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The role of chemical communication in sexual selection: hair-pencil displays in the diamondback moth, *Plutella xylostella*

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Theory suggests that if secondary sexual characteristics (or signals) are costly and females choose between mating partners, males should display more vigorously in the presence of competition. We investigated the use of chemical signals during courtship in the diamondback moth, *Plutella xylostella*. Males of *P. xylostella* have a hair-pencil gland on the tip of their abdomen, which allows for the release of chemical signals during courtship. Males detected potential competitors using chemical signals, but male investment in sexual signalling did not increase linearly in the presence of increasing numbers of competitors. The patterns of male display and mating behaviour among the parental population were not necessarily replicated among the next generation of males. Males of the parental generation that were exposed to one male competitor were more likely to display their hair-pencil than males exposed to zero or two competitors, but the display behaviour of the next generation of males (while higher overall) did not vary significantly across these treatments. Furthermore, males of the parental generation that acquired matings displayed their hair-pencil at a higher rate than males that did not obtain matings; again this pattern was not evident among the next generation of males. Finally, sons tended to be more likely to display their hair-pencil, and were significantly more likely to achieve a successful mating, than the previous generation of males. These intergenerational patterns suggest that the tendency to achieve copulations may have a genetic component.

The evidence that females use male secondary sexual characteristics, or signals, to influence their choice of mating partner (e.g. Darwin 1871) derives primarily from investigations into the role of visual and acoustic signals (Andersson 1994; Kelley 2004; Bennett & Thery 2007). Males of some species can rapidly adjust the intensity of their signal, often by varying the frequency with which it is delivered: for example, the speed of drumming in wolf spiders (Rivero et al. 2000), or the frequency and/or volume of vocalizations (e.g. McComb 1991). Male signals are typically costly to produce, and thus display frequency may provide a measure of male attractiveness. Accordingly, males may display to females more frequently or more vigorously when in the presence of rival males. For example, male midwife toads, *Alytes obstetricans*, increase their calling rate (Bosch & Marquez 1996) and male fowl, *Gallus gallus*, court more frequently (Wilson et al. 2009) in the presence of an intruder. While males may increase signal investment in the presence of rivals to increase the likelihood of copulating with the choosier female, more vigorous displaying may also reduce the duration of courtship and thus the probability of male interference. It is not known whether an increase in the vigour or frequency of display necessarily increases linearly with the number of male competitors; the display rate will depend upon the benefits, through copulation success, and the costs arising from signal production and/or the risk of predation. Additionally, there may be a trade-off between investment in signalling towards females and male-directed signals associated with male–male competition (see Berglund et al. 1996). Finally, rivals may actively inhibit male courtship signalling behaviour (e.g. Hirai et al. 1978; Aragon 2009).

Chemical signals, or pheromones, are arguably the most widespread form of communication (Wyatt 2003), yet their role in sexual selection is surprisingly poorly understood (Johansson & Jones 2007). Sex pheromones are usually produced by females to convey information to males about their locality, and were first reported by Fabré (1911) in the emperor moth, *Imbrasia belina*. However, these signals may also indicate the quality of a potential mating partner by providing information about reproductive history, fertility and genetic compatibility (see Johansson & Jones 2007). Much of our understanding of the evolution of pheromones comes from studies of Lepidoptera and Diptera (see Symonds & Elgar 2008), although the main focus of these studies was to

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identify the chemical structure and role of female-released, long-range mate and species recognition pheromones (Svensson 1996; Ptacek 2000). In contrast, the role of male-produced sex pheromones and their associated behaviours are poorly understood, and little is known about their role in either female choice or male–male competition (e.g. Johansson et al. 2005). Furthermore, the heritability of chemical signals that may have a role in sexual selection has rarely, if ever, been investigated, unlike visual or acoustic signals (e.g. Alatalo et al. 1997; Johansson & Jones 2007).

In some moths, males posses a hair-pencil gland located towards the tip of the abdomen that is everted during courtship (Birch et al. 1990). This gland contains many long, thin filaments that, when everted, allow for the evaporation of the male short-range sex pheromone (Birch 1970). It is common for males to beat their wings when they evert their hair-pencil to disperse the pheromone. The significance of hair-pencils and other organs associated with the release of male sex pheromones has not been extensively investigated. Hillier & Vickers (2004) suggested that the odours released from the hair-pencil gland of male tobacco budworms, *Heliothis virescens*, are associated with female mating preferences. However, their experiment could not distinguish between the combined effects of male behaviour, hair-pencil morphology and chemical signals.

The diamondback moth, *Plutella xylostella* (Plutellidae), is an excellent model system to explore the use of male sex pheromones in sexual selection. First, *P. xylostella* are an abundant agricultural pest worldwide (Talekar & Shelton 1993), often occurring in high densities and with limited adult dispersal within crop fields (Mo et al. 2003), thereby creating the potential for high levels of male–male competition (e.g. McLain 1982; Cade & Cade 1992; see also Head et al. 2007). Second, females of *P. xylostella* are largely monandrous, with only 20% of females remating (Wang et al. 2005), thereby ensuring that their opportunities to exercise mate choice are mainly confined to the preinsemination stage. Third, the chemical characteristics of female-derived sex pheromones, and the responses by males and females to these odours, have been extensively investigated (e.g. Dai et al. 2008). Finally, males of *P. xylostella* have a paired tuft hair-pencil gland (Justus & Mitchell 1999), which is everted (displayed) only in the presence of females (L. Davie, unpublished data), suggesting that males may use short-range sex pheromones during courtship. Whether male hair-pencil display behaviour is modified in the presence of other males is untested. Moreover, the reproductive biology of *P. xylostella* in general has not been extensively investigated.

We investigated the role of male–male competition in hair-pencil display behaviour, by exposing courting males and females to social environments that differed in the number of potential male rivals. We also compared the variation in male display behaviour across two generations.

METHODS

Culturing of Stock

Larval stages of *P. xylostella* were collected from the western suburbs of Melbourne during March and April of 2008. Larvae were obtained from wild *Brassica* (Brassicaceae) bushes by tapping the bush and collecting fallen larvae. Larvae were then reared at 24 °C (±1 °C), 45% (±5%) humidity with a 12:12 h light:dark regime. Larvae were kept in communal larval containers (17 × 11.5 cm and 4 cm high) and fed an ad libitum diet of *Brassica* leaves. Once larvae pupated, all parasitized pupae (Talekar & Yang 1991) were removed and discarded. Three cultures were established, using adults reared from the field-captured larvae. All cultures containing both larvae and adults were maintained in plastic breeding containers (17 × 14 cm and 14 cm high) with one side removed and replaced with fine gauze to allow for air transfer. Larvae were provided with fresh *Brassica* leaves (replaced every second day), and adults were provided with 10% honey solution (honey, 10%; ascorbic acid, 0.6%; sorbic acid, 0.1%; methyl paraben, 0.1%). After 2 weeks, all leaves and larvae were removed from the breeding container and placed in communal containers (17 × 11.5 cm and 4 cm high).

Containers were cleaned every day to remove moisture and fresh *Brassica* leaves were added. Pupae were removed from the communal containers and placed in separate 5 ml vials to control for adult mating status. Pupae were checked daily and, on eclosion, adults were supplied with 10% honey solution. All moths not required for experiments were returned to the breeding cultures. Adults used in experiments had eclosed between 24 and 36 h previously, thereby allowing any further maturation. Adults were weighed 1–3 h before trials commenced using a digital scale to four decimal places (mean weight ± SE of males = 2.49 ± 0.040 mg; females = 3.17 ± 0.070 mg).

Effects of Male Competitors on Male Courtship Displays

We tested the effect of male competition on courtship behaviour of virgin males by assigning males to one of three treatments that varied the potential for male competition. In each treatment, the test male was placed in a 30 ml vial with a randomly selected virgin female. This vial was then placed above another 30 ml vial with fine gauze separating the two vials. In the first treatment, the second vial remained empty as a control to examine male courtship behaviour in the absence of competitors. In the second treatment, one virgin male competitor was placed in the second vial to simulate low levels of male competition, while in the third treatment two virgin male competitors were placed in the second vial to simulate high levels of male competition. Test individuals had no previous exposure or contact with any conspecific individuals prior to the trials; however, competitor males were occasionally reused the following day owing to low numbers of males available in the culture. The gauze permitted diffusion of olfactory signals and limited visual contact, but prevented physical contact between test subjects and their competitors. Each treatment had 40 replicates.

Trials began 1 h after the onset of scotophase and ran for 30 min or until mating occurred. The time until a male began displaying his hair-pencil, the number of hair-pencil displays and the time until mating (if it occurred) were recorded. Hair-pencil displays varied in duration, and in the absence of any knowledge of the biological significance of this variation, we categorized these displays as 'long' (>2 s) or 'short' (<2 s) to identify any different functions of the duration of the display. The hair-pencil displays could be unambiguously categorized because the 'long' displays were typically much longer than 2 s. There were also qualitative differences between short and long hair-pencil displays: males engaging in long hair-pencil displays commonly turned in circles, a pattern that was not observed in short hair-pencil displays. As a result, males performing long hair-pencil displays were much more conspicuous than males performing short hair-pencil displays. It is likely that the costs, in terms of time and energy, differ between the two kinds of displays.

After trials were completed, competitor males were removed and discarded. Test males were kept isolated overnight with the same female to allow for mating to occur. These males were then killed and preserved in ethanol for later dissection. The following day, experimental females were placed in individual oviposition plastic containers (as above) with fresh *Brassica* leaves and 10% honey solution and allowed to oviposit. After 3 days, females were transferred to new oviposition containers with fresh leaves and 10%