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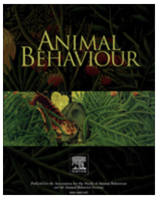

# Male mate choice by chemical cues leads to higher reproductive success in a bark beetle

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Darwin's sexual selection theory predicts that males should compete to gain access to females, while females should choose the best possible male. However, males can also be choosy, especially in monogamous mating systems with female-biased sex ratios and biparental care. A few studies show that in systems in which males are highly selective, chemical cues can lead to mating of individuals with high reproductive success. Using *Dendroctonus valens*, a monogamous bark beetle with typical biparental care, and Y-tube olfactometers, we provided males with a choice between two females and found strong evidence of odour-based male mate preference. We then tested whether this preference led to successful matings and if it had consequences for courtship displays and offspring fitness. Male and preferred female pairs had less aggressive courtship interactions, longer copulation times, constructed galleries faster, and had cleaner galleries. Preferred females had faster oviposition rates and larval development. Our study shows that, in *D. valens*, males choose females based on chemical cues and that the resulting male–female pairs have superior reproductive success.

Since Darwin (1871), sexual selection has been divided into intersexual selection (mate choice by one sex) and intrasexual selection (competition by the same sex for access to mates). In intersexual selection, the sex with higher investment in reproduction must be choosy (Andersson 1994), and in many cases females fulfil this role (i.e. conventional sex roles) (Berglund et al. 2005). Female mate choice is more likely to evolve because the number of offspring a female produces increases either minimally or not at all with the number of matings she achieves (Bonduriansky 2001). Male mate choice, nevertheless, does not evolve as easily as female mate choice (Bonduriansky 2001; Clutton-Brock 2007). In male mate choice, the number of offspring a male produces increases in proportion to the number of matings he achieves (Bonduriansky 2001). Under certain conditions, males are expected to discriminate carefully among alternative potential mates. These include limited opportunities to obtain additional matings (e.g. monogamy), high parental investment (e.g. parental care), low effort required to find mates (e.g. several potential mates are encountered simultaneously), and high variation in female quality (Bonduriansky 2001; Barry & Kokko 2010).

Successful mate selection requires efficient sensorial and behavioural mechanisms, including highly evolved vision systems for detection of specific colour patterns, elaborate sexual rituals and chemical cues (Andersson 1994). Chemical cues play an important role in mate choice in many taxa (Johansson & Jones 2007). In insects, the use of pheromones is almost ubiquitous and researchers have typically emphasized the role of pheromones as sex attractants (Lewis 1984; Roitberg & Isman 1992; Morgan 2004). It is often assumed that the use of chemical cues only leads to simple discriminations, for example, to determine whether another individual is a conspecific or a heterospecific (species recognition), or either a male or a female (mate recognition). However, a growing body of evidence indicates that chemical cues may be used by males or females to derive complex information about potential mates (Johansson & Jones 2007; Coleman 2009). Insect chemical cues have been shown to provide information regarding size, body condition, parasite load, immunocompetence, familiarity, reproductive status and degree of relatedness (Johansson & Jones 2007). Despite this, there is little evidence showing that, in systems in which males are highly selective, interindividual variation in chemical cues allows assessment of potential mates with potential consequences for reproductive success.

Chemical communication has long been recognized in bark beetles (Sauvard 2004; Blomquist et al. 2010). Some evidence suggests that recognition of chemical signals is probably the first

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cue to bring males and females together (Wood 1982; Blomquist et al. 2010) but there is no experimental evidence on the effects of chemical cues on male reproductive decisions in bark beetles.

The red turpentine beetle, *Dendroctonus valens*, native to North America and introduced to China in the early 1980s, is one of China's most destructive invasive pests of pine forests killing over 500 000 km$^2$ of Chinese oil pines, *Pinus tabulaiformis* (Yan et al. 2005). To overcome pine's natural defence mechanisms, *D. valens* attacks in large numbers (Smith 1971). Although the sex ratio of *D. valens* in natural populations is nearly 1:1, a skewed operational sex ratio (i.e. ratio of sexually active females to males; Emlen & Oring 1977) occurs during a mass attack, which varies from being female biased in the initial attack phase, to unbiased with an occasional male bias at the end of the attack phase (H. F. Chen & J. H. Sun, unpublished data). *Dendroctonus valens* is monogamous (i.e. the male pairs with only one female) and is characterized by a high level of cooperative biparental care (Smith 1971; Kirkendall 1983; Kirkendall et al. 1997; Liu et al. 2006). Females initiate boring through the outer bark of pines and excavate a small, circular nuptial chamber in the phloem (H. F. Chen & J. H. Sun, personal observation). Pheromones released by the female in the nuptial chamber typically attract a male within 3–5 days of colonization (Smith 1971; Liu et al. 2008). After excavating at least 2–3 cm of gallery, the pair begins to excavate a wider gallery in which eggs are oviposited (egg gallery). Egg gallery length has a positive correlation with offspring fitness because, as shown in other bark beetles, longer egg galleries give each newly hatched larva a larger area of fresh phloem on which to feed (Robins & Reid 1997; Pureswaran et al. 2006). Larvae of each family feed gregariously, that is, side by side in an irregular line, steadily moving forwards into fresh phloem (Smith 1971). Within a tree, multiple galleries occur and, often, neighbouring larval tunnels from different families meet. As a result, larval competition for phloem is fierce; in fact, this has been documented as an important fitness factor in bark beetles, including *D. valens* (Beaver 1974; Anderbrant et al. 1985; Anderbrant & Schlyter 1989; Kirkendall 1989).

An additional factor that plays a major role in all the aforementioned ecological interactions in *D. valens* is chemical communication. Recent studies reported that the hindguts of *D. valens* males and females contain five compounds (cis- and trans-verbenol, myrtenal, myrtenol and verbenone) and documented their effects on the beetle's colonization behaviour (Zhang & Sun 2006; Zhang et al. 2006, 2009; Liu et al. 2008). Furthermore, there is evidence suggesting that *D. valens* males may use chemical cues for mate recognition (Liu et al. 2006).

Given the importance of chemical cues in the mating system in *D. valens*, the occurrence of male mate choice, modulated via female chemical cues, would not be surprising. If evolution has favoured this type of sexual selection in *D. valens*, male–female pairs formed through this mechanism are likely to have fitness benefits. To test this hypothesis, we performed laboratory experiments to determine (1) whether males select females using chemical cues, (2) whether males' odour-based preference for females leads to successful matings, including consequences for courtship displays, and (3) whether the offspring of females selected by their odour have higher fitness.

## METHODS

### Study Subject

Laboratory experiments were conducted from 8 May to 30 June 2010 in a natural forest of *P. tabulaiformis* at the Beishe Mountain near the foot of the Luliang Mountains (37°48′N, 111°44′E; average elevation 1400 m). The onset of experiments coincided with the spring dispersal flight of newly emerged adult beetles (i.e. virgin females and males). In this period, which lasts 15 days, newly emerged virgin females fly to find a suitable tree to make galleries. To evaluate the condition of females, we dissected 132 field-collected females to determine presence of sperm in the spermathecal sac (details on this procedure are reported by Lissemore 1997). Only 3.8% (5/132) of females were mated. Beetles were collected daily from traps baited with *D. valens* kairomone lure (+)-(3)-carene. Sexes were separated based on the stridulation of males (Lyon 1958). Beetles were used within 2 days after capture. Prior to experiments, body length (anterior tip of head to posterior end of elytra) of each beetle was measured with an accuracy of 0.02 mm using a Vernier calliper. To factor out potential size effects, male mean body length was estimated ($6.72 \pm 0.06$ mm, $N = 427$) and males whose size fell within the mean ± SE were used.

### Experiment 1: Mate Choice with Live Females and Frass

Females collected from field traps were individually placed in labelled transparent plastic centrifuge tubes (12.5 cm height × 2.2 cm diameter). Five pieces of fresh phloem (each piece $11 \times 0.5 \times 0.3$ cm) were placed inside each tube for 36 h to simulate natural conditions of attack. This also allowed us to obtain female frass (i.e. faeces + phloem bore-dust) for our experiments. Female frass contains female-produced male volatile attractants (Liu et al. 2006; Shi & Sun 2010). To test male mate choice based on chemical cues alone, we carried out an experiment with two phases: phase 1 (tests with live females) and phase 2 (tests with frass produced by females from phase 1).

In phase 1, male preference for females was assessed using a glass Y-tube olfactometer, consisting of two female chambers, each being at the end of one arm, and one male start area at the beginning of the stem (Fig. 1). Wire-mesh walls (mesh size 2 mm) were placed in the female chambers to restrain the female to that area of the olfactometer. Unidirectional airflow was passed through the female chambers, at 150 ml/min, so male beetles had to walk upwind; air was filtered with activated carbon before entering the apparatus (Fig. 1). To factor out visual cues, the whole set-up was covered with a black blanket. Prior to each trial, females were first placed in the female chambers for 30 min for acclimatization before we placed the male in the male start area (Fig. 1). Males were given 10 min to make a decision after being released. We deemed that a choice was made when a male walked at least halfway into the chosen arm. Chosen females were labelled as P (preferred) and nonchosen females were labelled as NP (nonpreferred). Trials in which a male stayed in the male start area or did not walk into an arm further than halfway were discarded. Females were placed in either chamber (i.e. left or right) of the Y-tube randomly. Y-tubes were cleaned with acetone and dried for 5 h, to remove all possible chemical cues, before each trial. The whole set-up was maintained at $27 \pm 3$ °C and $60 \pm 12$% relative humidity during trials. A total of 181 trials were performed in this experiment. After phase 1, each individual was placed separately in a labelled vial with fresh phloem pieces for 48 h before phase 2.

To verify that male mate choice was based on chemical cues alone, we carried out phase 2 using the same set of beetles (one male and two females) in which the male had successfully made a choice in phase 1. Phase 2 was performed with the same Y-tube olfactometers used in phase 1, except that the females' frass was used instead of the females. In phase 2, frass was placed in opposite orientation (i.e. left or right) compared to the position of the female donor in phase 1. We used $100 \pm 5$ mg of frass from each female. We only used frass collected no more than 1 h prior to each trial. A total of 148 trials were conducted in phase 2.