

Effects of conspecific and heterospecific floral density on the pollination of two related rewarding orchids

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Abstract Variation in within-population floral density can affect interactions between plants and pollinators, resulting in variable pollen export for plants. We investigated the effects of conspecific and heterospecific floral densities on pollination success both of two related, self-compatible, nectar-rewarding orchid species in Ireland, *Spiranthes romanzoffiana* (rare and listed as endangered) and its congener, *S. spiralis* (more abundant and not of conservation concern). Floral densities, insect visitation rates, and orchid pollen transport were recorded in multiple quadrats in four populations of both orchid species over their flowering season. We found that conspecific and heterospecific co-flowering plant density affected pollination in both orchid species. For *S. romanzoffiana*, higher heterospecific density increased pollen removal. For *S. spiralis*, higher conspecific visitation increased pollen removal and increased heterospecific density decreased pollen deposition. In addition, increased conspecific density increased pollen

deposition in both species. This study shows that plants may interact to facilitate or compete for different components of the pollination process, namely; pollinator attraction, pollen removal and deposition. Such interactions have immediate consequences for endangered plant species, as increases in both conspecific and heterospecific co-flowering density may ameliorate the negative effects of rarity on pollination, hence overall reproductive success.

Keywords *Bombus* · Competition · Facilitation · Pollen export · *Spiranthes*

Introduction

Among animal-pollinated plants, it is estimated that less than 1% of pollen is exported to conspecific flowers (Harder and Thomson 1989; Harder and Johnson 2008). Such low pollen export has implications not only for the evolution of both pollen and plant mating systems (e.g., Harder and Barrett 2006; Harder and Johnson 2008), but also for the conservation of animal-pollinated plants (Kearns et al. 1998). Many ecological factors have been proposed to explain such low pollen transfer efficiency (PTE), for example: limited pollen pick-up by the pollinator (Sahli and Conner 2007), grooming by the pollinator (Harder 1990), and pollen discounting (Harder and Routley 2006). However, one major factor determining the fate

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of pollen is conspecific density (the number of flowering individuals per unit area) as this can influence pollination such that pollen limitation is reduced in plants which occur in higher density patches (Sih and Baltus 1987; Knight 2003). This is driven by pollinator foraging behavior: dense patches are preferentially selected because travel times between flowers are reduced, thus enhancing pollinator foraging efficiency (Heinrich 1979; Harder 1990; Mitchell et al. 2004). Increases in density are also likely to increase the probability that pollen will be transferred between conspecific individuals (Karron et al. 1995). However, increasing density can have potential negative effects on the pollination of individual plants via conspecific competition for pollinators (Ratchke 1983; Duffy and Stout 2008).

As plants often flower in communities with individuals of many species, heterospecific co-flowering plants may also affect the behavior of pollinators towards rare or isolated plants through interspecific competition and facilitation (Campbell and Motten 1985; Moeller 2004). A plant that occurs with low conspecific density within a patch alongside high density heterospecific co-flowering plants may suffer from interspecific competition for pollinator attention (Waser 1978; Ratchke 1983; Bell et al. 2005). Such individuals may also be prone to pollination limitation, resulting from not just reduced visitation rates, but also improper pollen transfer (IPT) (the transfer of pollen between co-flowering heterospecific flowers) and/or pollen wastage (Ratchke 1983; Stout et al. 1998; Wilcock and Neiland 2002). Alternatively, facilitation can occur whereby co-flowering conspecific or heterospecific plants form an increased floral display and all individuals receive increased visitation (Ratchke 1983; Moeller 2004). Such facilitation by co-flowering species has been recently demonstrated in both experimental (Ghazoul 2006) and natural populations (Johnson et al. 2003).

Orchids are useful model organisms for the study of the effects of floral density on pollination, as they share population characteristics with many angiosperms, such as occurring in patchy distributions in mixed floral communities (Neiland and Wilcock 1998; Duffy and Stout 2008) and can suffer pollen limitation within a flowering season (Johnson and Bond 1997). Since their pollen is aggregated into pollinia, it can be tracked directly in the field and

allows direct assessment of pollination success. In addition, competition may be particularly detrimental for orchids as IPT may result in the loss of the entire male component of an individual flower (Dressler 1981). The negative effects of IPT on female function may also be low due to a more precise pollen transfer in orchids. However, orchids that have sectile pollinia may have their pollen transferred among many flowers (e.g., Johnson et al. 2005). Competition and facilitation with heterospecific co-flowering plants may affect PTE in orchids strongly (Neiland and Wilcock 1999; Johnson et al. 2003), although the extent of competition may depend on their density and whether they provide a reward (Internicola et al. 2006).

Most studies of pollination of orchids have focused on nectarless species, with very few focused on nectar-rewarding species (e.g., Smithson 2002; Johnson et al. 2003; Internicola et al. 2006). The pattern of both intra- and inter-specific floral density on pollination are not clear and may be markedly different in rewarding species than in rewardless species. Because rewardless species depend on pollinators to feed on rewarding heterospecifics, inter-specific facilitation of pollination may be more predominant in natural populations of these species. For example, Johnson et al. (2003) found that co-flowering species can increase pollen removal and receipt in natural populations of the nectarless *Anacamptis morio*. Other studies showed increased fruit set when co-flowering with morphologically similar rewarding species (e.g., Juillet et al. 2007) and that the addition of an artificial nectar reward increases pollination success, regardless of local density of the orchid (Jersáková et al. 2008). However, field studies are lacking on the effects of conspecific and heterospecific density effects on the pollination success of rewarding orchid species.

Here, we investigate the pollination ecology of two nectar-rewarding orchid species to test whether conspecific and heterospecific density influence pollen export and import in natural populations. We expect increased pollen removal and deposition with increased density in both *S. romanzoffiana* and *S. spiralis*. We expect the pollination of *S. romanzoffiana* to be more affected by inter-specific effects as it flowers during peak flowering of many other plants, and occurs in fewer, smaller flowering populations. Heterospecific co-flowering plants may have a positive

or neutral effect on the pollination of *S. spiralis* because it occurs in larger conspecific populations and flowers later in the year than many of the other plants in the habitats in which it occurs, hence competition with heterospecifics is less likely to impact on pollination.

Specifically, we test the hypothesis that pollen removal and deposition in both *S. romanzoffiana* and *S. spiralis* are affected by:

- a) increased density of conspecific orchid flowers within populations (number of flowers per unit area)
- b) increased density of heterospecifics co-flowering within populations
- c) insect visitation rate to both the orchid and heterospecific co-flowerers.

Methods

Study species and populations

We investigated two members of the orchid genus *Spiranthes*, *S. romanzoffiana*, and *S. spiralis*. Both species are non-autogamous and share similar traits such as; self-compatibility, bee pollination, and offer a nectar reward, but differ in their flowering time and habitats in which they occur (Catling 1983; Willems and Lahtinen 1997). These species do not co-occur in Ireland. The pollen of both species is aggregated into a pair of sectile pollinia held together by a viscidium. The pollination mechanisms of these species have been described by Catling (1983) (*S. romanzoffiana*) and Darwin (1862) (*S. spiralis*). Both species are protandrous which may effectively reduce self-pollination and insects tend to forage acropetally (bottom to top) on inflorescences (Catling 1983). Medium- to long-tongued bees are the documented pollinators of both of these species (Catling 1983; Duffy and Stout 2008; Jacquemyn and Hutchings 2010). When bees probe the flowers, almost always both pollinia are removed together, and pollen is deposited as pollen sheets (massulae) on the stigma, with multiple flowers potentially receiving pollen from a single pollinium.

Spiranthes romanzoffiana (Cham.) is perennial orchid that is widespread in North America, yet confined to the fringes of Ireland and Britain in Europe (Lupton 2008). The typical habitat is damp

peaty meadows, pastures and lakeshores (Summerhayes 1968; Lupton 2008). In Ireland, *S. romanzoffiana* begins flowering in mid- to late-July and continues through to the end of August. The height of the inflorescence varies between 5 and 35 cm and can bear up to 30 tubular white flowers in a three-ranked arrangement. Fruit set is variable; it is 0% in European populations (Duffy and Stout 2008) while can be up to 75% in North American populations (Larson and Larson 1987). The reason for the lack of fruit set in European populations is not yet fully understood, but is not due to pollen limitation (Forrest et al. 2004; Duffy and Stout 2008). However, minute quantities of seeds are contained in the unripened fruits (Lupton 2008; KJ Duffy pers. obs.), which may maintain current populations. Hence, *S. romanzoffiana* is considered endangered in the Republic of Ireland and is protected by the Wildlife Act (1976) and the Wildlife Amendment Act (2000), under the Flora Protection Order (1999).

Spiranthes spiralis (L.) Chevall. is a small, long-lived perennial orchid with a distribution that ranges throughout Europe from North Africa as far north as Denmark to Russia in the east and Ireland in the west (Tutin et al. 1980). It is endangered in other parts of Europe, for example, the Netherlands (Jacquemyn et al. 2007) and France (Machon et al. 2003). It grows in relatively dry, nutrient poor meadows, or calcareous grassland. It occupies open grazed areas with a constant land-use. In Ireland, *S. spiralis* begins flowering in late August and continues through to mid-September. The height of the inflorescence varies between 5 and 25 cm and can bear up to 25 small, white flowers that are arranged as a spiral on the upper half of the flowering stalk. Fruit set can be variable; between 0 and 78% (mean: 35%) in the Netherlands (Willems and Lahtinen 1997). Seeds are wind-dispersed, but most fall in the vicinity of maternal plants (Machon et al. 2003).

This study was conducted in the west of Ireland in 2006. We selected four un-managed populations of varying size (based on the number of flowering orchids) of each of *S. romanzoffiana* and *S. spiralis* (Fig. 1). Populations of *S. romanzoffiana* were monitored from 25 July to 26 August; populations of *S. spiralis* were monitored from 2 September to 16 September, representing the flowering period for each species. Populations of *S. romanzoffiana* contained 14 (Carraig a moiltín), 21 (Knockmore), 71 (Loch

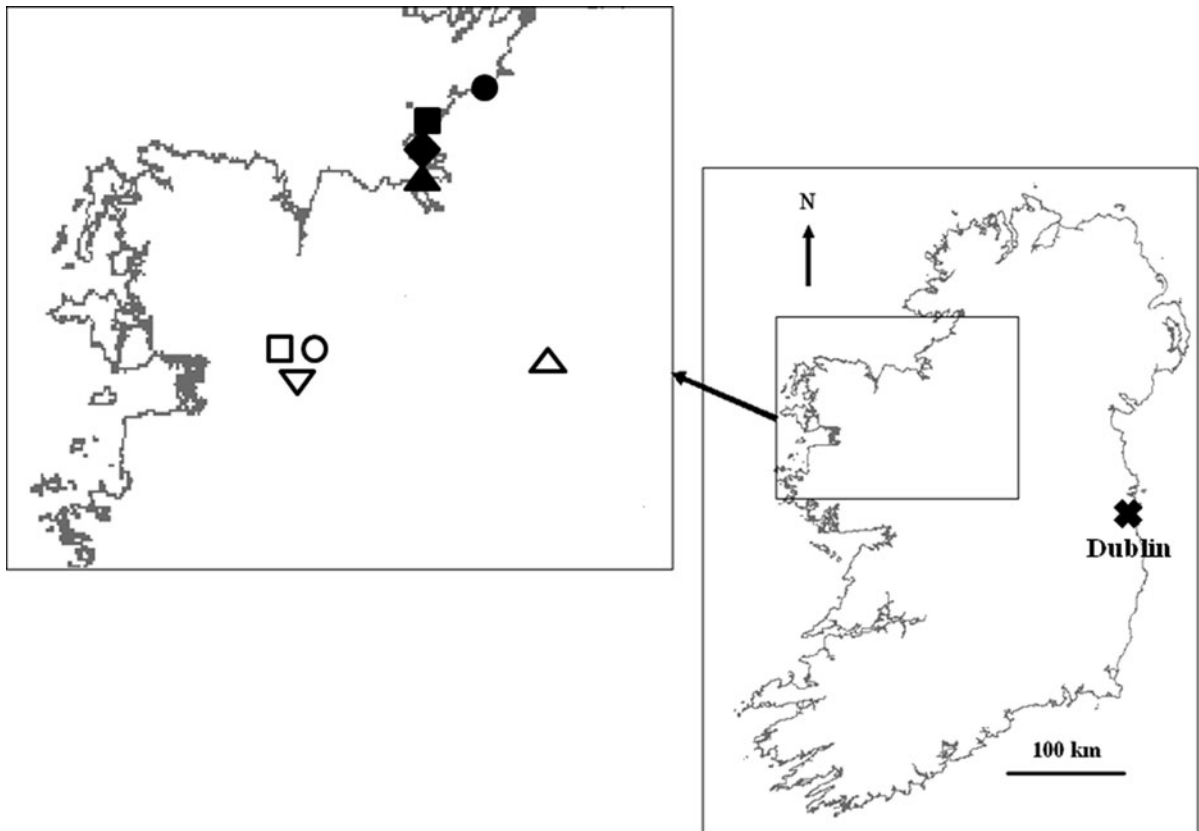


Fig. 1 Location of study populations of *Spiranthes romanzoffiana* and *S. spiralis* in the Republic of Ireland; *open symbols* indicate *S. romanzoffiana* populations; *closed symbols* indicate *S. spiralis* populations

Allen), and 102 (Loch Cullen) flowering individuals. Populations of *S. spiralis* contained 71 (Yellow Beach), 81 (Ballyconnell), approx. 250 (Strandhill), and approx. 500 (Mullachmore) flowering individuals. These populations were selected on the basis that they represent the range of population sizes of both species in the country (KJ Duffy pers. obs.). Other heterospecific co-flowering vegetation at *S. romanzoffiana* populations included: *Leontodon hispidus*, *Lythrum salicaria*, *Mentha aquatica*, *Potentilla erecta*, and *Prunella vulgaris*. Other heterospecific co-flowering vegetation at *S. spiralis* populations included: *Campanula rotundifolia*, *Leontodon autumnalis*, *Leucanthemum vulgare*, *Mentha aquatica*, *Senecio jacobea*, and *Trifolium repens*. As *P. erecta*, *L. vulgare*, and *S. jacobea* are species not visited by bees (Dramstad and Fry 1995) and were visited very infrequently in our study, we excluded these species from our calculations of heterospecific effects on pollination.

Visitation observations, pollinia removal and deposition

Observations were made in dry weather between 8:30 am and 6 pm over 10 days and 3–8 days in *S. romanzoffiana* and *S. spiralis* population, respectively. The floral densities of both orchids and each of three co-flowering heterospecific species were recorded in randomly selected 2 m × 2 m patches (10 replicates in each *S. romanzoffiana* population, 3–8 replicates in *S. spiralis* populations; Ballyconnell = 8, Mullachmore = 8; Strandhill = 7, Yellow Beach = 3), measured using a quadrat and measuring tape, of: a) flowering orchids and b) each of three neighboring co-flowering heterospecific species, on each of 10 days in all *S. romanzoffiana* populations and 3–8 days in *S. spiralis* populations in dry weather. This particular size of quadrat was selected because this size accounted for the variation in density in the field of both orchid species, bees often

forage within a few meters within patches, and allowed multiple independent observations in each population throughout the flowering period. Each patch was observed for 15 min and was only observed once during the season to avoid pseudoreplication. All insects entering patches, visiting flowers and probing for nectar and pollen, and the number of flowering units (individual flowers for *Spiranthes* spp.; individual flowers, inflorescences or capitula for co-flowering heterospecifics, depending on the species) visited by each individual were recorded. We identified bee visitors to species level (except *Bombus lucorum/terrestris* complex, whose workers are indistinguishable in the field; Edwards and Jenner 2005), and grouped other visitors as lepidopterans, syrphids or other dipterans. As observations of direct insect visitation to orchids can be infrequent (Tremblay et al. 2005) we also measured pollinia removal and deposition (presence/absence of massulae on the stigma) in *S. romanzoffiana* and *S. spiralis* using a 10× hand lens or a 12× head lens to examine all flowers within each patch at the end of each observation period. We calculated visitation rate per flowering unit per hour as the number of visits to flowering units in 15 min/total number of flowering units in the patch × 4. In *S. romanzoffiana* populations a range of taxa were observed visiting all flowers: *Bombus pascuorum* (36.4%), *B. lucorum/terrestris* (27.4%), *Apis mellifera* (16.3%), Syrphids (8.2%), *B. hortorum* (5.2%), Dipterans (5.9%), and Lepidopterans (0.6%). Similarly, we observed a range of taxa visiting all flowers in *S. spiralis* populations: *B. pascuorum* (33.8%), *B. lucorum/terrestris* (32.2%), *A. mellifera* (22.5%), Syrphids (8.1%), Dipterans (2.7%), and Lepidopterans (0.8%). We included only bees in our calculation of both conspecific and heterospecific visitation rates, as these were the only insects observed carrying *Spiranthes* pollen.

Data analysis

We tested for the relationship between: (i) density of conspecifics (ii) bee visitation rate to conspecifics, (iii) density of heterospecifics, and (iv) bee visitation rate to heterospecifics, on both the proportion of pollinia removed and proportion of pollinia deposited, separately for *S. romanzoffiana* and *S. spiralis*. The

composition of heterospecific co-flowering species did not vary much between populations of either orchid species; therefore we pooled the data and analyzed the visitation to all heterospecifics together. For both orchid species, we tested for collinearity between the explanatory variables by following the approach outlined by Zuur et al. (2009). We calculated variance inflation factors (VIF) for each fixed factor in all models and we omitted variables that showed significant correlation and high VIF values. We used mixed-effect models and included population as a random effect in each model. This was because we studied four populations for both orchids and our observation replicates were nested within-population. In addition, the response variables are binary (i.e., presence/absence of pollinia/massulae) and are likely to reach asymptote, therefore a binomial link function was used. Laplace approximation was used to generate the models. We found a significant correlation between heterospecific floral density and heterospecific visitation rate in *S. spiralis*, therefore we only included heterospecific floral density in the model of *S. spiralis* pollination. Fixed factors included in final analysis had VIF values <1.5, which is below the recommended threshold VIF value of 3 for evidence of collinearity between fixed factors (Zuur et al. 2009). To perform the mixed-model analyses, we used the lme4 package (Bates et al. 2009) in R 2.11.1 (R Development Core Team 2009).

Results

A total of 52 visits were observed to *S. romanzoffiana* flowers and 48 visits were observed to *S. spiralis* flowers during a total of 16.5 h of daytime observations. Bees (*Bombus* spp. and *Apis mellifera*) were the only visitors observed for both species. A total of 1,393 visits to co-flowering heterospecifics were recorded during a total of 30 h of daytime observations in *S. romanzoffiana* populations representing a range of insect taxa. A total of 323 visits to co-flowering heterospecifics were recorded during a total of 19.5 h of daytime observations in *S. spiralis* populations.

Pollinia removal and deposition occurred in all populations of both species. Among populations, *S. romanzoffiana* had a mean 0.31 flowers with pollinia removed per patch (range: 0.19–0.42) and a

Table 1 Mixed-effect models for factors influencing proportion of flowers with pollinia removed and proportion of flowers with massulae deposited in patches of *S. romanzoffiana*

Random Effect	Proportion pollinia removed				Proportion massulae deposited			
	Standard deviation				Standard deviation			
Population	<0.001				0.594			
Fixed Effects	Estimate	S.E. Estimate	<i>z</i>	<i>P</i>	Estimate	S.E. Estimate	<i>z</i>	<i>P</i>
Intercept	−1.457	0.195	−7.462	<0.001	−3.714	0.588	−6.311	<0.001
Conspecific density ^a	0.024	0.015	1.558	0.119	0.072	0.036	2.025	0.043
Conspecific visitation rate	0.089	0.094	0.946	0.344	0.052	0.179	0.292	0.770
Heterospecific density	0.015	0.007	2.061	0.039	0.006	0.017	0.360	0.719
Heterospecific visitation rate	−0.011	0.016	−0.660	0.509	0.037	0.035	1.084	0.278

^a Conspecific density was centred

mean 0.04 flowers with massulae deposited per patch (range: 0.02–0.06). Among populations, *S. spiralis* had a mean of 0.48 flowers with pollinia removed per patch (range: 0.44–0.54) and a mean of 0.12 flowers with massulae deposited per patch (range: 0.05–0.14). With *S. romanzoffiana*, there were positive relationships between pollinia removal and heterospecific density and pollinia deposition and conspecific density (Table 1; Fig. 2a, b). With *S. spiralis*, there was a positive relationship between the proportion of flowers with pollinia removed and the conspecific visitation rate (Table 2; Fig. 3a) and between pollinia deposition and conspecific density (Table 2; Fig. 3b). However, heterospecific density was negatively related to pollinia deposition (Table 2; Fig. 3c).

Discussion

In accordance with our expectation, there was increased pollen deposition with increased conspecific density in both *S. romanzoffiana* and *S. spiralis*. Also, there was increased pollen removal in *S. romanzoffiana* with increased heterospecific floral density, indicating facilitation of pollen removal. Increased visitation rate to flowers of *S. spiralis* increased pollen removal, while increases in heterospecific density reduced pollen deposition. Consequently, the results of this study show the variable nature of pollen export and import in hermaphrodite plants. As highlighted by Thomson (1982) there is no reliable way of predicting whether the interaction between any two plant species will be characterized by competition or facilitation for

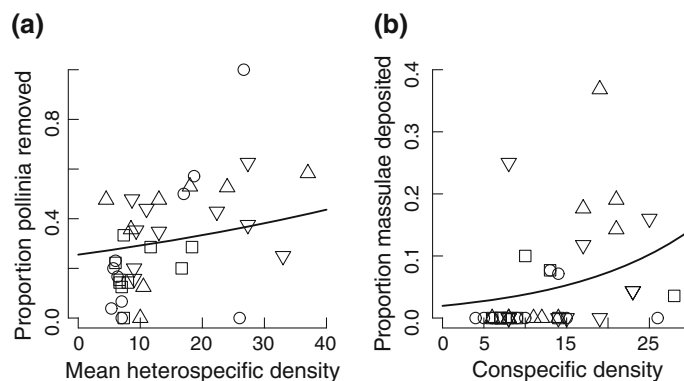
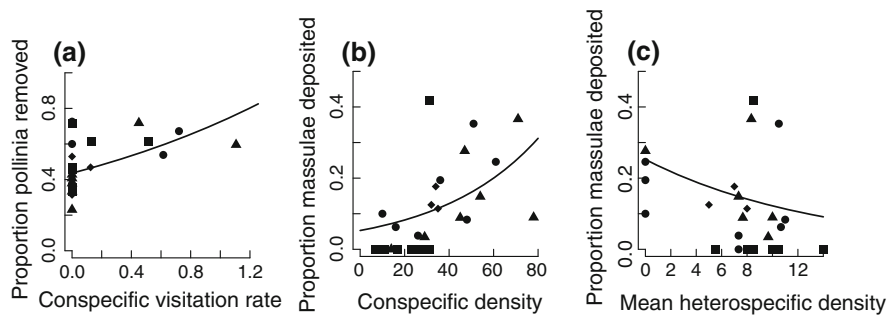


Fig. 2 The relationship between **a** density of heterospecific co-flowering species and proportion of flowers with pollinia removed and **b** conspecific density and the proportion of flowers with massulae deposited in *S. romanzoffiana*

populations (number of flowering plants in parenthesis): *open square* Carraig a moiltín ($N = 14$), *open circle* Knockmore ($N = 21$), *open triangle* Loch Allen ($N = 71$), *inverted open triangle* Loch Cullen ($N = 102$)

Table 2 Mixed-effect models for factors influencing proportion of flowers with pollinia removed and proportion of flowers with massulae deposited in patches of *S. spiralis*

Random effect	Proportion pollinia removed				Proportion massulae deposited			
	Standard deviation				Standard deviation			
Population	0.029				<0.001			
Fixed Effects	Estimate	S.E. estimate	<i>z</i>	<i>P</i>	Estimate	S.E. estimate	<i>z</i>	<i>P</i>
Intercept	−0.798	0.214	−3.739	<0.001	2.532	0.348	−7.268	<0.001
Conspecific density	−0.001	0.003	−0.336	0.736	0.022	0.005	4.001	<0.001
Conspecific visitation rate	0.536	0.215	2.491	0.013	0.172	0.350	−0.491	0.623
Heterospecific density	−0.002	0.017	0.090	0.928	−0.061	0.029	−2.060	0.039

**Fig. 3** The relationship between **a** conspecific density and proportion of flowers with pollinia removed, **b** conspecific density on the proportion of flowers with massulae deposited, and **c** heterospecific density on the proportion of flowers with

massulae deposited in *S. spiralis* (number of flowering plants parenthesis): *Filled diamond* Yellow Beach ($N = 71$), *filled square* Ballyconnell ($N = 81$), *filled triangle* Strandhill ($N = >250$), *filled circle* Mullachmore ($N = >500$)

pollination and this may differ between years (Dudash and Fenster 1997) and populations (Lázaro and Totland 2010). For example, a previous study on *S. romanzoffiana* revealed a negative relationship between pollinator visitation and conspecific inflorescence density (Duffy and Stout 2008). In this study, we found a positive effect of conspecific density on pollen deposition in both *S. romanzoffiana* and *S. spiralis* populations. This suggests intra-specific facilitation of pollination, and supports a previous study which showed that aggregated inflorescences of *S. spiralis* have greater fruit set than sparsely distributed ones (Willems and Lahtinen 1997), and probably reflects the optimization of foraging patterns by flower-visiting insects (Andersson 1988).

Pollen removal in *S. romanzoffiana* was higher when heterospecific density increased, but heterospecific density had no effect on pollen deposition. Visitors move from *S. romanzoffiana* to other co-flowering species, hence causing pollen wastage

and probably lose pollen while travelling between flowers (possibly via grooming or breakage of the sectile *Spiranthes* pollinia). Indeed, *Bombus pascuorum* and *B. lucorum/terrestris* were often observed visiting heterospecifics immediately after visiting *S. romanzoffiana* (KJ Duffy pers. obs.). When heterospecific co-flowering plants are more abundant, foragers may find them more profitable, causing foragers to focus on them (as majors) while occasionally including *S. romanzoffiana* in their foraging regime (as minors) (Heinrich 1979). It is possible that bees focus on *S. romanzoffiana* and *S. spiralis* for nectar and collect pollen from co-flowerers; nectar in bagged flowers was higher than in open flowers in *S. romanzoffiana* and *S. spiralis* (KJ Duffy unpub. data), suggesting depletion by insect foragers.

With *S. spiralis*, we found intraspecific facilitative effects, as there was an increase in pollen removal with increased conspecific visitation, and increased pollen deposition with increased conspecific density.

However, there was a decrease in pollen deposition when heterospecific density increased; hence increasing heterospecific density can negatively affect overall reproductive success in *S. spiralis*. In the Netherlands, Willems and Lahtinen (1997) found that the removal of co-flowering heterospecifics increased fruit production in *S. spiralis*, suggesting there was interspecific competition for pollinators. Although *S. spiralis* was rarely surrounded by dense flowering vegetation in our study populations, we detected a similar effect of heterospecific competition for pollen deposition. Also, it could be that *S. spiralis* flowers later in the season when fewer pollinators are available, thereby increasing competition for limited pollinator attention. We found a very low proportion of flowers had pollen deposited per patch in *S. romanzoffiana* populations (less than one in twenty on average), compared with *S. spiralis* populations (more than one in nine flowers on average). This is could be due to fewer co-flowering heterospecifics in *S. spiralis* populations compared with *S. romanzoffiana* or low numbers of conspecifics in *S. romanzoffiana* populations.

Fruit maturation failed to occur in *S. romanzoffiana* despite pollination, which makes it impossible to compare overall reproductive success with density. However, low numbers of embryo-containing seeds (~100–200 seeds) were found in the withered fruits, which makes it possible that *S. romanzoffiana* reproduces sexually (KJ Duffy pers. obs.). Whether these seeds are viable to establish new individuals in suitable habitats requires further long-term investigation. It may be that fruit failure is the result of genetic inbreeding. Indeed, Forrest et al. (2004) found low genetic diversity in Irish populations of *S. romanzoffiana* with microsatellite markers, which suggest autogamous reproduction or an extreme genetic bottleneck. However, Lupton (2008) used AFLP markers and microsatellite markers and showed that there is genetic variation and differentiation within and between Irish populations; hence examination of patterns of genetic variation in *S. romanzoffiana* requires further work. As *S. romanzoffiana* is endangered in Ireland and is known from very few populations, current populations therefore require monitoring and protection. Other co-flowering species may maintain pollinator species around *S. romanzoffiana* populations by providing other nectar or pollen rewards. Also, although *S. spiralis* is not

considered to be in decline in Ireland, it is rare in other European countries (e.g., the Netherlands; Jacquemyn et al. 2007), and therefore populations should be monitored to ensure severe population declines do not occur.

Although conspecific density has been shown to affect pollination in rewarding orchids (e.g., Brys et al. 2008; Duffy and Stout 2008; Johnson et al. 2009); this study shows that heterospecific co-flowering species may also play an important role in their pollination. Orchids are not known to provide a pollen reward for pollinators, therefore require co-flowering heterospecifics to provide such rewards (e.g., for pollen-collecting bees; Duffy and Stout 2008). Rewarding orchids may compete more effectively for pollinator attention compared with nectarless orchids, regardless of density, as they have a greater opportunity to attract and maintain pollinator fidelity, although they may suffer greater geitonogamous pollen transfer. In rewardless species, an increase in heterospecific co-flowering density may increase pollination of the orchid (i.e., facilitation) by attracting greater numbers of pollinators to patches within populations (e.g., Johnson et al. (2003)).

Further research on the effects of density on pollination in rewarding orchids in natural populations should focus on manipulative density experiments and the staining of pollinia with histochemical stains (Peakall 1989). Staining pollinia can allow for estimation of the contribution of geitonogamous versus xenogamous pollen movement to reproductive success. For example, Johnson et al. (2009) used histochemical stains to show that pollen transfer does not vary with population size in the orchid *Satyrion longicauda*; however, they found more there was self-pollination in small populations. Neither density manipulations or pollen staining were performed in our study as it would be extremely difficult to precisely count massulae on the stigmas of these *Spiranthes* species to get a reliable estimate of the contributions of geitonogamous pollen movement. *Spiranthes romanzoffiana* is endangered and transplanting individuals to manipulate density may negatively impact on population survival. Also, *S. spiralis* does not suffer a reduction in fruit set when self-pollinated by hand (Willems and Lahtinen 1997; Jacquemyn and Hutchings 2010). Although the quality of seed produced may be affected by self-pollination, recording stigmas with pollen deposited

was enough to quantify pollination success in these species.

In conclusion, even though plant species may be endangered and occur in few flowering populations, they may not necessarily suffer an overall reduction in pollination. The negative effects of low numbers and size of populations on pollination can be ameliorated by increases in within-population density and the presence of co-flowering heterospecific species. In addition, more abundant species may also suffer similar conspecific and heterospecific density effects on pollen transport, to endangered species. Increased knowledge in this area is important for developing our understanding of how insect-pollinated plant species respond to accelerating anthropogenic-mediated habitat reduction and modification.

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