unipuncta* (Haw.) (Lepidoptera: Noctuidae). J. Chem. Ecol. 11:207–215.

Foster, S. P. 2005. Lipid analysis of the sex pheromone gland of the moth *Heliothis virescens*. *A*rc. Insect Biochem. Physiol. 59:80–90.

———. 2009. Sugar feeding via trehalose haemolymph concentration affects sex pheromone production in mated *Heliothis virescens* moths. J. Exp. Biol. 212:2789–2794.

Foster, S. P., and C. P. Johnson 2010. Feeding and hemolymph trehalose concentration influence sex pheromone production in virgin *Heliothis virescens* moths. J. Insect Physiol. 56:1617–1623.

Friedlander, M., R. K. Seth, and S. E. Reynolds. 2005. Eupyrene and apyrene sperm: dichotomous spermatogenesis in lepidoptera. Ad. Insect Physiol. 32:206–308.

Fromhage, L., and J. M. Schneider. 2005. Virgin doves and mated hawks: contest behaviour in a spider. Anim. Behav. 70:1099–1104.

Gerhardt, H. C., and F. Huber. 2002. Acoustic communication in insects and anurans: common problems and diverse solutions. Chicago Univ. Press, Chicago.

Gökçe, A., L. L. Stelinski, L. J. Gut, and M. E. Whalon. 2007. Comparative behavioral and EAG responses of female obliquebanded and redbanded leafroller moths (Lepidoptera: Tortricidae) to their sex pheromone components. Eur. J. Entomol. 104:187–194.

Grafen, A. 1990. Biological signals as handicaps. J. Theor. Biol. 144:517–546.

Grant, A. J., and R. J. O'Connell. 2000. Responses of olfactory receptor neurons in *Utetheisa ornatrix* to gender-specific odors. J. Comp. Physiol. A. 186:535–542.

Greenfield, M. D. 1981. Moth pheromones: an evolutionary perspective. Fl. Entomol. 64:4–17.

Groot, A., C. Gemeno, C. Brownie, F. Gould, and C. Schal. 2005. Male and female antennal responses in *Heliothis virescens* and *H. subflexa* to conspecific and heterospecific sex pheromone compounds. Environ. Entomol. 34:256–263.

Harari, A. R., A. M. Handler, and P. J. Landolt. 1999. Size-assortative mating, male choice and female choice in the curculionid beetle *Diaprepes abbreviatus*. Anim. Behav. 58:1191–1200.

Harari, A. R., P. J. Landolt, C. W. O'Brien, and H. J. Brockmann. 2003. Prolonged guarding behaviour and sperm competition in the weevil *Diaprepes abbreviatus* (L.). Behav. Ecol. 14:89–96.

Hendricks, D. E., and T. N. Shaver. 1975. Tobacco budworm: male pheromone suppressed emission of sex pheromone by the females. Environ. Entomol. 4:555–558.

Hirai, K., H. H. Shorey, and L. K. Gaston. 1978. Competition among courting male moths: male-to-male inhibitory pheromone. Science 202:644–645.

Honěk, A. 1993. Intraspecific variation in body size and fecundity in insects—a general relationship. Oikos 66:483–492.

Iwasa, Y., A. Pomiankowski, and S. Nee. 1991. The evolution of costly mate preferences. II. The "handicap" principle. Evolution 45:1431–1442.

Jaffe, K., B. Mirás, and A. Cabrera. 2007. Mate selection in the moth *Neoleucinodes elegantalis*: evidence for a supernormal chemical stimulus in sexual attraction. Anim. Behav. 73:727–734.

Johansson, B. G., and T. M. Jones. 2007. The role of chemical communication in mate choice. Biol. Rev. 82:265–289.

Johnstone, R. A., J. D. Reynolds, and J. C. Deutsch. 1996. Mutual mate choice and sex differences in choosiness. Evolution 50:1382–1391.

Jurenka, R. 2004. Insect pheromone biosynthesis. Topics Curr. Chem. 239:97–131.

Kehat, M., and D. Gordon. 1977. Mating ability, longevity and fecundity of the spiny bollworm, *Erias insulana* (Lepidoptera: Noctuidae), Entomol. Exp. Appl. 22:267–273.

Kokko, H., and P. Monaghan. 2001. Predicting the direction of sexual selection. Ecol. Lett. 4:159–165.

Kokko, H., and B. B. M. Wong. 2007. What determines sex roles in mate searching? Evolution 61:1162–1175.

Kotiaho, J. S. 2000. Testing the assumptions of conditional handicap theory: costs and condition dependence of sexually selected trait. Behav. Ecol. Sociobiol. 48:188–194.

———. 2001. Costs of sexual traits: a mismatch between theoretical considerations and empirical evidence. Biol. Rev. 76:365–376.

———. 2002. Sexual selection and condition dependence of courtship display in three species of horned dung beetles. Behav. Ecol. 13:791–799.

Kvarnemo, C., and L. W. Simmons. 1999. Variance in female quality, operational sex ratio and male mate choice in a bushcricket. Behav. Ecol. Soc. 45:245–252.

Landolt, P. J., and R. P. Heath 1990. Sexual role reversal in mate-finding strategies of the cabbage looper moth. Science 249:1026–1027.

Lemington, S. 1983. Social preference for partners carrying 'good genes' on wild house mice. Anim. Behav. 31:325–333.

Light, D. M., and M. C. Birch. 1979. Electrophysiological basis for the behavioural response of male and female *Trichoplusia ni* to synthetic female pheromone. J. Insect Physiol. 25:161–167.

Lim, H., and M. D. Greenfield. 2007. Female pheromonal chorusing in an arctiid moth, *Utetheisa ornatrix*. Behav. Ecol. 18:165–173.

Lim, H., K. C. Park, T. C. Baker, and M. D. Greenfield. 2007. Perception of conspecific female pheromone stimulates female calling in an arctiid moth, *Utetheisa ornatrix*. J. Chem. Ecol. 33:1257–1271.

Liu, Y.-B., and K. F. Haynes. 1994. Temporal and temperature-induced changes in emission rates and blend ratios of sex pheromone components in *Trichoplusia ni*. J. Insect Physiol. 40:341–346.

Ljungberg, H., P. Anderson, and B. S. Hansson. 1993. Physiology and morphology of pheromone-specific sensilla on the antennae of male and female *Spodoptera littoralis* (Lepidoptera: Noctuidae). J. Insect Physiol. 39:253–260.

Lloyd, J. E. 1979. Sexual selection in luminescent beetles. Pp. 293–342 *in* M. S. Blum and N. E. Blum, eds. Sexual selection and reproductive competition in insects. Academic Press, New York.

Lo'pez, P., L. Amo, and J. Martin. 2006. Reliable signaling by chemical cues of male traits and health state in male lizards, *Lacerta monticola*. J. Chem. Ecol. 32:473–488.

Löfstedt, C. 1990. Population variation and genetic control of pheromone communication systems in moth. Entomol. Exp. Appl. 54:189–218.

Löfstedt, C., N. J. Vickers, W. A. Roelofs, and T. C. Baker. 1989. Diet related courtship success in the oriental fruit moth, *Grapholita molesta* (Tortricidae). Oikos 55:402–408.

Lucas, J. R., and R. D. Howard. 1995. On alternative reproduction tactics in anurans—dynamic games with density and frequency-dependence. Am. Nat. 146:365–397.

Miller, J. R., and W. L. Roelofs. 1980. Individual variation in sex pheromone component ratios in two populations of the redbanded leafroller moth, *Argyrotaemia velutinana*. Environ. Entomol. 9:359–363.

Miller, J. R., T. C. Baker, R. T. Cardé, and W. L. Roelofs. 1976. Reinvestigation of oak leaf roller sex pheromone components and the hypothesis that they vary with diet. Science 192:140–143.

Moore, A. J. 1988. Female preferences, male social status and sexual selection in *Nauphoeta cinerea*. Anim. Behav. 34:1160–1165.

Moore, A. J., N. L. Reagan, and K. F. Haynes. 1995. Conditional signalling strategies—effect of ontogeny, social experience and social status on the pheromonal signal of male cockroaches. Anim. Behav. 50:191–202.

Moore, P. J., N. L., Reagan-Wallin, K. F., Haynes, and A. J. Moore. 1997. Odour confers status on cockroaches. Nature 389:25.

Noguchi, H., and Y. Tamaki. 1985. Conspecific female sex pheromone delays calling behaviour of *Adoxophyes* sp. and *Homona magnanima* (Lepidoptera: Tortricidae). Japanese J. Appl. Entomol. Zool. 29:113–118.

Noldus, L. P., and R. P. J. Potting. 1990. Calling behaviour of *Mamestra brassicae*—effect of age and photo-period. Entomol. Exp. App. 56:23–30.

Nur, N., and Hasson, O. 1984. Phenotypic plasticity and the handicap principle. J. Theor. Biol. 110:275–297.

Olsson, M., T. Madsen, J. Nordby, E. Wapstra, B. Ujvari, and H. Wittsell. 2003. Major histocompatibility complex and mate choice in sand lizards. Proc. R. Soc. Lond., B (Suppl.) 270:S254–S256.

Ono, T., R. E. Charlton, and R. T. Cardé. 1990. Variability in pheromone composition and periodicity of pheromone titer in potato tuberworm moth, *Phthorimaea operculella* (Lepidoptera: Gelechiidae). J. Chem. Ecol. 16:531–542.

Otter, C. J. de, A. P. Bruins, H. Hendricks, and S. C. A. Bijpost. 1989. Palmitic acid as the component of a male-to-male inhibitory pheromone of the summer fruit tortrix moth, *Adoxophyes orana*. Entomol. Exp. Appl. 52:291–294.

Otter, C. J. de, A. De Cristofaro, K. E. Voskamp, and G. Rotundo. 1996. Electrophysiological and behavioural responses of chestnut moths, *Cydia fagiglandana* and *C. splendana* (Lep.: Tortricidae), to sex attractants and odours of host plants. J. Appl. Entomol. 120:413–421.

Owens, I. P. F., and D. B. A. Thompson. 1994. Sex-differences, sex ratios and sex-roles. Proc. R. Soc. Lond. Ser. B 258:93–99.

Palaniswamy, P., and W. D. Seabrook. 1985. The alteration of calling behaviour by female *Choristoneura fumiferana* when exposed to synthetic sex pheromone. Entomol. Exp. Appl. 37:13–16.

Parker, J. 1983. Mate quality and mating decisions. Pp. 141–166 *in* P. Bateson, ed. Mate choice. Cambridge Univ. Press, New York.

Phelan, L. 1997. Evolution of mate-signaling in moths: phylogenetic considerations and predictions from the asymmetric tracking hypothesis. Pp. 240–256 *in* J. C. Choe and R. J. Crespi, eds. Mating systems in insects and arachnids. Cambridge Univ. Press, Cambridge, U.K.

Phelan, P. L., and T. C. Baker. 1986. Male size related courtship success and intersexual selection in the tobacco moth, *Ephestia elutella*. *Experientia* 42:1291–1293.

Raina, A. K., J. A. Klun, and E. A. Stadelbacher. 1986. Diel periodicity and effect of age and mating on females sex pheromone titer in *Heliotjhis zea* (Lepidoptera: Noctuidae). Ann. Entomol. Soc. Am. 79:128–131.

Rantala, M. J., R. Kortet, J. S. Kotiaho, A., Vainilla, and J. Suhonen. 2003. Condition dependence of pheromones and immune function in the grain beetle *Tenebrio molitor*. Funct. Ecol. 17:534–540.

Rivero, A., R. V. Alatalo, J. S. Kotiaho, J. Mappes, and S. Parri. 2000. Acoustic signalling in a wolf spider: can signal characteristics predict male quality? Anim. Behav. 60:187–194.

Roelofs, W., J. Kochansky, R. T. Cardé, H. Arn, and S. Rauscher. 1973. Sex attractant of the grape vine moth, *Lobesia botrana*. Mitt. Schweiz. Entomol. Ges. 46:72–73.

Ryan, M. J. 1988. Energy, calling and selection. Am. Zool. 28:885–898.

Saad, A. D., and D. R. Scott. 1981. Repellency of pheromones released by females of *Heliothis armigera* and *H. zea* to females both species. Entomol Exp Appl. 30:123–127.

Schlyter, F., and G. Birgersson. 1989. Individual variation in bark beetle and moth pheromones—a comparison and an evolutionary background. Holarctic Ecol. 12:457–465.

Schal, C., R. E. Charlton, and R. T. Cardé. 1987. Temporal patterns of sex pheromone titers and release rates in *Holomelina lamae* (Lepidoptera: Arctiidae). J. Chem. Ecol. 13:1115–1129.

Schneider, D., S. Schulz, E. Priesner, J. Ziesmann, and W. Francke. 1998. Autodetection and chemistry of female and male pheromone in both sexes of the tiger moth *Panaxia quadripunctaria*. J. Comp. Physiol. A 182:153–161.

Segoli, M., A. R. Harari, and Y. Lubin. 2006. Limited mating opportunities and male monogamy: a field study of white widow spiders, *Latrodectus pallidus* (Theridiidae). Anim. Behav. 72:635–642.

Shelly, T. E., and A. M. Dewire. 1994. Chemically mediated mating success in male oriental fruit flies (Diptera: Tephritidae). J. Entomol. Soc. Am. 87:375–382.

Sorenson, P. W., and N. E. Stacey. 1999. Evolution and specialization of fish hormonal pheromones. Pp. 15–47 *in* R. E. Johnston, D. Muller-Schwarze, and P. W. Sorenson, eds. Advances in chemical signals in vertebrates. Kluwer Academic/Plenum Press, New York.

Svensson, M. G. E., M. Bengtsson, and J. Lofqvist. 1997. Individual variation and repeatability of sex pheromone emission of female turnip moth *Agrotis segetum*. J. Chem. Ecol. 23:1833–1850.

Teng, Z.-Q., and Q.-W. Zhang. 2009. Determinants of male ejaculate investment in the cotton bollworm *Helicoverpa armigera*: mating history, female body size and male age. Physiol. Entomol. 34:338–344.

Thornhill, R. 1980. Mate choice in *Hyllobitacus apicalis* (Insecta: Mecoptera) and its relation to some models of female choice. Evolution 34:519–538.

Torres-Vila, L. M., M. C. Rodriguez-Molina, and J. Stockel. 2002. Delayed mating reduces reproductive output of female European grapevine moth, *Lobesia botrana* (Lepidoptera: Tortricidae). Bull. Entomol. Res. 92:241–249.

Torres-Vila, L. M., J. Stockel, and M. C. Rodriguez-Molina. 1997a. Physiological factors regulating polyandry in *Lobesia botrana* (Lepidoptera: Tortricidae). Physiol. Entomol. 22:387–393.

Torres-Vila, L. M., J. Stockel, P. Lecharpentier, and M. C. Rodriguez-Molina. 1997b. Artificial selection in pheromone permeated air increases mating ability of European grape vine moth *Lobesia botrana* (Lep. Tortricidae). J. Appl. Ent. 121:189–194.

Velando, A., R. Beamonte-Barrientos, and R. Torres. 2006. Pigment-based skin colour in the blue-footed booby: an honest signal of current condition used by females to adjust reproductive investment. Oecologia 149:535–542.

Walther, B. A., and D. H. Clayton. 2005. Elaborate ornaments are costly to maintain: evidence for high maintenance handicaps. Behav. Ecol. 16:89–95.

Webster, R. P., and R. T. Cardé. 1982. Relationships among pheromone titre, calling and age in the omnivorous leafroller moth *(Platynota stultana)*. J. Insect Physiol. 28:925–933.

Weissling, T. J., and A. L. Knight. 1996. Oviposition and calling behaviour of codling moth (Lepidoptera: Tortricidae) in the presence of codlemone. Ann. Entomol. Soc. Am. 89:142–147.

Witzgall, P., and B. Frerot. 1989. Pheromone emission by individual females of the carnation totrix *Cacoecimorpha pronubana*. J. Chem. Ecol. 15:707–717.

Witzgall, P., M. Tasin, H.-R. Buser, G. Wegner-Kib, V. S. M., Mancebon, C. Ioriatti, A.-C. Backman, M. Bengtsson, L. Lehmann, and W. Francke. 2005. New pheromone components of the grapevine moth *Lobesia botrana*. J. Chem. Ecol. 31:2923–2932.

Worden, B. D., P. G. Parker, and P. W. Papas. 2000. Parasites reduce attractiveness and reproductive success in male grain beetles. Anim. Behav. 59:543–550.

Wyatt, T. D. 2003. Pheromones and animal behaviour. Cambridge Univ. Press, Cambridge, UK.

Xiang, Y.-Y., M.-F. Yang, and Z.-Z. Li. 2010. Calling behavior and rhythms of sex pheromone production in the black cutworm moth in China. J. Insect Behav. 23:35–44.

Yang, M.-W., S.-L. Dong, and L. Chen. 2009. Electrophysiological and behavioral responses of female beet armyworm *Spodoptera exigua* (Hübner) to the conspecific female sex pheromone. J. Insect. Behave. 22:153–164.

Zahavi, A. 1977. Cost of honesty—(further remarks on handicap principle). J. Theor. Biol. 67:603–605.

Zala, S. M., W. K. Potts, and D. J. Penn. 2004. Scent-marking displays provide honest signals of health and infection. Behav. Ecol. 15:338–344.

Associate Editor: U. Candolin