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Interactions between the sexually deceptive orchid *Spiculaea ciliata* and its wasp pollinator *Thynnoturneria* sp. (Hymenoptera: Thynninae)

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Males of the thynnine wasp *Thynnoturneria* sp. attempt to mate with female decoys in the flowers of the elbow orchid *Spiculaea ciliata*. Experimentally shifted orchids usually attract male wasps quickly, often within 2 minutes of presentation of the 'bait' orchids in appropriate habitat. Although the orchid effectively exploits the scramble competition mating system of the wasp, the insect is not totally at the mercy of the deceptive orchid. Fewer than half of all arriving males contact the column of the orchid flower, as required for orchid pollination. Moreover, the number of deceived visitors falls sharply over a short period and the number of wasp visitors does not rebound with the replacement of one bait orchid by another at that location. These observations suggest that patrolling wasp pollinators can discriminate to some extent between orchid decoys and female wasps, especially by learning to avoid particular locations that are associated with unrewarding flower decoys.

Keywords: Orchids, thynnine wasps, male behaviour, mating system, deception.

Introduction

The use of sexual deception by plants as a means to secure insect pollinators occurs only among the Orchidaceae (Wiens, 1978; Nilsson, 1992). This tactic is particularly common among the small terrestrial orchids of Australia (Stoutamire, 1983; Hoffmann and Brown, 1992; Bower, 1996). For example, many orchids in the subfamily Caladeniinae possess a highly modified flower petal, the labellum, that resembles to some extent the wingless female of a thynnine wasp; in addition, the labellum is endowed with secretory glands that apparently produce scents similar or identical to the sex pheromones released by sexually receptive female thynnines. As a result, males of some thynnine wasps fly to the orchid and attempt to carry away or to copulate with the labellar decoy, using the same behavioral responses that they exhibit toward conspecific females (Ridsdill-Smith, 1970; Alcock, 1981; Alcock and Gwynne, 1987). Some deceived males acquire pollinia from the orchid flower for transfer to another plant (Peakall and Beattie, 1996). Although relatively few cases of orchid-wasp interactions have been studied in any detail, the general rule

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appears to be that each of the female-mimicking orchids attracts pollinators primarily or exclusively from a single species of wasp (reviewed by Bower, 1996).

The elbow orchid *Spiculaea ciliata* Lindley is one of the many small, terrestrial orchids endemic to Western Australia that rely on sexual deception to secure the pollination services of a particular thynnine wasp (Erickson, 1978; Hoffmann and Brown, 1992). Males of a currently unnamed species of *Thynnoturneria* (Brown *et al.*, 1997) fly to and grasp the female decoy of the elbow orchid. Because the decoy is attached to the plant on a flexible stalk, the male thynnine's effort to carry it away fails. Instead, the wasp flies upward into the orchid column, where the insect becomes momentarily trapped by hooked projections on the column. During this stage of the interaction, the orchid's pollinia may become attached to the dorsum of the wasp's thorax. When the wasp escapes from the entrapping hooks, it may visit another elbow orchid and deposit the attached pollinia there.

Observations made of other species of orchids and their wasp pollinators have revealed possible counter-adaptations of the male wasps that may reduce the time and energy they expend on deceptive orchid decoys (Peakall, 1990; Handel and Peakall, 1993). The existence of these counter-adaptations is suggested by the rarity with which contacts between orchids and their wasp pollinators occur in nature. The only practical way in which to see wasps pouncing on female decoys involves cutting or picking orchids and then moving the plants to new locations. Moreover, in at least one wasp-orchid system, after orchid specimens have been shifted to new spots, the number of attracted males declines with great rapidity, indicating that males can learn the location (or distinctive odor) of deceptive orchids, so that they do not often return to the spot after an unrewarding encounter there (Peakall, 1990).

This paper reports similar experiments with the elbow orchid and its thynnine pollinator. I document that these male wasps quickly habituate to novel sources of sex pheromone provided by 'transplanted' bait orchids and that dishabituation does not occur when one bait orchid is replaced by another.

Materials and methods

Portions of the study were conducted in November 1995 and 1997 at Lilian Stokes Rock (Frank Hann National Park), Boyagin Rock Reserve and Pallarup Rocks Reserve in Western Australia. All three locations had populations of *S. ciliata* that numbered in the thousands. During the study, fewer than 10 mature elbow orchids were plucked from the ground at each site and placed upright in vials filled with locally collected sand and dirt. These specimens were then employed as bait orchids that could be shifted from location to location among the shallow soil patches lying over granite rocks within the reserve. When a vial containing an orchid was placed on the ground, records were made of the number of male wasps of *Thynnoturneria* sp. responding to presentation of the specimen (see figure 1). The males of this wasp are less than 1 cm in length with thin black bodies distinctively marked with yellow bands and bars.

Throughout all means are presented ± 1 *s.d.*

Results

Response to Spiculaea ciliata *by males of* Thynnoturneria *sp.*

In late November 1995, many male wasps were observed patrolling low over the ground at the Pallerup Reserve in places with large numbers of mature elbow

FIG. 1 A male of *Thynnoturneria* sp. mounted on a labellar decoy of an experimentally moved specimen of the elbow orchid *Spiculaea ciliata*. Note the pollinia attached to the dorsum of the thorax of the wasp. A second male (lower right) has also arrived and is approaching the unoccupied decoy above him.

orchids. On 25 November, a naturally growing orchid was selected for observation about 3 m distant from the spot where a bait specimen had recently succeeded in attracting at least one visiting wasp within 2 minutes of its presentation (figure 1). Each selected orchid was watched for 5 minutes. During this time, only two of eight naturally growing orchids were approached and touched by male *Thynnoturneria* sp.

The protocol was repeated on 29–30 November 1995 at Boyagin Rock Nature Reserve. At this site, only one of eight focal specimens was touched by a male wasp during the 5 minute observation blocks, although four orchids were closely approached by at least one wasp flying upwind to within 10 cm of a decoy flower before the wasp turned away.

Bait elbow orchids placed in a vial on the ground in a new location attracted male *Thynnoturneria* sp. more reliably than naturally growing specimens. Incoming males invariably flew upwind within a few cm of the ground in a tight zig-zagging flight. On 46 of 65 presentations (71%) of a bait orchid on 20–24 November 1997 at Pallerup Reserve, one or more male wasps made contact within 5 minutes. On

23 of the 46 cases involving male contact with a decoy, the first wasp arrived in less than one minute; the average time of arrival was 1.5 ± 1.3 minutes.

The first male to reach a bait orchid was often followed by other wasps in quick succession (figure 1). On 23 November 1995, bait orchids were distributed one at a time to 15 different sites separated by at least 3 m within a 7.5 m × 30 m area at Pallarup Rocks. Male wasps were netted as they arrived at each orchid over a 5 minute period. The captured wasps were then removed from the net one by one, marked with acrylic paints or liquid paper, and released. The mean number of captures per 5 minutes was 3.3 ± 2.1 ($n = 15$ trials).

At this study area, all bait orchids were removed after each 5 minute trial, but at three spots, specimens were returned to the ground at 20 to 30 minute intervals for additional observation. The total number of wasps taken at the three orchids was three, eight and 23, respectively, over eight 5 minute periods of capture, marking and release between 1130 to 1630. Five marked wasps were recaptured during these experiments, showing that at least some males patrolled overlapping home ranges in orchid habitat.

Evidence for learned avoidance of deceptive orchids

During any one 5 minute observation block, most wasps arrived at the bait orchids within the first two minutes, after which the probability of additional males landing on the orchid fell sharply. This pattern held for different locations in the same year and between years at the same location (figure 2).

If a bait orchid was left in place for a prolonged period, the number of visits remained low but did not end entirely. In one experiment conducted at Pallarup Rocks Reserve in November 1995, bait orchids were grouped in pairs, with one individual left on the ground for 60 minutes while its partner was picked up and removed after every 5 minute observation block, one of which occurred at the start of the hour, the second 25 minutes later, and the third at the end of the hour. After each hour-long trial, a new trial was conducted in which the specimens were assigned to the opposite category and moved to new locations. Thus, the bait orchid that had remained on the ground for the full hour now became the 'temporary' orchid that was removed from its presentation point after each observation period.

During the first 5 minute blocks, when both the 'temporary' and 'permanent' orchid specimens were made available for the first time, the rate of contacts by visiting males did not differ. But on the second and third observation blocks, the 'permanent' orchids were visited by significantly fewer males compared to the initial bout. The rate of contact visits fell to a lesser extent for those trials involving orchids that had been taken away from the presentation point for 25 minutes before they were offered again to patrolling male wasps (figure 3).

The tendency to avoid bait orchids after they had been on the ground for some time could have arisen because male wasps habituated to distinctive odor cues associated with a specific orchid or alternatively, because they learned to avoid particular locations associated with unrewarding odor sources. In an experiment conducted both at Frank Hann NP and Pallarup Reserve in late November 1997, one bait orchid was set out for 10 minutes and then a second specimen took its place for a final 5 minutes. The number of contacts did not rebound when the male wasps had access to the replacement orchid (figure 4), indicating that habituation was location-specific rather than orchid-specific.

Further evidence suggesting that male wasps can learn to avoid deceptive orchids

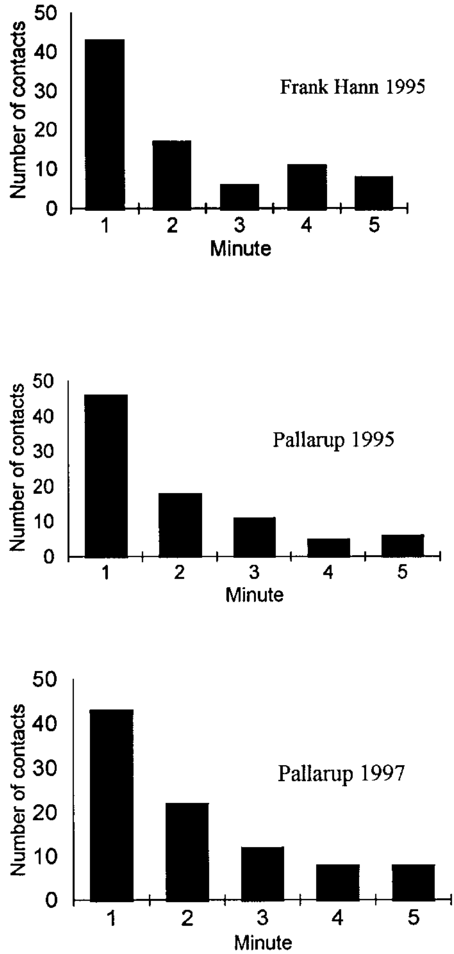

FIG. 2. The number of males contacting an elbow orchid over 5 minute presentations of bait specimens. (Top) Results of 10 trials conducted at Frank Hann National Park in November 1995. Differences in the mean numbers of contacts over the 5 minutes are statistically significant: ANOVA $F_{[4,45]} = 7.6$, $p < 0.001$. (Middle) Results of 16 trials at Pallarup Rocks Reserve in November 1995. Differences in the mean numbers of contacts over the 5 minutes are statistically significant: ANOVA $F_{[4,75]} = 5.4$, $p < 0.001$. (Bottom) Results of 36 trials at Pallarup Rocks Reserve in November 1997. Differences in the mean numbers of contacts over the 5 minutes are statistically significant: ANOVA $F_{[4,175]} = 11.5$, $p < 0.001$.

comes from frequent observations of individuals failing to respond maximally to decoys that they had approached. On 24 November 1997 at Pallarup Rocks, the responses of the first 50 males attracted to bait orchids were catalogued from mid-morning to early afternoon. Six of the 50 wasps came within 5 cm of the orchid only to veer away and leave without ever making contact. The other 44 (88%) touched or landed upon the orchid. Three of these perched for a few seconds before leaving without grasping a decoy. Nineteen wasps flew to and grasped a female decoy (figure 1) but released it almost at once. The remaining 22 males landed on the decoy and tried to fly off with it, resulting in their brief entrapment in the winged

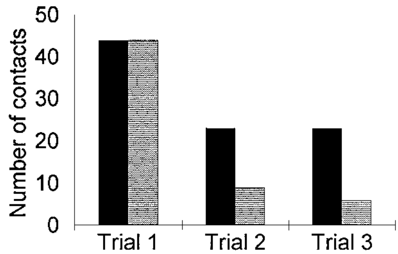

FIG. 3. The number of males contacting elbow orchids during three 5 minute presentations. Trial 1 occurred at the start of an hour, trial 2 took place 25 minutes later, and trial 3 at the end of the hour. The black bars provide data for 'temporary' bait orchids that were removed from the presentation point after each 5 minute observation block; grey bars present data for 'permanent' bait orchids that were left in place throughout the hour. Differences in the mean number of contacts across trials for the 'temporary' bait specimens were not statistically significant (ANOVA $F_{[2,21]} = 1.9$, $p > 0.10$) whereas the differences across trials for the 'permanent' bait orchids were significant (ANOVA $F_{[2,21]} = 11.9$, $p < 0.001$).

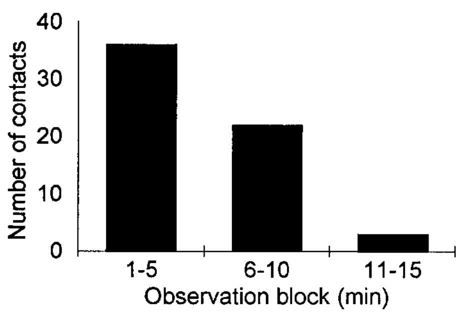

FIG. 4. The number of males contacting an elbow orchid during the first three 5 minute blocks following presentation of a bait specimen. After 10 minutes, the original bait specimen was replaced by a new individual. Differences in the mean numbers of contacts per 5 minute blocks are statistically significant: ANOVA $F_{[2,45]} = 22.2$, $p < 0.001$.

column of the orchid flower. Thus, only 44% of the interactions between the bait orchids and the wasps they attracted could have resulted in pollen acquisition or pollen transfer among orchids growing *in situ*.

Nevertheless, many male wasps were sufficiently deceived to acquire pollinia from at least one elbow orchid growing within their patrol routes. At Pallarup Rocks on 23 and 24 November, 14 of 21 males (67%) that landed long enough on bait orchids to permit close visual inspection carried pollinia from a previous orchid encounter.

Discussion

Interactions between males of the thynnine wasp *Thynnoturneria* sp. and the orchid *Spiculaea ciliata* follow the pattern reported for other wasp–orchid associations (e.g., Peakall, 1990; Bower, 1996). First, only one species of thynnine, *Thynnoturneria* sp., was attracted to elbow orchids. Thus, the plant apparently has a unique relationship with a single pollinator.

Second, male wasps were occasionally seen making contact with naturally growing orchids, but they were far more responsive to the transplanted bait orchids, as is true for other wasp–orchid associations (Peakall and Beattie, 1996). Bait orchids left in place for some time continued to be visited but at a very low rate. Thus, in nature patrolling wasps must rapidly habituate to the presence of an orchid that releases deceptive sex pheromone, although occasionally a naive male may enter an unfamiliar area and make contact with a previously visited orchid.

Third, the response to bait elbow orchids followed the pattern of visitation reported by Peakall (1990) for *Drakaea glyptodon*. Typically, bait orchids quickly attracted several males, occasionally as many as 10 or so, but then the contact rate dropped sharply and remained low, even if a new orchid was substituted for the original specimen. These results suggest that populations of patrolling thynnines are subdivided into small groups that repeatedly inspect a given location, restricting their flight paths to areas small enough so that should a female wasp begin releasing sex pheromone within the patrolled area, a male would be likely to detect and reach her within one or two minutes. Very rapid responses by males to pheromone-releasing females characterize thynnine mating systems (Ridsdill-Smith, 1970; Alcock, 1981; Alcock and Gwynne, 1987). The recapture of marked males in this study confirms that at least some males of this species have habitual patrol routes, overlapping with those of several other competitor males, just as has been documented for some other thynnines (Alcock, 1981).

Not all of the males that respond to the orchid actually contact the decoy in a way that could result in pollination. More than half of the males sampled in this study failed to complete the precopulatory pattern with labellar decoy, as is required if the male is to come into contact with the pollen-bearing component of the orchid. The same is true for other thynnines that have been observed interacting with deceptive orchids (Peakall and Beattie, 1996). These observations could be explained if unrewarding experiences with deceptive orchids increase the male threshold for responding vigorously to the deceptive sexual stimuli provided by labellar decoys.

Finally, the deception of the elbow orchid works for the same reason that it works for other similar species, namely because scramble competition for mates is extremely intense in male *Thynnoturneria* sp. and other thynnines. Given the great premium on speedy location and removal of receptive female wasps (e.g., Peakall, 1990; Alcock, 1981; Alcock and Gwynne, 1987), deceptive orchids can reliably exploit patrolling males for their own purposes. Judging from the responsiveness of male thynnines to bait orchids, most recently opened flowers will be contacted vigorously in short order. As the local patrolling thynnines rush to the source, some may remove pollinia from or apply pollinia to the stigma. If two such contacts are needed for pollination to be complete, the first flower to open on moderately isolated orchids seems certain to be visited by enough males. However, the ability of the wasps to habituate to specific locations raises questions about whether the second, third or fourth flowers produced by some individuals of *S. ciliata* are pollinated reliably as well as the reproductive success experienced by orchids growing in dense clumps as opposed to those that are some distance from their neighbors.

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