Pollinator attractiveness increases with distance from flowering orchids

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Orchids are extraordinary among plants because many species are pollinated through sexual duplicity by producing flowers that mimic female insects to lure unsuspecting males. Previous work showed that sexual deception by the orchid *Chiloglottis trapeziformis* can have a negative impact on its wasp pollinator *Neozeleboria cryptoides*. We report that female wasps may be capable of mitigating the cost of the orchids' deception. Although male wasps quickly habituated to areas planted with unrewarding flower decoys, we found that the effectiveness of the chemical cue used by the wingless females to attract males increases with increasing distance from an orchid patch. The apparent specificity of the males' site-based avoidance strategy means that females emerging in areas occupied by flowering orchids could, potentially, leave the orchid colony by walking to increase their attractiveness.

Keywords: pseudocopulation; pollination; thynnine; sexually deceptive orchid; reproductive mimicry

1. INTRODUCTION

Orchids are unique among plants in their use of sexual duplicity to achieve pollination (Nilsson 1992). The intended target of the orchids' sexual deception is usually a male insect (i.e. the 'operator'), which is lured to the flower (the 'mimic') through mimicry of the female (the 'model') (Wiens 1978). Although visual and tactile cues often play an important role in mediating the pseudocopulatory behaviour of the male needed to achieve pollination (Bergström 1978), chemical mimicry of the female sex pheromone is paramount in the orchids' sexual masquerade. This is because chemical mimicry is solely responsible for the long-range attraction of males to the flower (Kullenberg 1961; Peakall 1990; Schiestl *et al.* 1999). Most studies investigating the impact of sexually deceptive orchids on their pollinators have tended to focus on the behaviour of the males that are fooled. However, Wong & Schiestl (2002) suggested, using an Australian orchid–wasp association, that male and female insects can each be affected by the orchids' deceit.

The thynnine wasp, *Neozeleboria cryptoides* (Smith) is the sole pollen vector for *Chiloglottis trapeziformis* Fitz, a sexually deceptive orchid from southeastern Australia.

Like other thynnines (Ridsdill Smith 1970*a,b*; Alcock 1981; Alcock & Gwynne 1987; Peakall 1990), females are wingless and are dependent exclusively upon the use of pheromones (i.e. 'call') to attract flying males (Oakwood 1990; Wong & Schiestl 2002). In this regard, competition among males is intense with the operational sex ratio strongly biased towards males, and males respond rapidly to calling females (Alcock 1981; Alcock & Gwynne 1987; Peakall 1990; Wong & Schiestl 2002). Apart from the actual mating, female wasps also rely on males to carry them to their food source and to return them to a suitable oviposition site (Given 1954; Peakall 1990). Male wasps are incapable of discriminating between the chemical cues of female wasps and sexually deceptive orchids (Wong & Schiestl 2002). However, males habituate rapidly to unrewarding flower decoys and avoid areas occupied by orchids (Peakall 1990). Wong & Schiestl (2002) demonstrated that this could have a potentially negative impact on females attempting to attract mates in areas occupied by orchids. Specifically, females in the presence of orchids elicited fewer male approaches and no copulation attempts compared with when females were attempting to attract mates in the absence of orchids.

The demonstration of a negative impact on the pollinator is an important requirement for an evolutionary arms-race scenario between plant and pollinator (Stowe 1988). However, Wong & Schiestl (2002) suggested that the orchid's impact on the pollinator could also depend on other factors, such as the size and density of orchid colonies, the degree of overlap in the distribution of orchid and pollinator, the timing and frequency of flowering, as well as the flight time of the pollinator. In particular, they alluded to the possibility that a wingless female may be able to mitigate some of the negative consequences of sexual deception by walking out of areas occupied by orchids. Female thynnines have been observed walking up to 2 m above ground in a single bout and can also burrow horizontally for several centimetres below the surface (Ridsdill Smith 1970*b*). Moreover, anecdotal evidence suggests that females will move from a location if unsuccessful in their attempts at attracting a mate from a particular calling site (F. P. Schiestl and B. B. M. Wong, personal observations). The ability to mitigate the orchids' impact by walking out of an orchid patch, however, assumes that the attractiveness of the female sex pheromone increases with distance away from the orchid colony. The current study aims to test this assumption.

2. MATERIAL AND METHODS

All experimental work was carried out from September to October 2002 in a 5 ha patch of open *Eucalyptus* woodland in Canberra, Australia. The orchid, *C. trapeziformis*, did not occur naturally at the study site but the wasp pollinator, *N. cryptoides*, was common. Male wasps patrolled the wooded area for females, and copulating pairs visited *Eucalyptus* trees to feed on honeydew excreted by scale insects. The sex pheromone used by female wasps consists of only a single compound and is identical to the allomone produced by the orchid to mimic the chemical signal of female wasps (Schiestl *et al.* 2003). We used 10 μl of a synthetically available analogue applied onto a black bead to simulate a female wasp. The quantity of synthetic 'pheromone' used parallels that produced by real females, and was recently found to be equally attractive to male wasps as the sex pheromone of the female itself (Schiestl *et al.* 2003). Orchid flowers for experimental work were brought in from another area.

Orchids were introduced to the study area to simulate a colony of flowering plants (Wong & Schiestl 2002). The colony consisted of four flowers. Within each colony, individual flower stems were placed into separate water-filled plastic tubes set into the ground, with flowers spaced 20 cm apart in a circular grouping (Wong & Schiestl

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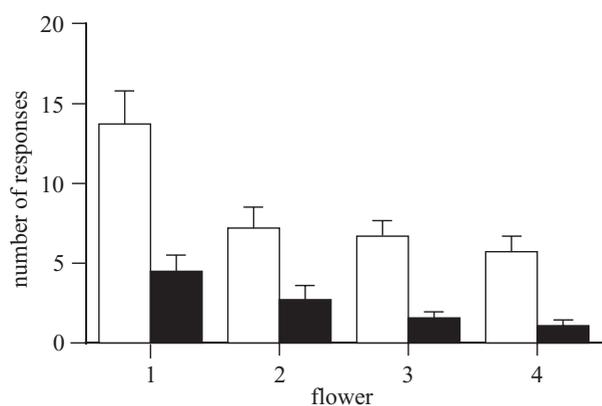
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Figure 1. Response of male wasps to orchid patches with increasing number of orchids (mean + s.e.) (open bars, approaches; filled bars, landings); $n = 13$.
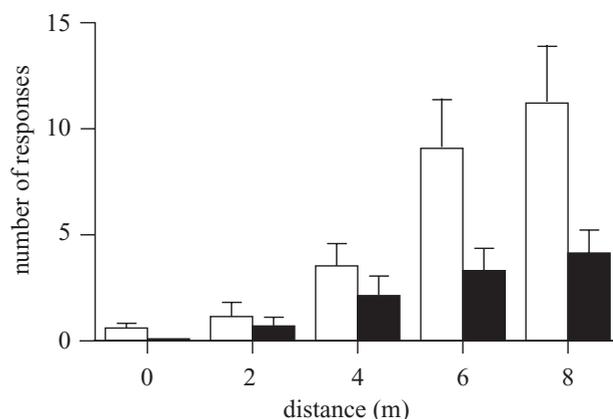

Figure 2. Response of male wasps to the 'dummy' female wasp with increasing distance from the centre of the orchid patch (mean + s.e.) (open bars, approaches; filled bars, landings); $n = 13$.

2002). Flowers were introduced one at a time at intervals of 2 min, and we recorded the number of male approaches and landings elicited by the newly placed flower (Wong & Schiestl 2002). Colonies were left for 2 h before the commencement of any testing with 'dummy' females. For each orchid patch, we tested the attractiveness of the dummy female five times, once each at distances of 0, 2, 4, 6 and 8 m from the centre of the colony. Each test was randomized with regard to both order and direction relative to the centre of the colony. Each test lasted for 2 min. During this time, we counted the number of male approaches and landings on the bead (Wong & Schiestl 2002). In total, we tested 13 dummies in 13 colonies, with colonies spaced at least 50 m apart; a new dummy female was used for each colony of orchids.

Data were analysed using the statistical package SPSS (Norusis 1993). We used Friedman tests to look for differences in the number of approaches and landings in response to increasing number of orchids. Friedman tests were also used to examine the number of approaches and landings in response to female dummies at different distances from the orchid patch.

3. RESULTS

When orchid flowers were offered to males sequentially, the number of male wasps visiting the colony decreased with increasing number of flowers and time (approaches: $\chi^2 = 14.46$, d.f. = 3, $n = 14$, $p = 0.002$; landings: $\chi^2 = 15.07$, d.f. = 3, $n = 14$, $p = 0.002$; figure 1).

We found a significant difference in the attractiveness of the dummies at different distances from the orchid colonies, with attractiveness increasing with distance. Specifically, more males approached the pheromone-impregnated bead and attempted copulation the further away the bead was relative to the centre of the orchid patch (approaches: $\chi^2 = 27.05$, d.f. = 4, $n = 13$, $p < 0.001$; landings: $\chi^2 = 7.14$, d.f. = 4, $n = 13$, $p < 0.001$; figure 2).

4. DISCUSSION

In an earlier study, Wong & Schiestl (2002) argued that the sexually deceptive orchid *C. trapeziformis* could negatively impact upon its wasp pollinator *N. cryptoides*. In common with previous results, we showed that male wasps initially responded rapidly to the presentation of orchid blooms. However, males also habituated quickly to unrewarding flower decoys and soon avoided areas occupied by orchids. This may be owing to the fact that males are unable to distinguish between the pheromones of females and the allomones of sexually deceptive orchids (Wong & Schiestl 2002; Schiestl *et al.* 2003). This lack of discrimination is supported in our study by the finding

that dummy females placed in the middle of the orchid patch did not stimulate an increase in the number of males to the orchid colony, as would be expected if males were capable of discriminating between orchids and females. This finding is explained by the fact that orchids and females emit the same compound to attract males (Schiestl *et al.* 2003). However, our results suggest that it may indeed be possible for females to mitigate the cost of emerging in the same areas as flowering orchids simply by walking out of the area, as proposed earlier by Wong & Schiestl (2002).

The utility of leaving a patch of orchids assumes that female attractiveness would increase with distance from the orchids, and in the present study we show that this is, in fact, the case. Specifically, we show that the number of approaches and copulation attempts increases with increasing distance away from the orchids. Earlier work on other species of thynnine wasps suggests that males may be capable of learning precise locations (Evans 1966) evidenced, for example, by the ability of males to return females very close to the site of initial encounter following copulation (Ridsdill Smith 1970*b*). The results reported in our study suggest that this learning may also be true for the site-based avoidance strategy of *N. cryptoides* males. However, our results also indicate that males could differ in the precision of their site-based avoidance since we did not observe a sudden uniform increase in attractiveness of female dummies when they were placed beyond some minimum threshold distance from orchids. An important finding in our results indicates that at a distance of 8 m from the centre of an orchid colony, the dummies appear to be as attractive to males as an orchid when it is first introduced to an area, which corresponds to a level of response consonant with what might be expected prior to male habituation. Female thynnines are capable of moving below and above ground (Ridsdill Smith 1970*b*; F. P. Schiestl and B. B. M. Wong, personal observations), and although there may still be some energetic costs associated with walking out of an orchid patch, the results of our current study suggest that females may have strategies for mitigating some of the negative impact of sexual deception (Wong & Schiestl 2002).

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