Are there fitness advantages in being a rewardless orchid? Reward supplementation experiments with *Barlia robertiana*

Ann Smithson[*] and Luc D. B. Gigord

School of Biological Sciences, University of Exeter, Hatherly Laboratories, Prince of Wales Road, Exeter EX4 4PS, UK

The Orchidaceae characteristically contain a very large number of species that attract pollinators but do not offer them any form of reward in return for visitation. Such a strategy is highly unusual in the plant kingdom. We conducted experiments in order to manipulate the reward strategy of the rewardless bumble-bee-pollinated orchid *Barlia robertiana* by adding sucrose solution to inflorescences. We found that supplementation decreased the probability of a pollinator removing pollinia by approximately ten times. Despite pollinators visiting many more flowers per inflorescence on supplemented plants, eight times fewer pollinia were removed from supplemented inflorescences during each visit. Pollinia deposition patterns were not significantly affected by supplementation and no geitonogamous deposition was recorded. In populations where inflorescences were supplemented for 20 days, pollinia removal was reduced by over half for supplemented inflorescences, whereas fruit set was unmodified by supplementation. We conclude that rewardlessness would increase total seed paternity, but not change either total seed maternity or the probability that offspring were outcrossed in this species. To the authors' knowledge this is the first time that there has been an unequivocal experimental demonstration of an evolutionary advantage for rewardlessness in the Orchidaceae.

Keywords: rewardlessness; Orchidaceae; bumble-bee; pollinia; geitonogamy; male function

1. INTRODUCTION

The Orchidaceae are unusual amongst animal-pollinated plants in that many species do not use any form of reward as an inducement for enticing pollinators into continued visitation. The ability to produce nectar is absent in many species (Ackerman 1986; Dafni 1987). Pollen is grouped into structures termed pollinia that are affixed to pollinators during visitation and, because pollinia are inaccessible to pollinators both during visitation and after removal, pollen does not normally function as a reward (reviewed in Johnson & Edwards 2000). While rewardlessness has occasionally been recorded in other plant families (reviewed in Dafni 1984), the 8000 species (around one-third of all known orchids) (Gill 1989) that are believed to be rewardless in the Orchidaceae is clearly exceptional. Rewardlessness occurs across all continents on which the Orchidaceae are found and in a wide range of genera. Rarely, some orchid species are pollinated through specific mimicry of other cues that could attract pollinators, e.g. another rewarding plant species or female insect (Dafni 1984). Most rewardless orchids fall into the category of 'non-model deceptive pollination' (Ackerman 1986) whereby the plant is pollinated by naive individuals sampling inflorescences while exploring their floral environment. The causes of the evolution of rewardlessness have been frequently discussed (e.g. Dressler 1981; Dafni 1984, 1987; Ackerman 1986; Gill 1989; Nilsson 1992; Johnson & Nilsson 1999) but there have been few experimental tests of these hypotheses.

Naive pollinators sampling rewardless orchids visit only a few flowers per inflorescence, visit a short sequence of inflorescences before departing the patch and quickly learn to switch to alternative sources of food (Dressler 1981; Dafni 1987; Smithson & Macnair 1997). This is expected to result in reduced pollinium removal, pollen deposition and seed set. The low reproductive success of some rewardless orchids has been widely cited, e.g. only 10% of *Cyprepedium acaule* set seed in any year (Gill 1989) and species comparisons indicate that fruit set is more strongly limited by pollinators in rewardless orchids (Neiland & Wilcock 1998). This presumption of the detrimental effects of rewardlessness to reproductive success has led to the suggestion that mutant reward-producing phenotypes will always invade a population of rewardless orchids and, thus, that rewardlessness is not evolutionarily stable (Gill 1989; Neiland & Wilcock 1998). Darwin (1904) considered the idea of rewardlessness so untenable in evolutionary terms that he dismissed the whole idea. Other authors believe that direct reproductive advantages must accrue to rewardless orchids (reviewed in Nilsson 1992). A high cost of nectar production could lead to an advantage for rewardlessness, but many rewardless orchids are pollinator limited not resource limited (Ackerman 1986; Nilsson 1992). The reduced number of flowers visited per inflorescence by pollinators on rewardless morphs could lead to reduced geitonogamous selfing (De Jong *et al.* 1993) and also an increase in pollen available for outcrossing (pollen discounting) (Dressler 1981; Holsinger *et al.* 1984; Johnson & Nilsson 1999). Thus, offspring quality is expected to be higher in rewardless morphs if selfing is costly and rewardlessness may then spread.

There is a need for testing these hypotheses experimentally, comparing rewarding and rewardless morphs within populations. To date no significant advantage for unrewarding orchids in terms of any reproductive function has been shown. Ackerman *et al.* (1994) found

[*]Author for correspondence (a.smithson@exeter.ac.uk).

that, by clipping off the spurs in order to render a rewarding species nectarless, male reproductive success was significantly reduced. However, experiments may be confounded by spur removal, as this reduces the probability of pollination in both rewarding and rewardless orchids (Neiland & Wilcock 1994). Johnson & Nilsson (1999) found that pollinators visited significantly more flowers per visit when unrewarding inflorescences are supplemented with nectar. Supplementing an unrewarding species only once with a large volume of nectar significantly increased pollinium removal and deposition 1 week later in a single population. However, nectar is normally continuously secreted in small amounts and, thus, this experiment may be unrealistic.

We report here on experiments that test for differences in reproductive fitness components between rewarding and unrewarding morphs within populations of the rewardless orchid *Barlia robertiana*. We supplemented inflorescences with 'nectar' and tested the effects on both long-term plant reproductive success and pollinator behaviour. We predicted that pollinium removal, pollen deposition and fruit set would be greater for supplemented inflorescences compared to controls, assuming pollinator limitation of reproductive success and self-compatibility. However, we also expected higher fruit quality for control inflorescences, since these fruits are less likely to be selfed. By comparing pollen deposition when pollinators arrived at inflorescences without pollinia, we could test whether geitonogamous depositions were greater for supplemented inflorescences.

2. METHODS

(a) *The study system*

Barlia robertiana is a robust, bumble-bee-pollinated, rewardless orchid species. It inhabits dry, sandy soils near the coasts of southern Europe. *Barlia robertiana* was studied in three populations during 1999 and 2000 in northern Spain: Begur A (41° 57.341′ N, 3° 11.843′ E), Begur B (41° 57.328′ N, 3° 11.723′ E) and Estartit (42° 3.845′ N, 3° 11.273′ E). Populations were selected in order to maximize the range of population sizes because variables such as visitation rates and the costs of selfing are expected to vary with population size. The population sizes were 636 (Begur A), 225 (Begur B) and 168 (Estartit) flowering plants in 1999 and the densities were 0.072 (Begur A), 0.042 (Begur B) and 0.051 (Estartit) flowering plants per square metre. Flowering occurs during February and March.

Extensive sampling of flowers using both 1 µl capillary tubes and filter paper wicks (60 inflorescences per population) failed to find any form of reward in any population, confirming recent studies suggesting that *B. robertiana* is rewardless (see Van der Cingel 1995). *Bombus lucorum* was recorded exclusively as the pollinator in the study populations, visiting on average 3.6 out of 27.6 flowers per inflorescence and 2.0 inflorescences sequentially before leaving or switching to another species. No form of sexual deception of pollinators could be inferred as the principal pollinators were queens and workers. The only rewarding coflowering plant species near each study population was *Rosemarinus officinalis*. There was no form of mimicry between *B. robertiana* and *R. officinalis* as a high degree of pollinator discrimination between the two was recorded (pollinators foraging on *R. officinalis* clearly rejected *B. robertiana* when it was offered in pots on 75% of occasions).

In each of the study populations we experimentally found significant pollinator limitation of both pollinia removal and fruit set (*ca.* 70% in each case), self-compatibility and no means for automatic selfing. In this species, there were two pollinia per flower that were normally removed as a pair of pollinaria, but pollen is deposited gradually onto stigmas in clumps (massulae).

(b) *Population supplementation experiments*

At the beginning of the flowering period during 1999, *ca.* 20 pairs of inflorescences, 0.3–2 m apart and matched for the traits described below, were selected haphazardly in each study population. One member of each pair was supplemented with 'nectar' (2 µl of 30% sucrose solution) in every open flower every day (early morning) for 20 days, while the other received a control manipulation. Sucrose was placed into the tip at the far end of the spurs of each flower using a 10 µl microsyringe. The sucrose solution did not have any significant negative effect on the flowers in terms of longevity or condition (cf. Ackerman 1981). Manipulations commenced when a minimum of five flowers were open on each inflorescence of both members of a pair. To prevent ants consuming the sucrose, the stem bases of each pair were surrounded with fluon (fluon GP1, Whitford Plastics, Runcorn, UK), which prevents insects gripping the stem. The fluon had no observable effect on the inflorescences during the experiments. We recorded the following traits for all inflorescences studied: the total number of flowers produced, inflorescence height (to the nearest 5 mm), lip length and width and spur length and width (to the nearest 0.1 mm).

After 20 days, pollinium removal was recorded and plants were bagged with green mesh bags in order to prevent pollinator access. Fruit set was counted after 1 month and fruits collected from inflorescences with fruits after a further 2 months. We took three fruits per plant from the bottom, middle and top of each inflorescence. Fruit length and width was measured in the field to the nearest 0.1 mm. The total seed weight per fruit was found in the laboratory using a five decimal places balance.

Experimental plants were marked and their precise positions recorded. We relocated all plants during 2000 and recorded their status (flowering, non-flowering or not emergent).

The numbers of pollinia removed, fruits set, fruit length, fruit width and seed weight were compared using ANOVA after $\log_{10}$-transformation. The effects of both treatment and population were fixed factors. Since inflorescence position influences pod size, we compared fruit length, width and seed weight using a partially hierarchical ANOVA, with treatment, population, plant and position within plants as factors, and with plant nested within populations. The statuses of experimental plants the following year were compared using a *G*-test in order to test whether there were changes in the probability of flowering.

Statistical analysis was performed using STATISTICAL PACKAGE FOR SOCIAL SERVICES (2001, SPSS, Inc., Chicago, IL, USA). Where repeated statistical tests were carried out, these were corrected using the sequential Bonferroni method (Rice 1989). Where means are reported, one standard error of the mean is also given.

(c) *Pollinator behaviour experiments*

During 2000, pollinator behavioural responses to rewarding and unrewarding orchids and resultant pollinia transfers were studied in detail in one population (Begur A). A pair of inflorescences was potted up from the study population in natural soil. One member of the pair was supplemented by placing 2 µl of 30% sucrose solution into the tip of the spur of each open
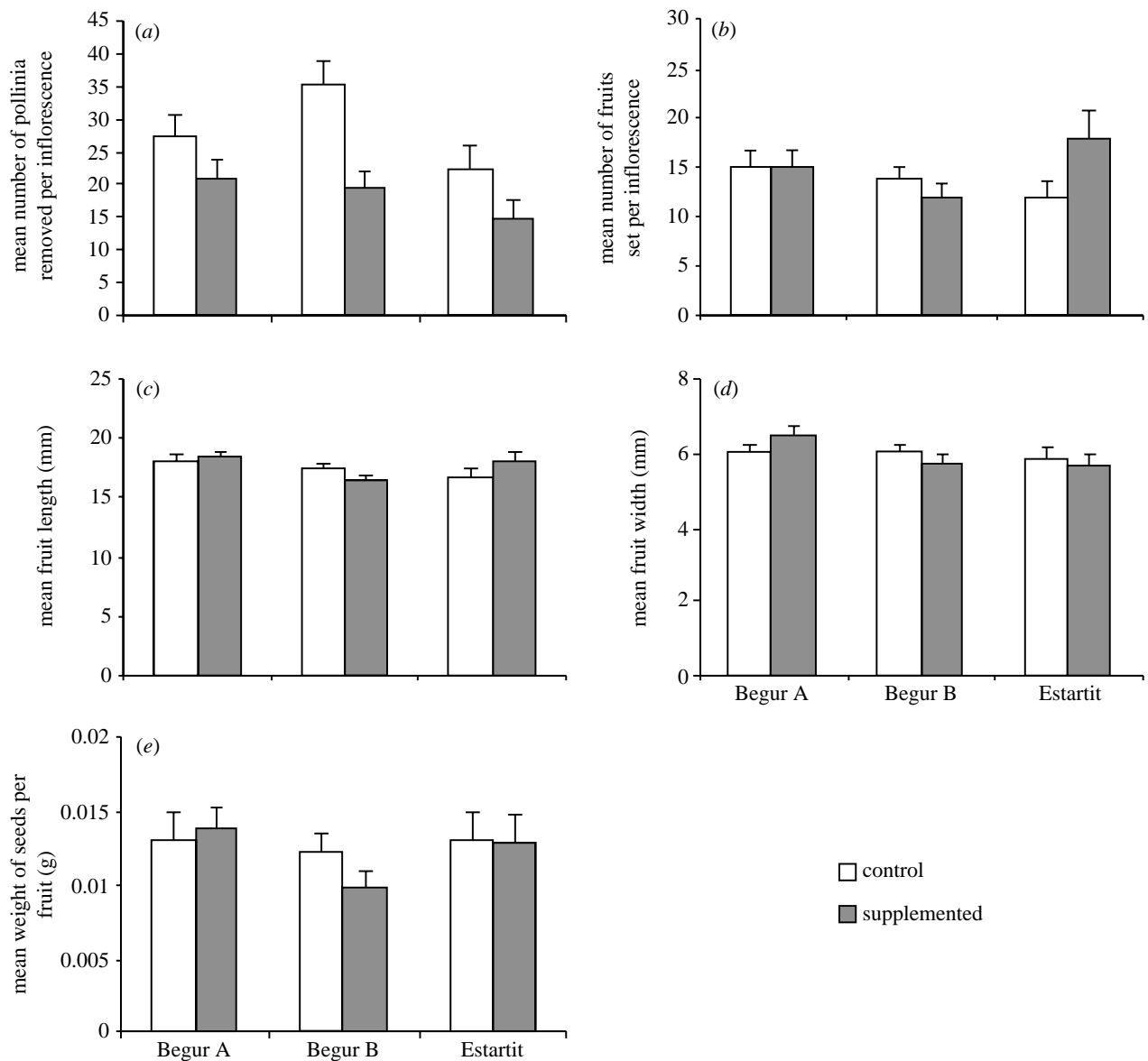
Figure 1. Responses of inflorescences of *B. robertiana* to supplementation of flowers with 2 μl of 30% sucrose solution each day for 20 days. The mean numbers of (*a*) pollinia removed, (*b*) fruits set, (*c,d*) fruit size and (*e*) seed weight per fruit are given in three populations for supplemented and control inflorescences. Bars indicate one standard error of the mean.

flower, while the other had a control manipulation. Pairs were identical in open flower number and measured and matched for other traits (inflorescence height, lip length and width, and spur length and width). Pairs were then offered side by side affixed to the end of a cane to a *B. lucorum* foraging on the coflowering plant species *R. officinalis*. Coin tosses were used to decide on the identity of the rewarding plant and the side of the cane into which the plants were placed. Only inflorescences without previous pollinia removals or depositions on open flowers were used. The experiments were conducted over a 3 week period and all plants were replaced into their positions within the population after use (all plants survived).

When a bee chose to visit the potted plants, we recorded its caste and counted the pollinaria that it was carrying, if any. The number and sequence of flowers visited on the inflorescence, the length of time spent per flower and whether pollinia were removed or pollen deposited were also recorded. Each tested pollinator was caught and marked on the thorax with

solvent-free correction fluid in order to prevent reuse. Flowers with depositions had their stigmas excised and collected, and subsequently the number of massulae deposited per stigma were counted under a dissecting microscope.

We tested for differences between inflorescence types in the mean number of flowers visited per inflorescence, the mean time spent per flower by pollinators, the mean number of pollinia removed per inflorescence visit, the probability of pollinium removal, the mean number of stigmas per inflorescence visit which received pollen depositions, the probability of pollen deposition and the number of massulae deposited per stigma using *t*-tests. Only the first inflorescence visited was included in this analysis. Probabilities were calculated by dividing the numbers of depositions or removals by the number of flowers visited. Data was either log_{10}-transformed or arcsine-transformed (probabilities) before analysis. We compared depositions onto the two inflorescence types made by pollinators that were not carrying pollinaria in order to test for differences in geitonogamy.

Table 1. Results of ANOVAs of pollinia removal and numbers of fruits set in the population supplementation experiment.

(Population and treatment were fixed factors and the table gives values of F and degrees of freedom (d.f.). The sample sizes indicate the total numbers of individual inflorescences compared within each of the three populations tested that survived at Begur A, Begur B and Estartit, respectively (experiments were started with 40 inflorescences at Begur A and B and 32 inflorescences at Estartit). The significances for F are indicated after sequential Bonferroni correction ($^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$).)

fitness measure	sample size	population		treatment		population × treatment	
		F	d.f.	F	d.f.	F	d.f.
numbers of pollinia removed	40, 39 and 32	2.04	2,105	118.44**	1,105	0.29	2,105
numbers of fruits set	39, 39 and 32	0.79	2,104	0.25	1,104	0.94	2,104

Table 2. Results of ANOVAs on fruit length, fruit width and seed weight from the population supplementation experiment.

(Treatment, population, position of pod within the inflorescence and plant are fixed factors with plant nested within populations. The significances for F are indicated after sequential Bonferroni correction (as for table 1). Degrees of freedom (d.f.) are also given. Split fruits were excluded from seed weight analysis.)

source	fruit length		fruit width		seed weight	
	F	d.f.	F	d.f.	F	d.f.
population	10.20***	2,234	12.55***	2,234	0.88	2,159
treatment	3.18	1,234	0.29	1,234	1.36	1,159
population × treatment	3.99	2,234	2.66	2,234	1.38	2,159
position	33.49***	2,234	17.19***	2,234	11.99***	2,159
plant	3.34***	49,234	5.11***	49,234	2.22***	47,159
population × position	0.31	4,234	0.28	4,234	0.10	4,159
treatment × position	0.63	2,234	0.15	2,234	1.38	2,159
population × treatment × position	2.44	4,234	0.85	4,234	2.33	4,159

3. RESULTS

(a) *Population supplementation experiments*

There were large and highly significant differences in pollinium removal between the two treatments (figure 1 and table 1), but these were not in the direction expected. Control inflorescences had on average over 50% more pollinium removal than supplemented inflorescences (control inflorescences $= 28.69 \pm 2.08$ and supplemented inflorescences $= 18.64 \pm 1.72$). Pollinium removal was higher for controls in all populations studied and there were no significant effects of population × treatment interaction (figure 1 and table 1). Again contrary to expectations, there were no significant effects of treatment or interactions with treatment on fruit set (figure 1 and table 1), fruit length, fruit width or seed weight per fruit (figure 1 and table 2).

There were no significant differences between supplemented and control inflorescences for the sizes of any of the traits recorded. Furthermore, ANCOVA revealed no significant interactions between traits and either pollinium removal or fruit set and the highly significant effects of pollinium removal remained with the inclusion of all six traits in the model ($F_{1,99} = 18.17$ and $p < 0.001$).

One hundred and eight out of 118 plants marked in 1999 were relocated in 2000. A comparison of the statuses in 2000 suggested no significant differences between

treatments (G-test of independence, $G_{\text{adj 2}} = 1.62$ and $p > 0.05$) (36% flowering, 62% non-flowering and 2% not emergent).

(b) *Pollinator behaviour experiments*

The results of these experiments are summarized in figure 2. Fifty-one successful visits were recorded by *B. lucorum*. The identity of the first inflorescence of a pair that the pollinators chose was random with respect to the presence of sucrose (26 bees chose control inflorescences first and 25 chose supplemented ones). As predicted, there were three times more flowers visited per inflorescence for supplemented than control inflorescences and this comparison is highly significant (t-test, $t_{49} = 3.76$ and $p < 0.001$). Similar results were found by observing pollinators in populations during 1999 where 3.60 ± 0.30 ($n = 38$ bees) flowers were visited for control inflorescences and 12.25 ± 5.45 ($n = 4$ bees) flowers were visited for supplemented inflorescences (Mann–Whitney U-test, $Z = 2.56$ and $p = 0.01$). The mean time bees spent on each flower in experiments did not differ significantly between treatments (t-test, $t_{48} = 0.51$ and $p > 0.05$).

The mean number of pollinia removed per inflorescence visit was over eight times larger for control inflorescences than supplemented ones and this comparison is again highly significant (t-test, $t_{49} = -4.40$ and $p < 0.001$). There were no pollen depositions onto any

flowers for either supplemented or control inflorescences when incoming bees carried no pollinaria. Where incoming pollinaria were present, the number of depositions made per inflorescence visit was not significantly different for the two treatments (*t*-test, $t_{13} = 0.44$ and $p > 0.05$). There were no significant effects of the presence or absence of incoming pollinaria on other factors such as pollinia removal ($F_{1,47} = 0.01$ and $p > 0.05$) or the number of flowers visited per inflorescence ($F_{1,47} = 0.02$ and $p > 0.05$). There were no significant differences in the numbers of massulae deposited per stigma between supplemented and control inflorescences (*t*-test, $t_{51} = 0.42$ and $p > 0.05$).

There was a significant effect of bee caste on pollinia removal (two-way ANOVA, $F_{1,47} = 23.83$ and $p < 0.05$). Queens made more removals on average than workers. However, both castes showed the same responses to the two treatments (two-way ANOVA caste × treatment interaction, $F_{1,47} = 2.51$ and $p > 0.05$). Pollen deposition effects were not tested across castes of bees due to the low number of bees with incoming pollinaria ($n = 15$).

Figure 2 also compares the probability of pollinium removal and deposition per flower visited as a measure of the efficiency of pollen transfer. The probability of pollinium removal is ten times higher for control than supplemented flowers and this comparison is highly significant (*t*-test, $t_{49} = 4.95$ and $p < 0.001$). The probability of pollen deposition on a flower is slightly but not significantly greater for controls compared to supplemented inflorescences (*t*-test, $t_{13} = 0.84$ and $p > 0.05$). The results of ANCOVAs testing for the effects of floral traits on the probabilities of removal and deposition showed that there were no significant effects of floral traits or interactions with treatment.

4. DISCUSSION

Our results did not agree with our *a priori* predictions. In contrast, we show, for the first time to the authors' knowledge, that there may be a strong reproductive advantage for rewardlessness in an orchid species. The probability of pollinium removal was decreased by an order of magnitude for supplemented inflorescences in the pollinator behaviour experiments and this resulted in eight times more pollinia being removed per inflorescence visit for controls. These results occurred despite the fact that pollinators visited significantly more flowers on supplemented inflorescences. The probability of pollen deposition and the numbers of stigmas onto which pollen were deposited were not significantly affected by supplementation and these even tended to be slightly higher for control inflorescences. The results of supplementation within populations confirmed the pollinator behaviour experiments in that there was a highly significant decrease in pollinium removal on supplemented inflorescences across all populations. Further, there were no significant changes in fruit set or fruit quality for the two treatments and no differences in flowering probabilities the following year. We did not find the predicted increase in geitonogamous pollen depositions for supplemented inflorescences as no geitonogamous depositions were recorded in the experiments regardless of treatment.
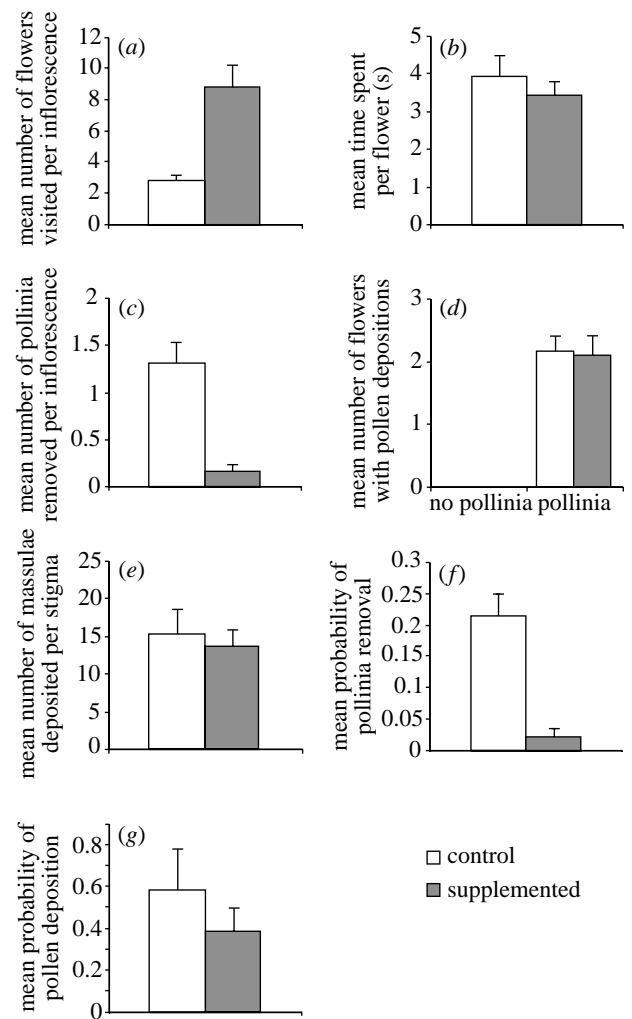
Figure 2. Results of pollinator behaviour experiments with *B. robertiana* inflorescences. The mean numbers of (*a*) flowers visited per inflorescence, (*b*) time spent per flower, (*c*) pollinia removed, (*d*) flowers with pollen depositions, (*e*) number of massulae per stigma and the probabilities of (*f*) pollinia removal and (*g*) deposition are given for inflorescences supplemented with 2 μl of 30% sucrose solution and for control inflorescences. Bars indicate one standard error of the mean. The mean number of massulae per pollinium was 45.48 ± 6.66 ($n = 27$).

These results are unlikely to be artefactual. Supplementation in itself did not adversely affect inflorescences in terms of shortening flower life. Inflorescences did not differ significantly in floral traits, nor did floral traits significantly interact with treatment. Bees were not visually assessing the rewards present in flowers nor was there any evidence that mimicry could have influenced pollinator behaviour. We found that the flowering time of some inflorescences exceeded the 20 day experimental period, resulting in a small but significant decrease in pollinium removal ($F_{1,110} = 16.10$ and $p < 0.001$) and fruit set ($F_{1,110} = 33.95$ and $p < 0.001$) for experimental plants compared to open controls. However, there was no evidence that pollinator selection changed at the end of the flowering season (the patterns of selection on traits for experimental and control plants were identical). It is unlikely that the small volume of supplemented sucrose in

itself would cause a change in probe depth by bees, and we estimated that any changes would be similar to the standard deviations in spur length across populations.

How then can the patterns observed be explained? Field observations suggested that the enhanced pollinium removal on rewardless plants was due to bee search behaviour. When nectar was not located in an initial proboscis extension into the spur, active searching continued. Research on rewarding plants shows that bumble-bees may spend more time searching for nectar on novel flowers when compared to experienced bees, even if nectar is not successfully located initially (Laverty 1994). On *B. robertiana*, we observed a change of body position on the flower as the bee thrust its body more strongly into the flower and perpendicularly to the lip in order to search the spur more actively. This push movement appeared to result in the viscidia, the sticky pad at the base of the pollinaria, becoming firmly attached to the body of the bee. When a reward was located easily in the supplemented flowers, the bee's body position remained parallel to the lip, possibly because this allowed it to move on to the next flower more rapidly, but apparently reducing the chance of coming into contact with the viscidia. The lack of change in pollen deposition probability and amount may indicate that deposition is less crucially related to bee behaviour, possibly due to the large stigmatic surface area or projecting position of the pollinia. Other authors have found that variation in flower morphology can critically affect the amount of pollen removed from a flower by bees, while pollen deposition is not so affected (Galen & Stanton 1989; Young & Stanton 1990). After pollinarium removal, the caudicle holding the pollinium above the body of the bee bends forward, resulting in a suitable position for deposition. The absence of geitonogamous pollen depositions probably reflects a slow bending time, suggesting low geitonogamy rates in this species (Johnson & Nilsson 1999; Johnson & Edwards 2000).

Do these results indicate potential fitness differences between rewarding and unrewarding morphs if these were found in populations of *B. robertiana*? The lack of differences in pollen deposition, fruit set and fruit quality suggests that maternal function is unlikely to differ between the two morphs. The lack of geitonogamy suggests there are unlikely to be differences in the proportion of selfed progeny between the two morphs. The increased numbers and probability of pollinia removed per visit suggest that the numbers of seeds sired on other individuals could be significantly greater for the rewardless morph. We did not test this hypothesis directly, but the number of pollinaria attached to a pollinator arriving at an inflorescence is significantly and positively related to the numbers of flowers receiving pollen depositions ($F_{1,20} = 4.81$ and $p < 0.05$). The numbers of pollinia removed and those subsequently deposited onto other plants were highly positively correlated in the orchid *Aerangis ellisii* (Nilsson *et al.* 1992). Thus, we suggest that rewardlessness could have evolved through male advantage.

Can these experiments shed light on why rewardlessness is widespread in the Orchidaceae? A male function advantage is, to us, potentially a more parsimonious explanation than alternative hypotheses (Nilsson 1992)

such as evolution through reduced geitonogamy (Dressler 1981) as there is no reliance on a high cost of selfing. We predict from these results that rewardlessness would be most likely to evolve in generalist pollinators such as bumble-bees whose searching behaviour causes this pattern of increased pollinium removal. In his review of orchid pollination strategies Nilsson (1992) concluded that the mechanism resulting in the evolution of rewardlessness is linked to the possession of pollinia, and we suggest that it is the link between pollinator behaviour and all-or-nothing pollinium removal that may have resulted in rewardlessness being such a common strategy in the Orchidaceae.

REFERENCES

Ackerman, J. D. 1981 Pollination biology of *Calypso bulbosa* var. *occidentalis* (Orchidaceae): a food-deception system. *Madroño* **3**, 101–110.

Ackerman, J. D. 1986 Mechanisms and evolution of food-deceptive pollination systems in orchids. *Lindleyana* **1**, 108–113.

Ackerman, J. D., Rodriguez-Robles, J. A. & Meléndez, E. J. 1994 A meager nectar offering by an epiphytic orchid is better than nothing. *Biotropica* **26**, 44–49.

Dafni, A. 1984 Mimicry and deception in pollination. *A. Rev. Ecol. Syst.* **15**, 259–278.

Dafni, A. 1987 Pollination in *Orchis* and related genera: evolution from reward to deception. *Orchid Biol. Rev. Perspect.* **4**, 79–104.

Darwin, C. 1904 *The various contrivances by which orchids are fertilized by insects*, 2nd edn. London: J. Murray.

De Jong, T. J., Waser, N. M. & Klinkhamer, P. G. L. 1993 Geitonogamy: the neglected side of selfing. *Trends Ecol. Evol.* **8**, 321–325.

Dressler, R. L. 1981 *The orchids—natural history, and classification.* Cambridge, MA: Harvard University Press.

Galen, C. & Stanton, M. L. 1989 Bumble bee pollination and floral morphology: factors influencing pollen dispersal in the Alpine sky pilot, *Polemonium viscosum* (Polemoniaceae). *Am. J. Bot.* **76**, 419–426.

Gill, D. E. 1989 Fruiting failure, pollinator inefficiency, and speciation in orchids. In *Speciation and its consequences* (ed. D. Otte & J. A. Endler), pp. 458–481. Sunderland, MA: Sinauer.

Holsinger, K. E., Feldman, M. W. & Christiansen, F. B. 1984 The evolution of self-fertilization in plants: a population genetic model. *Am. Nat.* **124**, 446–453.

Johnson, S. D. & Edwards, T. J. 2000 The structure and function of orchid pollinaria. *Plant Syst. Evol.* **222**, 243–269.

Johnson, S. D. & Nilsson, L. A. 1999 Pollen carryover, geitonogamy, and the evolution of deceptive pollination systems in orchids. *Ecology* **80**, 2607–2619.

Laverty, T. M. 1994 Bumble bee learning and flower morphology. *Anim. Behav.* **47**, 531–545.

Neiland, M. R. M. & Wilcock, C. C. 1994 Reproductive ecology of European orchids. In *Proceedings of the 14th World Orchid Conference* (ed. A. Pridgeon), pp. 138–147. London: HMSO.

Neiland, M. R. M. & Wilcock, C. C. 1998 Fruit set, nectar reward, and rarity in the Orchidaceae. *Am. J. Bot.* **85**, 1657–1671.

Nilsson, L. A. 1992 Orchid pollination biology. *Trends Ecol. Evol.* **7**, 255–259.

Nilsson, L. A., Rabakonandrianina, E. & Petterson, B. 1992 Exact tracking of pollen transfer and mating in plants. *Nature* **360**, 666–668.

Rice, W. R. 1989 Analysing tables of statistical tests. *Evolution* **43**, 223–225.

Smithson, A. & Macnair, M. R. 1997 Negative frequency-dependent selection by pollinators on artificial flowers without rewards. *Evolution* **51**, 715–723.

Van der Cingel, N. A. 1995 *An atlas of orchid pollination, European orchids.* Rotterdam, The Netherlands: A. A. Balkema.

Young, H. J. & Stanton, M. L. 1990 Influences of floral variation on pollen removal and seed production in wild radish. *Ecology* **71**, 536–547.