THE ROYAL SOCIETY

How an orchid harms its pollinator

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Certain orchids produce flowers that mimic the sex pheromones and appearance of female insects in order to attract males by sexual deception for the purpose of pollination. In a series of field experiments, we found that the sexually deceptive orchid, *Chiloglottis trapeziformis*, can have a negative impact on its wasp pollinator *Neozeleboria cryptoides*. Male and female wasps, however, were affected differently by the orchid's deceit because of their different roles in the mimicry system. Male wasps could not discriminate between the chemical cues of orchids and female wasps, a vital signal in long-range attraction. Males, however, learn to avoid areas containing orchids. This strategy has implications for females attempting to attract mates in areas occupied by orchids. Compared with circumstances when females were on their own, females in the presence of orchids elicited fewer male approaches and no copulation attempts. Females in a large orchid patch also elicited fewer male approaches than females in a small patch. The nature of the orchid's impact on its wasp pollinator indicates an arms race evolutionary scenario in this interaction between plant and pollinator.

Keywords: arms race; pseudocopulation; deceptive pollination; thynnine; reproductive mimicry

1. INTRODUCTION

Many organisms mimic others in order to increase their own fitness, but mimicry can also have important fitness consequences for the other partners in the system. A typical mimicry system involves a model that is imitated by the mimic and a signal receiver or operator that reacts to the mimic (Wickler 1968). For the model and the operator, the effects of the mimic can be beneficial, neutral or deleterious (Wickler 1968; Stowe 1988). These effects, in turn, may impose strong selective pressures that potentially generate reciprocal evolutionary interactions. A better understanding of how mimicry affects the partners in a system may therefore reveal important information about evolutionary interactions (Wiens 1978; Gilbert 1983; Stowe 1988). In this regard, although most of what is known about mimicry has resulted from work done on animal systems, mimicry in plants also has much to contribute (Roy & Widmer 1999).

One of the most remarkable examples of reproductive mimicry in plants occurs exclusively in orchids and involves the use of sexual deception to effect pollination (Nilsson 1992). In these systems, the female of the pollinator species usually acts as the model, with the orchids' intended target, the male, acting as the operator (Wiens 1978). The orchids' sexual masquerade often is achieved through a combination of chemical, visual and tactile cues (Dafni 1984). Chemical mimicry of the female sex pheromone is probably the most important factor because of its role in the long-range attraction of male insects to flowers (Kullenberg 1961; Peakall 1990; Schiestl *et al.* 1999). At close range, visual and tactile mimicry of the female mediates the pseudocopulatory behaviour of the male needed to effect pollination (Bergström 1978).

The orchids' impact on pollinators remains unresolved

(see Stowe (1988) for a review). Although there has been considerable interest in sexually deceptive pollination systems, studies looking at the effects of mimicry on the pollinators have, until now, focused exclusively on the behaviour of the operator (e.g. Peakall 1990; Handel & Peakall 1993; Peakall & Handel 1993; Alcock 2000). The effects of mimicry on the actual models in the systems, the female insects, have been completely overlooked. Although the operator's behaviour can offer important insights into the impact of sexual deceit on pollinators, it is important to recognize that male and female insects can each be affected in different ways because of their different roles in the interaction. It is useful, therefore, to also consider how female insects are affected by the orchids' deception.

Thynnine wasps in the genus *Neozeleboria* are the pollen vectors for sexually deceptive orchids in the genus *Chiloglottis* (Oakwood 1990; Handel & Peakall 1993; Peakall & Handel 1993; Bower 1996; Mant *et al.* 2002). The reproductive biology of thynnines is likely to magnify the differential effects of sexual deception on the two sexes. Female wasps are wingless so they are entirely dependent upon the use of pheromones to attract flying males (Ridsdill Smith 1970*a*,*b*; Alcock 1981; Alcock & Gwynne 1987; Peakall 1990). Besides the actual mating, female thynnines also rely on males to carry them to their food source and to return them to a suitable oviposition site after copulation (Given 1954; Peakall 1990). Several studies on a number of thynnine–orchid associations have shown that male wasps habituate rapidly to areas occupied by orchids and subsequently avoid these locations (e.g. Peakall 1990; Handel & Peakall 1993; Alcock 2000). By avoiding areas occupied by unrewarding flower decoys, it is assumed that male wasps are able to discriminate, to some extent, between orchids and female wasps (Alcock 2000). This assumption, however, remains to be tested. Females of several thynnine species have previously been observed trying to attract mates among colonies of sexually deceptive orchids (C. C. Bower and R. Peakall, personal

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communications). If males cannot discriminate, then females emerging in the same areas as flowering orchids might have to emit pheromones ('call') for longer periods of time and expend more energy in order to attract a mate or, at worst, may be unable to attract any males. Here, we investigate the effects of sexual deception on male and female wasps using the orchid, *Chiloglottis trapeziformis* Fitz and its pollinator, *Neozeleboria cryptoides* (Smith). Specifically, we test whether male wasps can discriminate between orchids and female wasps, and compare the attractiveness of females calling in areas with and without orchids.

2. MATERIAL AND METHODS

All experimental work was carried out during September 2001 in a 5 ha patch of open *Eucalyptus* woodland in Canberra, Australia. The orchid did not occur naturally at the study site but the wasp was common. Male wasps patrolled the wooded area for females and copulating pairs visited *Eucalyptus* trees to feed on honeydew excreted by scale insects. Copulating females were netted at the food source, gently separated from the males and placed into individual plastic vials. Orchid flowers for experimental work were brought in from another area.

To test whether patrolling males could discriminate between the olfactory cues produced by conspecific females and orchids, we set up a dual-choice test. An orchid flower was placed into one of two opaque glass chambers with central holes at the top and the bottom. A female wasp was placed into the other chamber. Individual females were coaxed into calling (Peakall 1990) by allowing them to crawl onto the top of a toothpick secured vertically inside the chamber using cotton wool. Air was simultaneously pumped through the bottom of each chamber and out through the opening at the top. A black bead was placed above the opening at the top of each chamber. This provided the visual cues necessary for inducing landing by males. The bead and the use of opaque glass for the chambers controlled for the effect of visual stimuli from the orchid and the female. We placed the chambers 5 cm apart on the ground and then counted the number of males that landed onto each chamber's bead over a 5 min sampling period. We tested a total of eight orchid–female pairs within the study area at different locations spaced at least 20 m away from any previous test site. Each orchid and female was tested once.

We conducted a separate experiment to test the attractiveness of females calling alone and in the presence of flowering orchids. *Chiloglottis trapeziformis* often occurs in quite dense colonies of up to several dozen plants (Oakwood 1990; B. B. M. Wong and F. P. Schiestl, personal observation). Orchids were introduced to the study area to simulate two colonies (small and large) of flowering plants, 50 m apart. The small and the large colony contained five and 10 flowers, respectively. Within each colony, individual flower stems were placed into separate water-filled plastic tubes set into the ground with each flower spaced 20 cm from the next in a circular grouping. 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 (Peakall 1990). Both colonies were left for 2 h before the commencement of any testing with calling females. In contrast to the previous experiment, both visual and tactile cues of females and orchids were available. We counted the number of approaches and landings elicited by females over a 3 min period when calling (i) on her own, (ii) in the small orchid colony, and
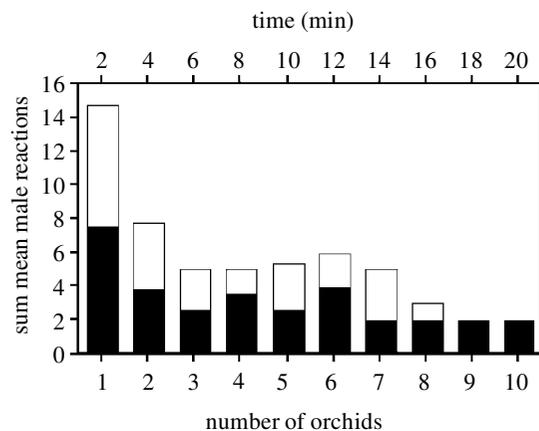

Figure 1. The number of male wasp visits to the orchid patch with increasing time and number of orchids (black bars, approaches; white bars, copulation attempts).

(iii) in the large orchid colony. Each female was tested once per treatment with the order randomized. When a female was tested in an orchid colony, she was placed at the centre of the circular array of flowers. The area where solitary females were tested was at least 200 m away from the two orchid colonies. As in the previous experiment, when testing females on their own, each female was tested at a spot at least 20 m from any sites where females had previously been tested.

The data were analysed using the statistical package Spss (Norusis 1993). A Wilcoxon signed ranks test was used in the two-choice experiment to compare the number of landings elicited by female wasps and orchids. We used a Friedman test to look for differences in male approaches and landings in response to calling females with and without orchids. Subsequently, for *a posteriori* multiple comparisons, we used Wilcoxon signed ranks tests with the level of significance set at $p < 0.017$.

3. RESULTS

Male wasps in the two-choice test could not discriminate between the odours emitted by calling female wasps and flowering orchids as the number of landings elicited by one was not significantly more than the other (mean no. of landings ± s.e.: on female = 2.63 ± 0.86, orchid = 2.50 ± 1.20; $n = 8$, $p > 0.05$).

When orchid flowers were offered to the males sequentially in the same area, the mean number of male visits to the orchid colonies decreased with increasing number of orchids and time (figure 1). This indicates that males avoid the area with the orchids rather than individual flowers.

The mean numbers of male approaches and landings for females calling alone were 8.2 ± 1.3 and 3.62 ± 1.07, respectively. Although some males approached calling females in the small orchid colony (mean no. of males ± s.e. = 1.06 ± 0.24), no males were attracted to the big orchid patch. In both orchid patches, calling females did not elicit any landings by males (figure 2). There was a significant difference in both the number of male approaches and landings under the three treatments (approaches: $\chi^2 = 17.18$, d.f. = 2, $n = 9$, $p < 0.001$; landings: $\chi^2 = 16$, d.f. = 2, $n = 9$, $p < 0.001$). In both the small and large orchid patches, females attracted significantly fewer male approaches than when calling alone (alone ver-
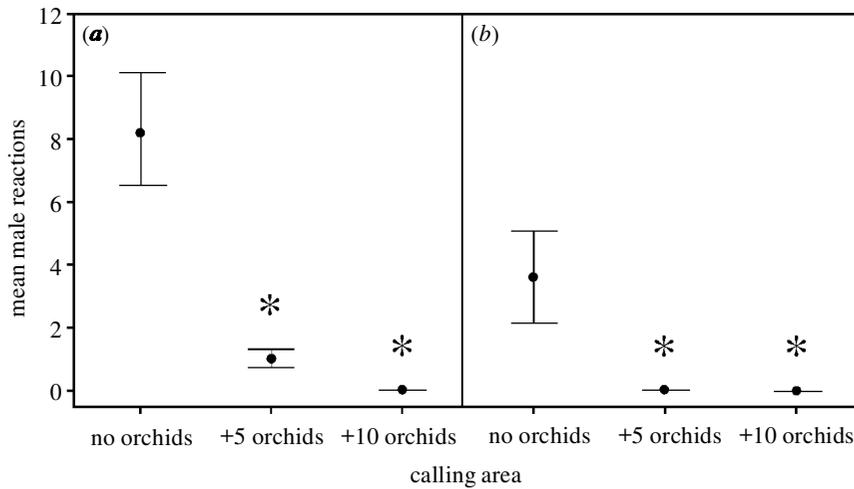
Figure 2. Reaction of male wasps to females calling in areas with and without orchids (mean ± s.e.) (*a*) approaches, and (*b*) copulation attempts. Asterisks indicate significant differences between male reactions to females in the absence of orchids and male reactions to females in the presence of orchids.

sus small orchid colony: $p = 0.008$; alone versus large orchid colony: $p = 0.008$; figure 2). However, females were still able to elicit more approaches in the small colony compared with the large ($p = 0.016$; figure 2). The number of male landings was also significantly higher for females calling alone (alone versus small orchid colony: $p = 0.012$; alone versus large orchid colony: $p = 0.012$; figure 2).

4. DISCUSSION

We have demonstrated for the first time, to our knowledge, that the sexually deceptive orchid, *C. trapeziformis*, can negatively affect its pollinator, a thynnine wasp, by making the attraction of mates more difficult for female wasps, the models in this system.

In sexually deceptive mimicry systems, the olfactory stimulus is paramount because it is solely responsible for long-range mate attraction (Kullenberg 1961; Dafni 1984). This also appears to be the case in long-range attraction of *N. cryptoides* males to both females and orchids. Like other thynnines (Peakall 1990; Alcock 2000), competition for mates is high and, in our study, males responded rapidly by approaching and attempting to copulate with females that were calling on their own. The orchids exploit this feature of the *N. cryptoides* mating system by mimicking the sex pheromone of the female to attract patrolling males to the flowers. The effectiveness of this chemical mimicry is demonstrated in our experiment by the finding that male wasps could not discriminate between the olfactory cues produced by orchids and female wasps in the dual-choice test. This inability to distinguish between the chemical signals of the model and mimic affects *N. cryptoides* males and females in different ways because of their different roles in the mimicry system.

For the male, visits to unrewarding flower decoys probably has little impact on the reproductive success of individual wasps (Peakall 1990). Sexually deceptive pollination systems are characterized by low rates of actual encounters between males and orchids (Peakall 1990; Peakall & Handel 1993; Alcock 2000; but see Handel &

Peakall 1993). In *C. trapeziformis*, as few as 13% of wasp visits may actually result in pseudocopulation (Oakwood 1990). Furthermore, even when contact is made between a sexually deceptive orchid and its pollinator, ejaculation is rarely observed (Peakall 1990). Energetic costs for the males, it is argued, would be akin to an unsuccessful late response to a female (Peakall 1990). Furthermore, as demonstrated in our study, male wasps habituated rapidly to the presence of orchids.

We showed that the number of male visits to the orchid patches decreased with time. This response is consistent with those reported in many other sexually deceptive mimicry systems (Peakall 1990; Handel & Peakall 1993; Peakall & Handel 1993; Alcock 2000; Ayasse *et al.* 2000). In thynnine wasps, avoidance is probably mediated by learning and avoiding the specific locations where orchids were previously encountered (Peakall 1990). It is unlikely that males were learning to associate particular chemical cues with an unrewarding orchid encounter (Peakall 1990) as was shown in the European *Ophyrs–Andrena* association (Ayasse *et al.* 2000). Specifically, our results did not show a resurgence in the number of males visiting the orchid patches when calling females were introduced. This might be expected if males were learning to avoid previously visited flower decoys based on individual chemical recognition (Ayasse *et al.* 2000). We can exclude the possibility that females calling in the orchid patches were somehow unattractive to males, as the same females tested on their own successfully elicited approaches and copulation attempts.

This site-based avoidance strategy may have important fitness consequences for female wasps. In contrast to the European *Ophrys*-pollinator system, where females are capable of flight, wingless females of Australian thynnines are dependent upon males to locate them in order to copulate and to carry them to their food source. During our 5 min trials, few males entered the orchid patches and approached a calling female and none attempted to copulate. The number of approaches elicited by females were also fewer in the larger orchid patch compared with the smaller patch. By contrast, solitary females elicited significantly more approaches and copulation attempts. The

high speed at which male wasps responded to the presence of calling females outside an orchid patch characterizes thynnine mating systems (Peakall 1990; Alcock 2000; Mant *et al.* 2002). Females emerging in the same area as the flowering orchids may, at the very least, have to expend more energy in calling or call for longer periods of time before a male is able to locate her. We do not discount the possibility that wingless females may be capable of walking out of areas occupied by orchids. Presumably, however, this would still impose some energetic costs. At the very worst, especially in large orchid patches, females may be unable to elicit any copulations as a consequence of the males' site-based avoidance strategy.

Our results have important implications for understanding the evolutionary dynamics between sexually deceptive orchids and their pollinators. The demonstration of a negative impact on the pollinator satisfies the requirement for a coevolutionary arms-race scenario. Specifically, according to an argument espoused by Stowe (1988), if pollinators are harmed by sexual deception, selection would be expected to favour males capable of discriminating between orchid flowers and calling females (Stowe 1988; Holen *et al.* 2001). This, in turn, would place selective pressure on the orchid to enhance and maintain the similarities between model and mimic, because any deviation from the model would allow males to discriminate against the orchid. Selection on the odour signal probably led to the elimination of common floral or 'green leaf' volatiles not present in the original model signal that male thynnines may otherwise be able to detect. The presence of these compounds is relevant because sexual deception is a pollination syndrome that is believed to have evolved from a food-deceptive system where floral odours are prevalent (Kores *et al.* 2001). If this is the case, selection against the persistence of non-mimetic components in the orchid's chemical signal is supported by preliminary findings that indicate a lack of any floral odour compounds in *C. trapeziformis* flowers (F. P. Schiestl, unpublished data). Although our study has identified the important element of 'harm' required under Stowe's hypothesis, further work is needed to test its validity in the current system, with special focus on the mating system of the wasps and ecological factors, such as the size and density of orchid colonies, the degree of overlap in the distribution of orchid and pollinator and the timing and frequency of flowering, as well as the flight time of the pollinator.

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