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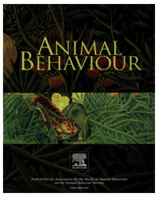

Mating success is predicted by the interplay between multiple male and female traits in the small hairy maggot blowfly

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Intersexual selection drives the evolution of reproductive traits, including ornamentation and courtship behaviour. However, most studies attempting to explain the relationships between reproductive traits are oversimplified because traits are evaluated independently, and assessment of mate choice is restricted to one sex. Further research is required to understand the extent to which interactions between traits influence mating decisions in both sexes. The small hairy maggot blowfly, *Chrysomya varipes*, offers an ideal opportunity to examine how interactions between foreleg ornamentation (males have conspicuous white and black hairs, whereas females have only black hairs) and complex courtship behaviour influence mating success in males and females. This study involved mate choice trials between single females and males that varied in foreleg ornamentation. Courtship investment was quantified for males, and ornamentation and body size were quantified for both sexes. Mating success was predicted by time invested in courtship, but not by either male and female ornamentation or body size alone. However, there was a significant interaction between courtship investment, ornamentation and body size. At any given courtship investment, males with more ornamentation had an increased probability of mating success. Females with more ornamentation also had an increased probability of mating success, because males invested more courtship in more ornamented females. Furthermore, the lightest males had an increased probability of mating success compared to the heaviest males, as lighter males invested more in courtship. Female weight, however, did not significantly affect the probability of mating. The results suggest that male foreleg ornamentation in *C. varipes* acts as an amplifier of the visual courtship display, adding to a small but growing body of evidence that multiple male and female traits can interact to influence mating decisions.

Intersexual selection (mate choice) leads to variation in reproductive success, thereby driving the evolution of traits such as elaborate ornaments and intensive courtship displays (Andersson, 1994; Darwin, 1859; 1871). However, the importance of male secondary sexual traits, and their link with increased acceptance by females, is still heavily debated because data from female mate choice studies are often contradictory. Although there is a large body of evidence that females can prefer individual male traits (Collins & Luddem, 2002; Longpre, Koepfinger, & Katz, 2011; Loyau, Petrie, Saint Jalme & Sorci, 2008; Petrie, 1994), these studies have almost invariably been based on experiments designed to test variation in one trait alone. However, elaborate sexual ornaments often accompany complex courtship displays, making it likely that selection acts on interactions between both of these traits (Candolin, 2003). It has therefore been argued that empirical studies have provided conflicting results because the influence of sexually selected traits is evaluated independently of other traits (Hunt, Breuker, Sadowski, & Moore, 2009). Recent behavioural studies have shown that females evaluate a number of different male traits, and that the interaction between traits may obscure preferences for single traits (Hebets, Stafstrom, Rodriguez, & Wilgers, 2011; Kekäläinen, Valkama, Huuskonen, & Taskinen, 2010; Kodric-Brown & Nicoletto, 2001; Lehtonen, Rintakoski, & Lindström, 2007; Patricelli, Uy, & Borgia, 2003). These studies indicate that selection by female choice acts on multiple male traits, highlighting the value of inclusive analyses (incorporation of multiple traits) in mate choice studies.

Theoretically, preferences for multiple traits may arise through a specific preference selecting for multiple traits or through multiple preferences each of which coevolves with a trait (Brooks & Couldridge, 1999). Furthermore, selection may operate on different traits either in concert or antagonistically. Interactions between traits may in fact occur in several ways. First, the attention

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a potential mate pays to one trait could depend on the expression of another trait (Candolin, 2003). For example, one trait may need to exceed a threshold value before the receiver pays attention to another trait (sequential interaction). Second, one trait may amplify the message of another. In particular, courtship displays may expose and amplify ornamental traits and vice versa (Hasson, 1989). Third, one trait may influence the cost and expression of another trait (Johnstone, 1996). For example, a large body size (which is often used to discriminate between mates) makes an individual more conspicuous and therefore increases predation risk during courtship (Reynolds, 1993).

Although empirical studies are beginning to explore the interaction between multiple reproductive traits more deeply, some of these studies have been unable to find evidence for the interplay between multiple traits and its effect on mate choice (e.g. Birkhead, Fletcher, & Pellatt, 1998; Shamble, Wilgers, Swoboda, & Hebets, 2009; Sheldon & Ellegren, 1999). One explanation for divergent findings may be that most studies investigating the effects of multiple male traits have ignored both the synchronous effect of multiple female traits and the effects of male mate choice. There is now considerable theoretical and empirical evidence to indicate that males show mating preferences when the costs of mating are high (Wong & Jennions, 2003) or when females vary greatly in either fecundity (often indicated by body size) or genetic quality (Bonduriansky, 2001; Edward & Chapman, 2011). As such, male mate choice should be factored into studies investigating the evolution of ornamentation and courtship behaviour. Another explanation for divergent findings may be that many of the model systems that have been studied are complicated. Specifically, most model systems potentially have various mechanisms underpinning female choice (e.g. direct material benefits and genetic benefits). It would be fruitful for further research to focus on systems in which the number of mate choice mechanisms in operation is limited. These types of systems may allow the relative influence of each trait, and their interactions, to be more easily evaluated.

The small hairy maggot blowfly, *Chrysomya varipes*, provides an excellent model for experimentally investigating how mate choice is influenced by the interaction between ornamentation and courtship. *Chrysomya varipes* is endemic to and widespread throughout Australia and adjacent Pacific islands. It is an obligate inhabitant of animal carcasses, in the vicinity of which it accumulates and engages in courtship behaviour prior to mating. Males possess striking ornamentation in the form of white hairs on their forelegs, which are used as part of their highly stereotyped courtship behaviour (Norris, 1959). Furthermore, males do not force mating, and only provide females with sperm, so any benefits of female mate choice will only come through indirect genetic mechanisms. In regard to male mate choice, females do not provide any maternal care, so males might only benefit by discriminating against (1) small females of low fecundity, (2) nonvirgin females that represent a high risk of sperm competition or (3) genetically inferior or incompatible females.

The general aim of this study was to determine the interaction between multiple sexually selected traits, and their influence on mating success in both male and female *C. varipes*. The specific aim was to test the hypothesis that the probability of mating success will be affected by an interaction between male courtship investment, ornamentation and body size in both sexes.

METHODS

Fly Collection and Culturing

Flies were trapped using a 'Western Australian' trap (Vogt & Havenstein, 1974) between 0900 and 1600 hours on 7 and 9 January 2013, at the University of Wollongong, Australia. Following capture, flies were kept at a temperature of 23 °C (±1 °C) with a 12:12 h light/dark cycle in a constant temperature room in the University's Ecological Research Centre. Adult flies were maintained in plastic cages (300 × 500 mm and 250 mm high) with a fly screen lid. They were provided with a constant supply of granulated raw sugar, and water delivered via a dental wick. They were also provided with a small portion (ca. 100 g) of kangaroo mince (Luddenham Pet Meats, Sydney, Australia) held in a plastic weigh boat, as both a food source and an oviposition medium. After eggs were laid, the meat was removed and isolated in a plastic rearing container (130 × 190 mm and 70 mm high) with a fine mesh top. The bottom of the container was covered with wheaten chaff as a pupation substrate, and the weigh boat containing the maggots was placed on top of the chaff. Extra kangaroo mince (ca. 200 g) was given to the maggots to ensure that food was not limiting. Upon pupation, the rearing container holding the pupae was relocated into a larger cage, permitting the free movement of adult flies after their eclosion. The flies were sexed within 24 h of emergence and males and females kept in separate cages (300 × 500 mm and 250 mm high) to ensure that all flies used in experiments were virgins.

Mating Experiments

To quantify courtship behaviour, mating trials were conducted in a standardized experimental arena (described below). The F1 generation was used in all mating experiments, during which virgin flies were aged 12 (±2) days. The behaviour of flies was recorded with a CCTV recording system and eight Samsung SCB-2000P digital cameras under standard laboratory light and temperature conditions (23 ± 1 °C). All trials took place between 1000 and 1700 hours on 29, 30 and 31 January 2013. Male and female flies (168 of each sex) were randomly selected and placed in mixed-sex pairs into a sealed transparent petri dish (85 × 15 mm) containing approximately 0.5 g of kangaroo meat positioned centrally. Kangaroo meat was provided because in nature females typically aggregate around the carcasses of vertebrates, such as kangaroos, and the meat also provided the flies with moisture to prevent their desiccation. Food did not appear to alter courtship behaviour, as a pilot study revealed that males were equally likely to display in the presence or the absence of meat (all $P > 0.05$; data not shown). Behavioural trials ran until mating occurred, or for a maximum of 20 min. Eight behavioural trials ran concurrently, with each trial recorded using a separate video camera. The experimental arenas were surrounded with cardboard barriers to ensure that the flies in each trial were unable to see each other and interact with their neighbours.

Behavioural Analysis

Videorecorded mating trials were analysed using the behavioural analysis software package EthoLog 2.2 (Ottoni, 2000). Only trials in which courtship behaviour occurred ($N = 73$) were used for analysis. For each experimental replicate, the behaviour of the male was described and quantified from the time of courtship initiation until mating (or until 20 min, whichever occurred first). Each courtship behaviour (categories given in Table 1) was quantified as the proportion of time spent by the male in that behaviour from the time of first courtship until mating (or completion of the trial). Total courtship investment (hereafter simply referred to as 'courtship investment') was calculated as a fraction of the observation period (i.e. 20 min or until mating) and was inclusive of all courtship behaviours. Males that spent proportionally more time courting were defined as having a higher courtship investment (Ejima & Griffith, 2007). Following mating trials, males and females were immediately euthanized via freezing and then preserved in 70% ethanol.