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Original article

The effects of plant density and nectar reward on bee visitation to the endangered orchid *Spiranthes romanzoffiana*

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ABSTRACT

Density can affect attraction of pollinators, with rare plants receiving fewer pollinating visits compared with more common co-flowering species. However, if a locally rare species is very attractive in terms of the rewards it offers pollinators, it may be preferentially visited. *Spiranthes romanzoffiana* is a nectar rewarding, geographically rare, endangered orchid species which forms small populations in Ireland, co-flowering with more common, florally rewarding species. We examined visitation rates to *S. romanzoffiana* and two nectar rewarding co-flowering species (*Mentha aquatica* and *Prunella vulgaris*) in the west of Ireland. These three plant species were visited by three bee species (*Bombus pascuorum*, *B. hortorum* and *Apis mellifera*). *B. pascuorum* was the most common visitor, while *A. mellifera* was least common. Our results suggest that individual *S. romanzoffiana* inflorescences compete intra-specifically for visitation from pollinators at high densities. The relationship between visitation to *S. romanzoffiana* and total floral density appeared to be positive, suggesting interspecific facilitation of pollinator visitation at high densities. Nectar standing crop varied through the season, among species and between open and bagged flowers. Nectar standing crop was not correlated with visitation in *S. romanzoffiana*. Despite relatively high visitation, *S. romanzoffiana* produced no mature fruit during this flowering season. The lack of fruit maturation in this species may be a major factor causing its rarity in Europe.

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1. Introduction

The density at which plants occur within a population can affect their reproductive success. Low density and fragmented populations of outcrossing, seed-producing plants may suffer from limited seed recruitment due to a number of factors including: (i) a low number of potential mates (Alexandersson and Ågren, 1996), (ii) inbreeding depression caused by mating with closely related individuals (Fritz and Nilsson, 1994) and (iii) reduced attractiveness to pollinators (Sih and Baltus,

1987; Eriksson and Ehrlén, 1992; van Treuren et al., 1993) and pollination limitation (Burd, 1994; Larson and Barrett, 2000; Spira, 2001). Reduced fecundity of plants in small populations will put them at greater risk from environmental change and other stochastic processes (Ellstrand and Elam, 1993; Kearns et al., 1998). Plant density can be considered at two scales: either the number of individuals in a local area (i.e. per population) (Kunin, 1993) or the number of populations in a geographic area (Hartley and Kunin, 2003). Rare plants may be either locally rare (a small number of individuals per

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population) or geographically rare (a low number of populations in a region), or both. In this paper, we examine how small-scale density affects pollination success of a geographically rare species within a locally abundant population.

Many rare plants, whether locally or geographically rare, occur in mixed arrays, often sharing pollinators with more common co-flowering species. Consequently, we must also consider the relative density and impact of co-flowering species on the pollination of rare plants. Individuals of a plant species that occur at low density may compete interspecifically with co-flowering species for pollinator attention (Waser, 1978; Rathcke, 1983; Bell et al., 2005). This is because many pollinators tend to prefer abundant plant species and large floral displays in order to maximise foraging efficiency (Heinrich, 1979; Goulson, 1994; Stout et al., 1998; Mitchell et al., 2004). In addition, plants that occur at a low frequency and in a mixed arrays are more likely to suffer from improper pollen transfer (IPT) (the transfer of pollen to a stigma of a different species) and pollen wastage (Rathcke, 1983; Goulson, 1994; Stout et al., 1998). Ultimately this can result in pollination limitation (Wilcock and Neiland, 2002). Alternatively, if a species is geographically restricted, but locally abundant, individuals may also face intra-specific competition for pollinator attention (Campbell and Motten, 1985; Kunin, 1993; Brown and Mitchell, 2001). In this case, although the population as a whole may receive higher rates of pollinator visitation, individuals may compete with one another for visitation, and mate with closely related individuals, which increases the likelihood of inbreeding (Ellstrand and Elam, 1993; Karron et al., 2004). On the other hand, facilitation may occur whereby co-flowering conspecific or heterospecific plants form an increased floral display and all individuals receive increased visitation (Rathcke, 1983; Geer et al., 1995; Moeller, 2004). This is the “magnet species” effect (Lavery, 1992), and is known mainly in nectarless orchids (e.g. Johnson et al., 2003; Juillet et al., 2007).

In addition to plant density, the quantity and quality of reward provided by a plant can affect the foraging behaviour of pollinators and consequently plant reproductive success. The quantity of nectar reward can affect how many visits a plant receives (attractiveness to pollinators) (Klinkhamer and van der Lugt, 2004) and the duration of visits (Pleasants and Zimmerman, 1979; Pleasants, 1989). However, Rodriguez-Robles et al. (1992) found that nectar availability was not a good predictor of effective pollinator visitation. The quality of nectar reward can affect the pollinator species attracted to individuals, for example, bumblebees prefer concentrated nectar, as dilute nectar can be heavy to carry and inefficient to store (Waser, 1983; Wilson et al., 2006). The quality of nectar reward of a rare species compared with more common co-flowerers may further affect its pollination success.

Terrestrial orchid populations often occur in mixed arrays, at variable densities, and with variable nectar rewards (approximately one-third of the Orchidaceae provide no nectar at all) (Nilsson, 1992; Ackerman et al., 1994; Neiland and Wilcock, 1998). Hence orchids are particularly susceptible to competition with co-flowering species and IPT (Rathcke, 1983; Alexandersson and Ågren, 1996; Neiland and Wilcock, 1999).

This can be particularly detrimental for orchids as their pollen amasses as pollinia and so IPT may result in the loss of an individual flower's entire male complement (Dressler, 1981; Johnson et al., 2005).

Spiranthes romanzoffiana is a nectar rewarding orchid species with an uneven amphi-Atlantic distribution (Forrest et al., 2004). Individuals produce lateral buds in the autumn, which over-winter and develop into the following year's vegetative or flowering parts (Forrest et al., 2004). *S. romanzoffiana* may reproduce vegetatively by infrequent production of twin (or, rarely, three to four) lateral buds, which can lead to the formation of multiple inflorescences (Summerhayes, 1968). It occurs widely in North America where it is pollinated by medium- and long-tongued bumblebees and solitary bees (Catling, 1983) but in Europe, it only occurs in Britain and Ireland. The percentage of flowers that set fruit has been reported at relatively high levels (>75%) in North America (Larson and Larson, 1987). Greenhouse hand pollination experiments in North America demonstrated that the *S. romanzoffiana* was incapable of agamospermy (seed production without pollen) (Catling, 1982). However, this species is capable of autogamy (within flower self-pollination) with 64% fruit set, with both geitonogamy (within plant self-pollination) and xenogamy (outcrossing) pollination treatments, yielding 100% fruit set (Catling, 1982). In Britain and Ireland, *S. romanzoffiana* has never been reported to set fruit (Forrest et al., 2004). However, Forrest et al. (2004) demonstrated that northern Scottish populations (from the Hebridean Islands) are genetically distinct from Irish populations. They suggest that reproduction (hence successful seed set) could be historical or current but infrequent. It is possible that infrequent seed set events may have been overlooked (Forrest et al., 2004) or that the rarity of the species and associated pollinator limitation is responsible for the lack of fruit set.

Little is known of the pollination ecology of orchids in Ireland in general and *S. romanzoffiana* in particular. In Ireland, *S. romanzoffiana* is known from only nine 10 × 10 km squares (Preston et al., 2002) in the north and west of the country, and often occurs in very small populations. Hence this species can be considered both locally and geographically rare. *S. romanzoffiana* is protected in the Republic of Ireland by the Wildlife Act (1976) and the Wildlife Amendment Act (2000), under the Flora Protection Order (1999). In this study, we investigate the impacts of inflorescence density and nectar production by *S. romanzoffiana* and co-flowering species on pollinator visitation in Ireland. Specifically, we tested the following hypotheses:

1. Pollinator visitation to *S. romanzoffiana* is affected by its density and relative frequency compared with common co-flowering species.
2. Pollinator visitation to *S. romanzoffiana* is affected by nectar rewards, specifically:
 - (a) *S. romanzoffiana* has nectar reward that is depleted by insect visitors
 - (b) nectar levels vary over the flowering season
 - (c) there is a relationship between nectar standing crop and pollinator visitation rates.
3. Fruit set in *S. romanzoffiana* is related to visitation rates.

2. Materials and methods

2.1. Study site

This study was conducted in a population of approximately 200 flowering *S. romanzoffiana* inflorescences (mean no. flowers per inflorescence (\pm SD) = 15.94 ± 5.51 ; $n = 50$) in a $1 \text{ km} \times 0.5 \text{ km}$ area in Co. Mayo, Ireland. This population was selected for study as it is the largest known population (other populations contain <50 , though occasionally up to 100, flowering individuals; K.J. Duffy, personal observation) and plants formed naturally discrete patches of varying densities. The study site is adjacent to a lakeshore and is regularly flooded during winter months but remains dry during the summer. Other common flowering vegetation at the site included *Mentha aquatica*, *Prunella vulgaris*, *Anagallis tenella*, *Leontodon hispidus*, *Lythrum salicaria*, and *Parnassia palustris*. *P. vulgaris* and *M. aquatica* were selected for further study as potential competitors/facilitators because they are also small herbaceous plants displaying flowers at the same time, in the same patches and at a similar height as *S. romanzoffiana* and flowers are visited by the same insect species foraging for nectar. Other co-flowering species were visited by the same species but to a much lesser extent (K.J. Duffy, personal observation) and did not form dense patches alongside *S. romanzoffiana*. This study was carried out between July 27th and September 1st 2005.

2.2. Pollinator visitation

Six patches ($3\text{--}4 \text{ m} \times 3\text{--}4 \text{ m}$) within the *S. romanzoffiana* population were selected according to natural density of *S. romanzoffiana* (ranging from 0.33 to 3.25 inflorescences per m^2). Twenty-minute observations were carried out in these patches between 08.30 and 19.30 h on 16 days over the flowering season. Observations were only made on days without rain or strong winds (mean temperature taken at ground level during observations (\pm SE) $19.7 \pm 0.18^\circ\text{C}$; range $16\text{--}21^\circ\text{C}$; % cloud cover during observations (\pm SE) $43.6 \pm 4.4\%$; range 0–100%). Observations of each patch were made randomly throughout the day to minimise the effect of time of day on data collected in a particular patch. All insects entering patches and visiting flowers, probing for nectar or collecting pollen, and the number of inflorescences visited by each individual, were recorded. The relationships between the total number of visits to *S. romanzoffiana* (per inflorescence per hour) and density of *S. romanzoffiana* (inflorescences per m^2) and, separately, total flower density (inflorescences per m^2), were analysed using curve estimation regression analysis. In addition, we controlled for the effect of *S. romanzoffiana* density in observational patches using partial correlation coefficients to examine the relationship between density of co-flowering species and visitation to *S. romanzoffiana*. These analyses were performed using SPSS 14.0.1[®] (SPSS Inc., 2005).

2.3. Pollinia removal and deposition

In order to measure pollinia removal and deposition in *S. romanzoffiana*, we examined 30 flowers from different inflorescences

selected at random throughout the population on the 8th of August and 30 flowers on the 20th of August (which corresponds with the beginning and middle of flowering by *S. romanzoffiana*) for the presence or absence of pollinia.

2.4. Nectar measurements

On each day pollinator observations were made, 10 randomly selected flowers of *S. romanzoffiana*, *P. vulgaris*, and *M. aquatica* were sampled for nectar standing crop. Nectar was sampled using $1 \mu\text{l}$ glass micropipettes (Drummond “Microcaps”, Drummond Scientific Co., USA). On three days during the flowering season (one day at each of the beginning, middle and end), nectar was taken from flowers that had been bagged for 24 h. Sugar concentration was measured using a hand-held refractometer modified for small volumes (Ceti-Digit-080, Carl Stuart, Ireland). To test whether sugar concentration differed among focal species, we used a Kruskal–Wallis test, with significant terms analysed with Mann–Whitney tests with Bonferroni correction. To test whether standing crop varied over the flowering season among focal species, nectar volumes were compared according to species and period in the season (beginning, middle, and end). Two days within each of the beginning, middle and end of flowering season were selected and analysed using a three-factor mixed model ANOVA ($n = 10$) with day nested within period of season. To examine differences in nectar standing crop and volume in bagged flowers among species, we used a three factor ANOVA ($n = 10$), according to species, treatment (bagged vs. unbagged) and day. Nectar volume data were $\ln(x + 1)$ transformed prior to analyses where necessary (i.e. when variances were heterogeneous), and were analysed using GMAV 5 (Underwood and Chapman, 1997). Significant terms were analysed using post hoc Student–Newman–Keuls (SNK) tests to determine which means differed significantly from each other. To test for the effect of visitation on nectar standing crop we used Pearson correlation on means calculated from the pooled nectar standing crop and visitation rates over the flowering season. These data were analysed using SPSS 14.0.1[®] (SPSS Inc., 2005).

2.5. Fruit production

At the end of the flowering season fruits were examined randomly from 1000 individual *S. romanzoffiana* flowers.

3. Results

3.1. Pollinator visitation

A total of 1571 visits were observed to the three focal plant species in 21 h of observations over the flowering season. *Bombus pascuorum* (medium-tongued bumblebee) (81.47% of all visits \pm SE 1.84%), *B. hortorum* (long-tongued bumblebee) (17.59% of all visits \pm SE 1.2%), and *A. mellifera* (short-tongued honeybee) (0.94% of all visits \pm SE 0.15%) were the only diurnal insects recorded visiting flowers of *S. romanzoffiana*, *P. vulgaris*, and *M. aquatica*. *B. pascuorum* was the most frequent visitor to all focal plant species. When data from all patches are pooled, the three focal species received similar

visitation rates per inflorescence per hour (*S. romanzoffiana*: 0.52 visits per hour \pm SE 0.083; *P. vulgaris*: 0.64 visits per hour \pm SE 0.102; and *M. aquatica*: 0.73 visits per hour \pm SE 0.073). Several Lepidopterans were present in the habitat but were not observed to visit the focal plant species. Visits by bumblebees occurred mainly between 10:30 and 17:00 h. We noted that visitors foraged for up to a maximum of 60 s per *S. romanzoffiana* inflorescence and visited a maximum 10 *S. romanzoffiana* inflorescences in sequence. There was a significant negative relationship between density of *S. romanzoffiana* and visitation per *S. romanzoffiana* inflorescence per hour (visitation rate = $-0.28 \ln(\text{density}) + 0.55$, $R^2 = 0.6628$, $F_4 = 7.86$, $p = 0.049$; Fig. 1a). The relationship between total floral density and visitation to *S. romanzoffiana* appeared to be positive, except in the patch with the highest floral density (Fig. 1b). However, none of the curve estimation regression analyses fitted these data. In addition, when *S. romanzoffiana* density was controlled for, there was no relationship between density of co-flowering species and visitation to *S. romanzoffiana* ($r = 0.165$; $p = 0.068$).

3.2. Pollinia removal and deposition

We found pollinia had been removed from 9/30 flowers on the 8th August and 14/30 on the 20th August. Pollinia had been deposited on 1/30 stigmas on the 8th August and 7/30 on the 20th August. Flowers with their own pollinia intact (i.e. those

where the pollinia had not been removed) were not observed to have received pollinia deposition on their stigmas. Pollinia (though not always entire pollinia) were observed on 19 individuals of *B. pascuorum* in flight and 4 individuals of *B. hortorum*. Pollinia were visible on the proboscis and/or thorax of these species from within a 1 m distance from the observer. No pollinia were seen on *A. mellifera* despite individuals being observed within similar distances. The mean corolla length of *S. romanzoffiana* (\pm SE) in this population is 9.9 mm \pm 1.2 mm ($n = 50$), which may be too long for the tongue of *A. mellifera* (mean tongue length 6.5–6.7 mm; Alpatov, 1929).

3.3. Nectar levels

There were significant differences in standing crop among the three focal plant species, with most nectar found in *S. romanzoffiana*, and least in *M. aquatica* (Table 1, Fig. 2). There was no significant difference in nectar standing crop through the flowering season in any focal species (Table 1). When we examined differences in nectar standing crop volume in bagged flowers among species, we found a significant interaction between plant species, treatment (bagged vs. unbagged) and day (Table 2). SNK post hoc tests revealed that all three species had more nectar in bagged compared with open flowers on day 2 (Fig. 3a, b and c). However, *P. vulgaris* had more nectar in open than bagged flowers on day 1. There were other variations among sampling days, but with no consistent pattern. *S. romanzoffiana* had more nectar in bagged flowers on all days compared with *P. vulgaris* ($p < 0.01$) and more than open flowers of *M. aquatica* on one sampling day. There was significant variation in nectar concentration among focal species ($H_2 = 18.64$; $p < 0.001$). Mann-Whitney post hoc tests with Bonferroni correction revealed both *S. romanzoffiana* ($U = 117$; $p < 0.001$) and *P. vulgaris* ($U = 54$; $p = 0.003$) had a higher sugar concentration than *M. aquatica*, but there was no difference between the concentration of *S. romanzoffiana* and *P. vulgaris* ($U = 375$; $p = 0.689$). Mean sugar concentration (\pm SE) in *S. romanzoffiana* was $24 \pm 0.4\%$ ($n = 43$), $20 \pm 0.3\%$ ($n = 16$) in *M. aquatica*, and $23 \pm 0.6\%$ ($n = 18$) in *P. vulgaris*. No significant correlation was found between mean standing crop and mean number of visits over the flower season of *S. romanzoffiana* ($r = -0.25$; $p = 0.35$) nor in *M. aquatica* ($r = -0.05$; $p = 0.854$), however there was a significant positive correlation between

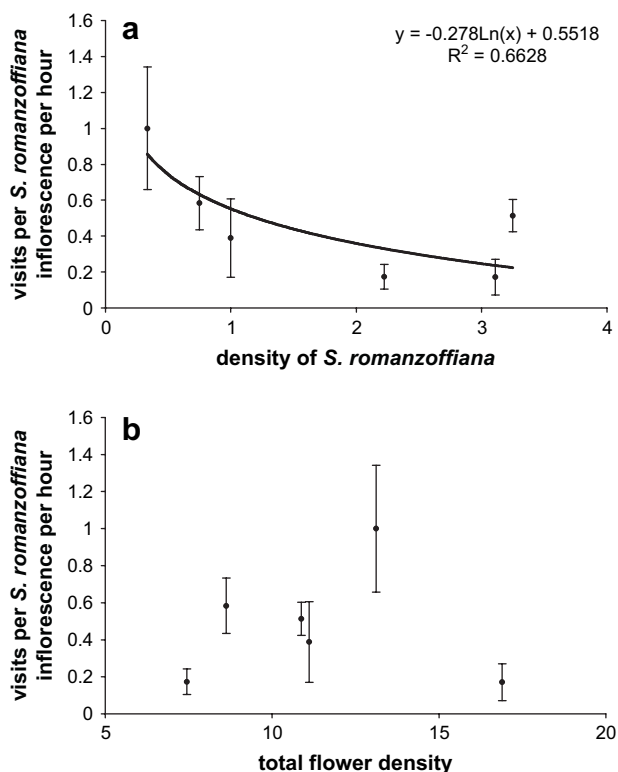


Fig. 1 – Mean number of visits to *S. romanzoffiana* (per inflorescence per hour) (\pm SE) according to (a) density of *S. romanzoffiana* in patches (inflorescences/m²) and (b) total floral density in patches (inflorescences/m²).

Table 1 – Three factor mixed model ANOVA of nectar standing crop (μ l/flower) according to focal plant species (*S. romanzoffiana*, *P. vulgaris* and *M. aquatica*), time in season (beginning, middle and end) and day (nested within time during season)

Source of variation	df	SS	MS	F	p
Species	2	1.53	0.765	57.09	0.0001
Time in season	2	0.054	0.027	5.38	0.1017
Day (time in season)	3	0.015	0.005	0.76	0.5158
Species \times time in season	4	0.103	0.029	1.93	0.2249
Species \times day (time in season)	6	0.08	0.013	2.02	0.0653
Error	162	1.072	0.006		

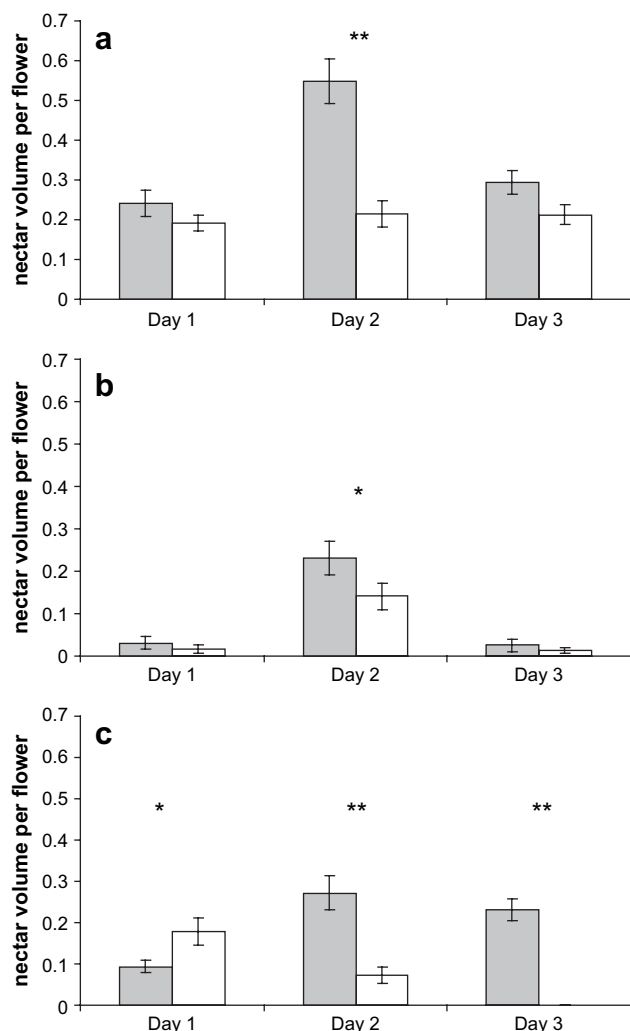


Fig. 2 – Mean nectar volume per flower (μl) taken on three days during the flowering season (±SE): (a) *S. romanzoffiana*, (b) *M. aquatica*, (c) *P. vulgaris*. Shaded bars represent bagged flowers; open bars represent standing crop. Significant differences according to SNK tests: *p < 0.05; **p < 0.01.

mean standing crop and mean number of visits to *P. vulgaris* ($r = 0.609$; $p = 0.012$).

3.4. Fruit production

Fruit maturation was not observed in any *S. romanzoffiana* plants in the population. Capsules were observed to go flaccid and wither within one week after flowers withered.

4. Discussion

4.1. Pollinator visitation and pollinia removal and deposition

Despite being nationally rare, *S. romanzoffiana* was relatively abundant in the study site and was frequently visited by

medium-tongued (*B. pascuorum*) and long-tongued (*B. hortorum*) bumblebees, and occasionally by short-tongued honeybees (*A. mellifera*). This is the first time that insect visitors to this orchid species have been recorded in Ireland. Only bumblebees were observed to pick up pollinia, and presumably these species act as the primary pollinators of *S. romanzoffiana*. However, although we cannot rule out the possibility that nocturnal insects also visit flowers, most records show that members of *Spiranthes* are adapted to bee pollination and even though some *Lepidoptera* may visit flowers, none have been recorded carrying pollinia (Catling, 1983; van der Cingel, 1995).

Orchids generally flower at low densities (Tremblay et al., 2005) and inflorescence density has been shown to be an important factor that can influence pollinator activity on orchids (Meléndez-Ackerman and Ackerman, 2001; Internicola et al., 2006; Juillet et al., 2007). Indeed, Juillet et al. (2007) showed that the nectarless *Traunsteinera globosa* has increased reproductive success in the presence of high densities of the rewarding *Trifolium pratense*. In this study, we found that at low density, *S. romanzoffiana* attracted more visits per inflorescence per hour than at high density. This inverse relationship suggests that in higher density patches, individual *S. romanzoffiana* inflorescences compete intraspecifically for pollinator attention. However, when we considered total floral density within patches (i.e. the total density of all three focal plant species), there appeared to be a trend for an increase in visitation to *S. romanzoffiana* with increasing total density, except at very high densities. This suggests that heterospecific inflorescences may facilitate visitation to *S. romanzoffiana*, up to a point, above which *S. romanzoffiana* competes with heterospecifics for pollinator attention (Rathcke, 1983). Indeed, previous studies have found that the presence of co-flowering species can increase pollinator visitation to rare species (Kwak et al., 1991; Geer et al., 1995; Gibson et al., 2006). Recently, Ghazoul (2006) experimentally demonstrated interspecific facilitation for pollination with *Raphanus raphanistrum*: this species received higher pollinator visits and had increased seed set in the presence of co-flowerers than when it occurred alone. In addition, Ghazoul (2006) found that the balance between facilitation and competition was dependent on relative density of co-flowerers. As well as experiencing

Table 2 – Three factor ANOVA comparing nectar volume (μl/flower) in three plant species (*S. romanzoffiana*, *P. vulgaris* and *M. aquatica*), two treatments (bagged or unbagged), on three days

Source of variation	df	SS	MS	F	p
Plant species	2	1.0252	0.5126	17.63	0.0104
Treatment	1	0.1983	0.1983	0.00	<0.0001
Day	2	0.4869	0.2434	8.37	0.0372
Treatment × day	2	0.2162	0.1081	8.06	0.0395
Treatment × plant species	2	0.0536	0.0268	2.00	0.2504
Day × plant species	4	0.1163	0.0291	5.31	0.0005
Treatment × plant species × day	4	0.0536	0.0134	2.45	0.0483
Error	162	0.8866	0.0055		

Variances were significantly heterogeneous and so data were transformed using $\ln(x + 1)$ to reduce heterogeneity (Cochran's $C = 0.1518$, $p > 0.05$).

competition for pollinator visitation, plants that occur in mixed arrays at high densities may suffer from increased IPT (Heinrich, 1979; Stout et al., 1998). We noted that focal plant species shared individual visitors and that switching between focal species occurred regularly at high densities (K.J. Duffy, personal observation). However, levels of IPT were not quantified because it was not possible to sample stigmas of *S. romanzoffiana* due to its conservation status.

Despite relatively high visitation rates to *S. romanzoffiana*, only a small number of visitors may effectively pollinate the plants. Effective pollinators need to pick up pollinia from the anther and deposit it on a receptive stigma (Tepedino et al., 1999). We believe effective pollination occurs in *S. romanzoffiana* in this population because pollinia were found on the proboscis/thorax of visiting *B. pascuorum* and *B. hortorum* and on the stigmas of randomly checked flowers. Pollinia removal was greater than pollinia deposition suggesting that flower visitors are not entirely efficient at transferring pollinia. Previous studies have shown low pollen transfer efficiency is common in orchids; with pollinia removal in temperate, nectar rewarding species at 51.9% ($n = 19$ studies) and fruit set at 41.8% ($n = 58$ studies) (Tremblay et al., 2005). No pollinia were observed on stigmas of flowers that had their own pollinia intact: this is due to a protandrous mechanism to encourage outcrossing in *S. romanzoffiana*, which involves the position of the stigma changing when pollinia are removed from a flower (Catling, 1983; Sipes and Tepedino, 1995).

4.2. Nectar levels

Randomly selected *S. romanzoffiana* flowers contained more nectar (standing crop) than co-flowerers. Since all three species received a similar rate of insect visitation, this suggests that either *S. romanzoffiana* has a higher nectar production rate, or that insects do not completely drain *S. romanzoffiana* flowers of nectar, but do drain co-flowerers. Examination of bagged *S. romanzoffiana* flowers suggests the former, particularly in relation to *P. vulgaris* (there was more nectar in bagged flowers of *S. romanzoffiana* than bagged *P. vulgaris*). When we examined nectar depletion by comparing bagged and open flowers, we found evidence for nectar depletion (i.e. a difference between bagged and unbagged flowers) in all three species, supporting observations that insects utilised all three species in this site. Since few orchids offer a pollen reward for visitors (because pollen is amassed in pollinia) (Tremblay et al., 2005 and references therein), *P. vulgaris* and *M. aquatica* (along with the other co-flowering species in the population) may be pollen sources for the bee visitors in addition to providing a nectar reward. Nectar sugar concentrations of *S. romanzoffiana* and *P. vulgaris* were similar throughout the flowering season (between 20 and 25%), with *M. aquatica* offering a less concentrated reward. However, these nectar concentrations are attractive for bumblebees and consistent with other nectar producing orchid species (e.g. 18–23% for *Satyrion hallackii*; Johnson, 1997).

Nectar standing crop and visitation rates were not correlated in *S. romanzoffiana* or *M. aquatica* but they were positively related in *P. vulgaris*. This is unusual because increased visitation should result in reduced nectar standing crops. Perhaps an increase in visitation causes an increase in nectar

production in *P. vulgaris*, or an increase in production may be causing the increase in visitation. Whether this directly affects visitation to *S. romanzoffiana* was not tested in this experiment and merits further investigation.

4.3. Fruit set

As orchids contain many dust-like seeds, fruit set is the conventional method to measure reproductive success (Neiland and Wilcock, 1998). Neiland and Wilcock (1998) show that fruit set for nectariferous orchids in Europe is approximately 63%. Despite hand pollination (Duffy and Stout, unpublished data) and relatively high visitation over the flowering season, examination of more than 1000 flowers revealed that *S. romanzoffiana* fruit failed to swell in this population. This supports previous observations of no fruit set in this species in Europe, but our findings do not support the suggestion that *S. romanzoffiana*'s failure to produce fruit is due to pollinator limitation. Recent research has demonstrated that seeds, containing viable embryos, are present in the unripened capsules (O'Connor, 2006) and this warrants further investigation. Antlfinger and Wendel (1997) measured the role of floral photosynthesis on reproductive effort in *Spiranthes cernua* over its flowering season, with the highest rates of photosynthesis occurring when the plants were in bud ($9.2 \mu\text{mol CO}_2/\text{m per s}$) and the lowest for the infructescence ($0.2 \mu\text{mol CO}_2/\text{m per s}$). Capsules of *S. romanzoffiana* were observed to wither within a week after flowering. A rapid reduction of photosynthesis at the end of flowering may affect the formation of mature fruits, though this is unlikely. Willems et al. (2001) show that experimentally shaded plants of *S. spiralis* developed more seeds than control plants. The reserves for fruit formation come from the current tuber, with seed maturation occurring when the infructescence is dying. The mechanism for the failure of fruit maturation in European *S. romanzoffiana* needs to be understood urgently for future conservation.

5. Conclusions

In conclusion, *S. romanzoffiana*, as well as being nationally rare, often occurs in small and low density populations. However, flowers are probably not pollination limited, as they are visited frequently by bumblebees and offer substantial nectar rewards to these floral visitors. The failure of fruits to mature in this species may have some other cause. In addition, pollinator sharing among the three flowering species examined here may contribute to overall reproductive success.

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