Floral diversity and the facilitation of pollination

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Summary

1 Multiple-species floral displays have been hypothesized to facilitate pollination by attracting a greater number and/or diversity of pollinators. Here I present experimental confirmation of pollination facilitation among coflowering plants that have morphologically distinct flowers.

2 Pollinator visits to *Raphanus raphanistrum*, a self-incompatible herbaceous plant, increased when it occurred with one or a combination of *Cirsium arvense*, *Hypericum perforatum* and *Solidago canadensis* than when it occurred alone.

3 Enhanced visitation to *R. raphanistrum* in mixed species plots was reflected by increased seed production.

4 Facilitative effects in pollination were conditional on the density and evenness of the floral mixture and graded into competition as the relative abundance of *R. raphanistrum* declined in a two-species mixture.

5 Previously proposed mechanisms for facilitative interactions cannot explain facilitation among florally distinct plant displays. An alternative mechanism of differential but complementary floral rewards is proposed to explain facilitative attraction of pollinators.

6 Facilitation of, and competition for, pollination has implications for regeneration by seed of rare or isolated plants, and of mitigating Allee effects that afflict such populations.

Key-words: Allee effects, bees, floral display size, hoverflies, plant reproduction, plant–pollinator interactions, pollinator competition, pollinators, positive interactions, *Raphanus raphanistrum*, seed set

Introduction

Competition among plants for pollinator services can determine floral community structure through competitive exclusion and differentiation of floral forms or phenologies (Campbell 1985; Stone *et al.* 1998; Fishman & Wyatt 1999; Caruso 2000; Brown & Mitchell 2001; Brown *et al.* 2002). However, plant species within many communities have substantially overlapping flowering periods and share generalist pollinators (Smith-Ramirez *et al.* 1998; Gross *et al.* 2000), suggesting that, where these diverse plant communities persist, plant reproduction is not limited by pollinator availability. Many plant populations do, however, appear to be pollinator limited (Burd 1994; Wilcock & Neiland 2002), and the pollination-mediated Allee effects that have repeatedly been demonstrated in small or isolated populations (Groom 1998; Ghazoul 2005) imply

competition for pollinator services. Alternatively, it has been proposed that shared floral displays might mitigate such Allee effects and that coflowering plants may, instead of competing, actually facilitate pollination (Brown & Kodric-Brown 1979; Waser & Real 1979; Rathcke 1983; Callaway 1995). This hypothesis implies that the pollination and reproductive success of plants in species-rich swards are enhanced over that of plants in less diverse but equally sized floral displays. A diverse floral display may provide mutual benefits if pollinator attraction or pollination stability are increased over less diverse displays (Schemske 1981; Rathcke 1983; Roy 1994).

The convergence of floral morphologies among some coflowering heterospecifics has been explained as a mutually beneficial pollinator attraction strategy (Bobisud & Neuhaus 1975; Schemske 1981) based on increased floral visitation to large floral displays (Augspurger 1980; Dejong *et al.* 1992; Podolsky 1992). Evidence for such indirect, positive interactions is currently limited to species, often cogenerics, that have morphologically similar displays (Thomson 1978; Moeller 2004), or to deceit-pollinated plants that

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provide no floral resources to pollinators (Laverty 1992; Johnson *et al.* 2003). Positive interactions among the many coflowering plants that share pollinators, but differ in their floral morphologies and colours, have yet to be explored, and explaining their coexistence remains a challenge for ecology.

Facilitation among florally distinct species may occur if pollinators do not discriminate between floral types. This would result in visitation responses similar to those described for intraspecific or convergent floral displays, and would enhance pollination success if pollinator attraction were an accelerating function of the collective display size. Of course many pollinators can and do discriminate among flowers (Thomson 1981; Thomson & Chittka 2001), but if discrimination is partial (i.e. pollinators are generalists but tend to show preferences to particular floral types), then pollination of rare, or less rewarding, species may be enhanced by the increased attraction of pollinators to highly rewarding 'magnet' species with which they occur (Laverty 1992; Johnson *et al.* 2003). Alternatively, diverse floral displays may attract pollinators disproportionate to the display size, for instance if pollinators seek multiple resources, such as pollen, nectar, mates or prey, each provided at different plants, or if resources are acquired more efficiently or predictably in plant mixtures. The latter, however, seems unlikely unless resources are temporally or spatially patchy in monospecific stands.

A diverse and species-rich floral mixture would reduce a rare-species disadvantage generated by frequency-dependent pollinator foraging, simply because differences in the relative abundances of flowering species are reduced. Additionally, diverse displays are expected to attract a greater variety of pollinators and relatively rare plants are likely to benefit by virtue of the sampling effect: as the number of pollinating species rises there is an increasing likelihood of a pollinator showing preference for (or at least not discriminating against) rarer species. Interference interactions among pollinator species at the most rewarding flowers may also displace pollinators to other, possibly rarer, flowers more than their floral frequencies would suggest.

These scenarios depict asymmetrical interactions where only the rare or less apparent species secure the benefits of improved pollination, while 'facilitator' species receive no benefits and are even possibly disadvantaged through reduced pollinator visitation or pollen quality. However, pollinator diversity may also contribute to pollination service stability, to the benefit of all coflowering plants that share pollinators. Displays that almost exclusively attract honeybees, for example, may be subject to periods of extremely low visitation should honeybees recruit to other foraging locations (Goulson 2003). Pollination services provided by a diverse pollinator community are less sensitive to changes in the behaviour or abundance of individual species and are more likely to remain stable under changing climatic conditions as pollinator species replace each other along a temperature gradient according to their thermal preferences (Willmer 1983).

Rathcke (1983) proposed that facilitation and competition among plants for pollination services are opposite extremes of a continuum that is a function of population density or, equally, population size or relative abundance. Such population attributes could therefore determine whether interactions among plants are positive or negative, and thus whether Allee effects, to which low density populations in high diversity communities are susceptible, are reinforced or mitigated (Moeller 2004; Ghazoul 2005). Understanding pollination dynamics in terms of the balance between competition and facilitation has relevance to the conservation of both plant and pollinator communities, particularly among rare species in degraded communities. Yet facilitation of pollination among coflowering heterospecifics, and particularly among species with distinctly different flowers, remains controversial and largely unexplored, except for the special case of non-rewarding flowers pollinated by deceit (Laverty 1992; Johnson *et al.* 2003).

In this study interspecific unidirectional facilitation of reproductive success was investigated by assessing pollination and seed set of flowers of a focal species, *Raphanus raphanistrum*, grown in single-species stands and in mixtures with three other species that have morphologically contrasting flowers. The balance between competition and facilitation under a range of relative densities was also investigated in a simpler two-species system. The central hypothesis is that reproduction of *R. raphanistrum* is facilitated by coflowering species, and that positive effects are replaced by negative interactions as species evenness declines.

Methods

Raphanus raphanistrum (Brassicaceae) is a self-incompatible native annual herb that bears white flowers. It occurs naturally in species-rich mixtures of herbaceous plants and is visited by a wide range of generalist pollinators, including social and solitary bees, wasps, flies and occasionally butterflies and other insects. It is therefore an ideal focal plant for this study as it is very likely to share pollinators with other coflowering species. Furthermore, self-incompatibility ensures a dependence on pollination that is readily translated into a measure of plant fitness by scoring fruit and seed set. Each flowering *R. raphanistrum* typically has between four and ten open flowers at any one time, and each flower lasts for *c.* 1 day. At Silwood Park, Berkshire, UK, *R. raphanistrum* occurred naturally in a fallow field that had been ploughed in March 2004. By July in the same year an almost uniform cover of *R. raphanistrum* (between 15 and 20 plants metre^{-2}) mixed with scattered *Cirsium arvense* at much lower densities (3–5 metre^{-2}) had established throughout the western side of the field. About 30 m away on the eastern side of the field *C. arvense* and *R. raphanistrum* had more similar

densities of between six and ten plants metre^{-2}. Within this field two experiments were established to investigate, first, the effects of local plant diversity on pollinator visitation and seed set of *R. raphanistrum*, conducted in the western half of the field, and secondly, the response of pollinators to the relative floral density of *R. raphanistrum*, established in the eastern half.

Facilitation of pollination was assessed by scoring the abundance of pollinators and subsequent fruit and seed set at *R. raphanistrum* plants grown alone and in mixtures with three other species, creeping thistle *Cirsium arvense* (Asteraceae), goldenrod *Solidago canadensis* (Asteraceae) and perforate St John's wort *Hypericum perforatum* (Clusiaceae). These species were selected because they occurred naturally with *R. raphanistrum* in local fields and field margins and have similar flowering periods, yet their floral morphologies are distinctly different, ensuring that facilitation would not simply be due to an inability of pollinators to distinguish between flowering species. *R. raphanistrum* has four-petal white flowers about 20 mm across. The flowers of *H. perforatum* are also 20 mm across but have five golden-yellow petals and many protruding stamens; the lilac flower heads of *C. arvense* are 15–20 mm wide; and *S. canadensis* has yellow flower heads that are only 5 mm across with very short ray florets. These species also have broadly equivalent floral display sizes as the first three have flowers that are of similar size and number (typically between three and ten for each species), while the smaller flower heads of *S. canadensis* are presented in greater numbers (around 50 on the single panicle). Furthermore, all four species are visited by a wide range of generalist pollinator taxa, principally bees, wasps, hoverflies and butterflies.

POLLEN LIMITATION OF REPRODUCTION

The dependency of *R. raphanistrum* seed set on pollinator visits, as opposed to resource availability, was addressed by testing for pollen limitation of seed set through hand-pollination experiments. Four plants were selected in each of 25 experimental plots, described below. These plots contained 50 *R. raphanistrum* plants and had not yet received the treatment plants (see

below). Freshly dehisced pollen collected from plants outside the experimental area was applied, using a fine paintbrush, to the stigmas of four flowers on two of the selected plants. Four flowers on the remaining plants were labelled but not otherwise treated. All hand-pollinations were applied between 10.30 and 14.00 on 12 July 2004, which was a warm and sunny day with much pollinator activity. The degree of pollen limitation was calculated by comparing fruit set and seed set of hand-pollinated flowers with open-pollinated flowers.

FACILITATION THROUGH FLORAL DIVERSITY

The objective of this experiment was to test whether pollination and seed set of *R. raphanistrum* was enhanced by coflowering species at constant floral density. Twenty-five 3.6×3.6 m plots, arranged in a 5×5 block design and separated by a 2-m strimmed buffer were demarcated in the fallow field where *R. raphanistrum* was growing naturally. Within each plot, *R. raphanistrum* plants were randomly thinned to leave 50 plants *in situ*. All other plants bearing flowers, including *C. arvense*, were removed. These prepared plots were randomly allocated to eight treatments (Table 1). The first treatment simply placed a further 24 potted *R. raphanistrum* on the surface of each plot. Seven other treatments comprised the addition of 24 potted plants belonging to *H. perforatum*, *S. canadensis* or *C. arvense*, or a balanced combination of two or all three of these species. Each plot therefore contained 74 plants (50 naturally growing *R. raphanistrum* plus 24 potted plants) so that flowering plant density was maintained constant at each plot and total floral display was broadly similar across plots. Each treatment was replicated three times except for the *Raphanus*-only treatment, which had four replicates.

The number and species identity of pollinators visiting the 50 naturally growing *R. raphanistrum* in each plot were recorded during 2-min observation periods conducted twice daily (am and pm) for seven consecutive days from 17 to 23 July 2004. Following a brief pilot study, a 2-min observation period was deemed long enough to quantify visitation and sufficiently brief to prevent multiple counts of the same pollinator; several pollinators could be readily followed and

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Table 1 Combinations of floral displays used in the experiment to assess facilitation of pollination through floral diversity. Treatments refer to the number of potted individuals of each species added to 50 naturally growing *Raphanus raphanistrum* in 3.6×3.6 m plots. The total number of plants in each plot is 74

Floral sp. richness	Replicates	*R. raphanistrum*	*H. perforatum*	*S. canadensis*	*C. arvense*
1	4	24	0	0	0
2	3	0	24	0	0
2	3	0	0	24	0
2	3	0	0	0	24
3	3	0	12	12	0
3	3	0	12	0	12
3	3	0	0	12	12
4	3	0	8	8	8

distinguished across the plots for this length of time. Visitation frequency results were analysed using generalized linear models assuming a Poisson error as appropriate for count data. Rarefaction curves to compare pollinator species richness among treatments were generated using EstimateS software Version 7.5 (Colwell 2000). Differences among treatments were evaluated by comparing the observed diversity of the smaller pollinator communities with the 95% confidence limits of the richest community (Magurran 2004).

On 25 July the proportion of *R. raphanistrum* flowers that formed fruit was scored from 30 sequentially produced flowers on each of eight randomly selected plants within each plot. Flowers that did not produce fruit left an obvious scar at the point of attachment to the stem and could be easily scored as such. Seed set per fruit was determined from the four apical (most recently matured) pods per plant and from 16 randomly selected plants in each plot. Fruit and seed data are directly relevant to the period of pollinator observation as fruit and seed develop within a few days of floral anthesis and flowers themselves last only a single day (personal observation). The number of seed per fruit was analysed using a linear mixed-effect model to account for nesting.

FACILITATION AND COMPETITION FOR POLLINATORS UNDER CHANGING CONDITIONS OF RELATIVE ABUNDANCE

In the second experiment, run simultaneously with the first, facilitation and competition for floral visitors were examined in two-species mixtures (*R. raphanistrum* and *C. arvense*) where the floral density of the second species was allowed to vary. Seven 3.6×3.6 m plots were thinned so that each contained only 50 *R. raphanistrum* and 50 *C. arvense*. The number of *C. arvense* inflorescences in each plot was allowed to vary naturally as the inflorescences opened and dehisced. The abundance of flower visitors to *R. raphanistrum* was recorded (as above) during 2-min observations twice daily over 8 days and scored against the number of open *C. arvense* inflorescences. Immediately after each observation period all flower visitors within the plot were counted over a 2-min period, regardless of whether they were visiting *R. raphanistrum* or *C. arvense*, and the proportion visiting *R. raphanistrum* was calculated based on the assumption that total flower visitors did not differ between the two sequential observation periods.

Breakpoint regression was used to analyse the number of flower visitors to *R. raphanistrum*. Following inspection of the distribution of residuals from a generalized linear model regression applied to the entire data set, the breakpoint was set at 32 *C. arvense* flower-heads, the value that resulted in the least residual deviance (Crawley 2002). The observed proportion of flower visitors that visited *Raphanus* plants (number visiting *Raphanus* plants divided by the total number of flower visitors within the plot) was compared with the values

expected under the null hypothesis that visitors do not discriminate between flowers but rather visit flowers based on their relative abundance alone. Arcsin transformation was applied to these proportion data and a linear model used to compare observed and expected values.

Results

POLLINATION LIMITATION

A total of 788 seeds were obtained from 200 hand-pollinated flowers compared with 525 seeds from the same number of open-pollinated flowers (nested ANOVA, $F = 114.0$, d.f. = 1, $P < 0.005$). This 50% increase in seed set of hand-pollinated flowers implies that seed set was pollen limited even under weather conditions that were highly favourable for pollinator activity. The lower seed set of open pollinated flowers can be attributed to both a lower proportion of open pollinated flowers developing into fruit (69.5% compared with 90.5% fruit set by hand-pollinated flowers) and fewer seed in each fruit: 3.8 ± 0.13 in open-pollinated fruit vs. 4.3 ± 0.12 in hand-pollinated fruit ($t = 1.99$, d.f. = 86, $P < 0.01$).

FLORAL DIVERSITY

A total of 2952 insects in 800 min were observed visiting *R. raphanistrum* across the 25 plots. The most common pollinators, accounting for 61% of flower visitors, were bees *Bombus lapidarius*, *B. pascuorum* and *B. terrestris*, *Apis mellifera*, *Colletes* sp. and *Halictus* sp., with the hoverflies *Episyrphus balteatus*, *Syrphus ribesii*, *Metasyrphus corallae* and *Sphaerophoria scripta* accounting for 38% of flower visitors. Occasional visits to flowers by butterflies (*Pieris brassicae*) and sphecid or eumenid wasps accounted for the remaining 1%.

Numbers of pollinators visiting *R. raphanistrum* were significantly higher in plots to which any one of the three coflowering species had been added (Fig. 1). The identity of the coflowering species, i.e. *S. canadensis*, *H. perforatum* or *C. arvense*, made little difference to the outcome, with each enhancing visits to *R. raphanistrum*. In three-species mixtures, facilitation was enhanced additively when *H. perforatum* and *C. arvense* were added to *R. raphanistrum*, but the significant negative interactions between the effects of *H. perforatum* and *S. canadensis* and of *C. arvense* and *S. canadensis* indicated early saturation of the facilitatory effect of floral diversity (Fig. 1; Table 2).

Thirty-two per cent of *R. raphanistrum* flowers developed into fruit but no significant difference was observed among plots ($F_{7,199} = 1.271$, $P = 0.26$). By contrast, the number of seeds per fruit was higher in plots to which *S. canadensis* or *H. perforatum* had been added, but there was no additional increase when combinations of these plants were added (Fig. 2, Table 3).
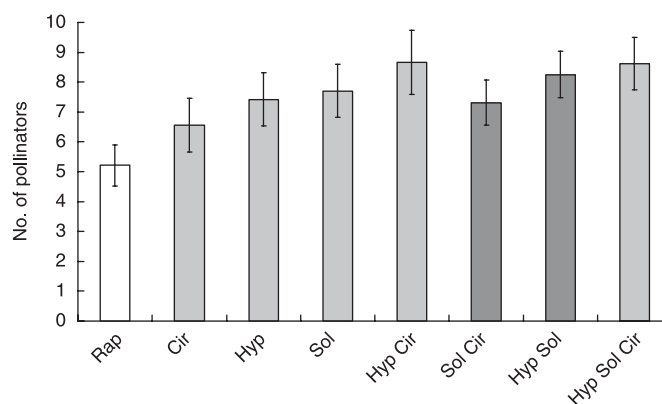
Fig. 1 Number of pollinators visiting *R. raphanistrum* in single and mixed species plots (mean and standard error). The control plot (labelled 'Rap' at the left of the graph) consisted of 74 *Raphanus raphanistrum* plants only. Treatments consisted of the addition of 24 plants of *Cirsium arvense* (Cir), *Hypericum perforatum* (Hyp), *Solidago canadensis* (Sol), or balanced combinations of these, to plots containing 50 *Raphanus* plants. Shaded bars are significantly different to the control, and heavily shaded bars indicate significant negative interaction effects among added species. Significance values are given in Table 2.
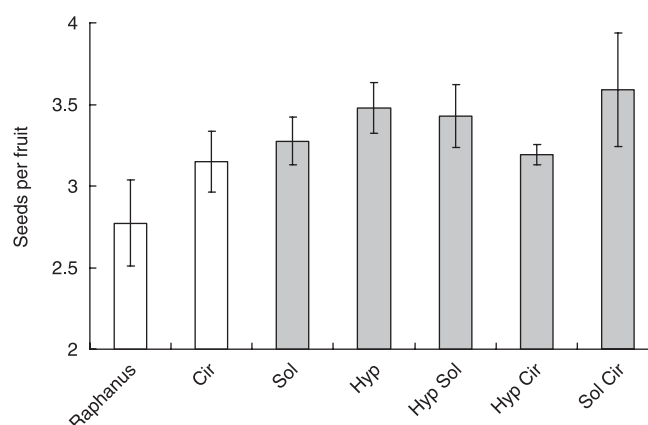

Fig. 2 Seed per fruit among *R. raphanistrum* plants in single and mixed species plots. The treatments refer to the addition of *H. perforatum* (Hyp), *S. canadensis* (Sol) or *C. arvense* (Cir) singly or in combination, and are compared with the control consisting of *R. raphanistrum* plants only. There are no significant interaction effects. Shaded bars are significantly different to the control at the 0.05 level.

Table 2 Generalized linear model of pollinator visits to plots with different floral displays. Significance tests carried out using the *F*-distribution to account for overdispersion of the data. After accounting for date and time of day, addition of either *H. perforatum* (Hyp), *S. canadensis* (Sol) or *C. arvense* (Cir) to *R. raphanistrum* resulted in a significant increase in pollinator visits. Significant Sol:Cir and Hyp:Sol interactions indicate negative (non-additive) interaction effects of these floral combinations on pollinator visits (see Fig. 1), while the three-way interaction of Hyp:Cir:Sol is not significantly different to the expected additive outcome

Terms	d.f.	Residual deviance	Residual d.f.	*F*-value	*P*(> *F*)
Null		744.2	399		
Time (am/pm)	1	726.5	398	17.63	0.001
Date	7	666.7	391	8.55	0.001
Hyp	1	629.8	390	36.93	0.001
Cir	1	622.6	389	7.17	0.007
Sol	1	607.5	388	15.06	0.001
Hyp:Cir	1	607.5	387	0.02	NS
Sol:Cir	1	599.8	386	6.66	0.009
Hyp:Sol	1	593.1	385	7.72	0.005
Hyp:Cir:Sol	1	591.8	384	1.33	NS

Table 3 Results of nested mixed effects model (plants nested within plots) showing significance of treatment effects on seed set per fruit of *R. raphanistrum* from plots to which *H. perforatum* (Hyp), *S. canadensis* (Sol) or *C. arvense* (Cir) had been added either singly or in combination, compared with control plots that contained *R. raphanistrum* only. There are no significant interaction effects

Terms	Seeds/ fruit	SE	d.f.	*t*-value	*P*-value
Intercept (*Raphanus*)	2.77	0.26	1400		
Hyp	3.48	0.15	18	2.296	0.035
Sol	3.28	0.14	18	2.231	0.039
Cir	3.15	0.19	18	1.146	NS
Hyp:Sol	3.43	0.19	18	−1.480	NS
Hyp:Cir	3.19	0.06	18	−1.234	NS
Sol:Cir	3.59	0.35	18	−0.610	NS

C. arvense had no effect on seed production, reflecting the fact that it facilitated insect visitation the least.

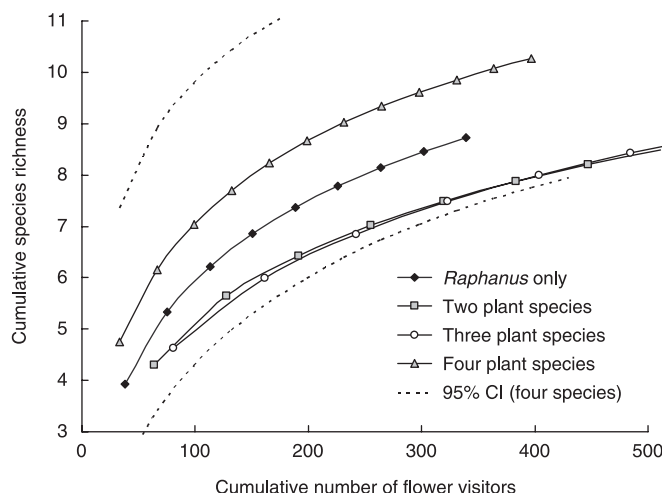There was no significant relationship between floral diversity and flower visitor richness, as reflected by

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Fig. 3 Rarefaction curves of cumulative pollinator species for the four plant richness treatments. All curves lie within the 95% confidence limits of the rarefaction curve of the richest pollinator community (found in the treatment with four plant species), indicating no difference in pollinator richness among treatments. The two shorter curves represent the actual sampling effort (number of flower visitors observed) for these treatments.
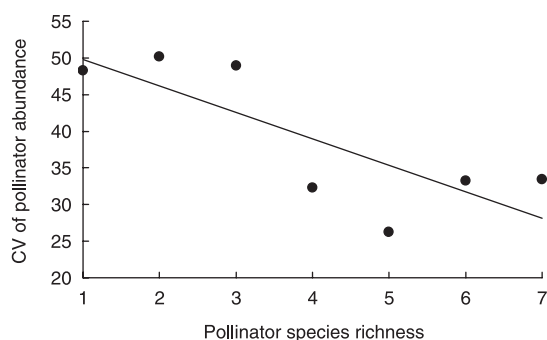

Fig. 4 The coefficient of variation in the number of pollinators at *R. raphanistrum* declines with increasing species richness of floral visitors ($F_{1,5} = 8.39$, $P = 0.034$).

rarefaction curves that lie within the 95% confidence intervals of that of the richest community (Fig. 3). The variability in insect abundance across plots and dates did, however, decline with increasing insect species richness (Fig. 4; $F_{1,5} = 8.39$, $P = 0.034$) indicating increased stability of pollination service at diverse floral displays.

RELATIVE ABUNDANCE

In *Cirsium–Raphanus* mixtures insect visits to *R. raphanistrum* flowers were positively correlated with the abundance of *C. arvense* inflorescences at relatively low densities (i.e. less than 32 *C. arvense* inflorescences; Fig. 5, $z = 6.89$, d.f. = 117, $P < 0.0001$). As *C. arvense* inflorescences became more abundant the frequency of flower visits to *R. raphanistrum* declined to a point (at approximately 300 *C. arvense* inflorescences) where all flower visitors were monopolized by *C. arvense* (Fig. 5, $z = 7.661$, d.f. = 117, $P < 0.0001$). This pattern of visitation departed significantly from the expected null pattern based on visitation frequency being directly related to the proportion of *R. raphanistrum* flowers in the floral mixture (Fig. 6, $R^2 = 0.6486$, $F_{1,117} = 218.8$, $P < 0.0001$).
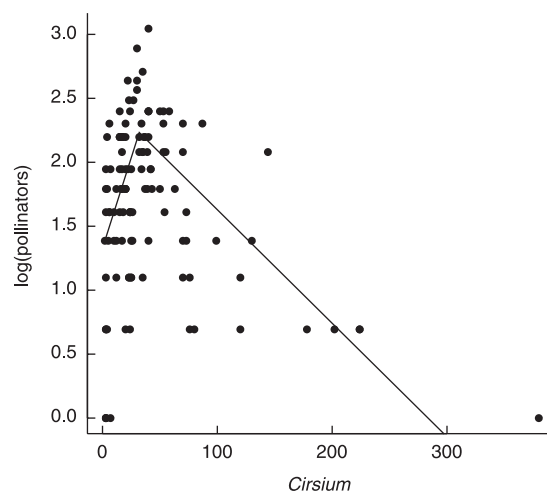

Fig. 5 Number of pollinators visiting *R. raphanistrum* flowers at different densities of *C. arvense* inflorescences. Values greater than the intercept suggest facilitation of pollinator visitation to *R. raphanistrum* by *C. arvense*, while values below the intercept indicate negative interactions. Breakpoint regression line shown with break set at 32 *Cirsium* flower heads.

Discussion

Plants can respond to competition for shared pollinators by adjusting breeding systems (Fishman & Wyatt 1999) or partitioning pollinators in time (Stone *et al*. 1998). Many diverse coflowering communities, such as herbaceous meadows and alpine pastures, do not, however, appear to be structured by competition for pollinators, either because pollinators do not limit fitness or, alternatively, because plants that share pollinators interact positively to enhance pollination services. Evidence for such positive interactions among species, often congenerics, that bear similarly structured flowers is accumulating (Gross *et al*. 2000; Moeller 2004) or
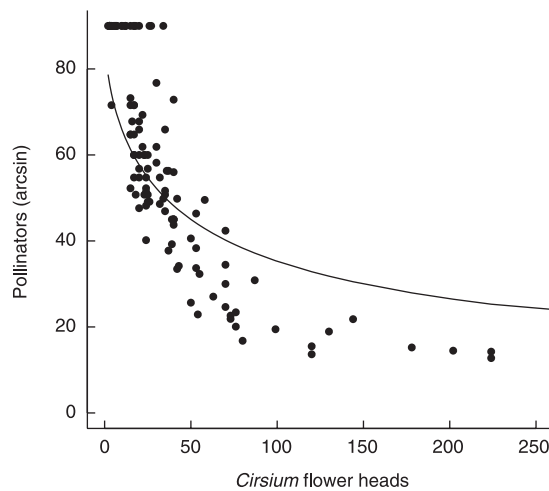
Fig. 6 Expected and observed values for the proportion of insect visits to *R. raphanistrum* flowers under varying *Cirsium arvense* floral densities. Expected values are represented by the line, with observed data denoted by the black circles. Observed and expected values are significantly different ($R^2 = 0.6486$, $F_{1,117} = 218.8$, $P < 0.0001$).

has been implied (Schemske 1981; Motten 1986; Rathcke 1988), but these studies have limited capacity to explain the coexistence of plants that have distinctly different floral shapes and colours subject to discrimination by pollinators. In seeking to broaden the relevance of positive interactions in mitigating Allee effects among small populations (or among rare species within mixtures) a demonstration of facilitative effects among morphologically diverse flowers is required. This study provides support for the hypothesis that multispecies floral displays can facilitate pollination leading to increased fitness of coflowering species, although it says nothing about the actual mitigation of Allee effects, which remains to be demonstrated.

The increased number of insects visiting *R. raphanistrum* flowers in mixed-species plots demonstrates heterospecific facilitation of pollination. Positive interactions are observed under a constant collective display size and, more importantly, the facilitating species have distinctly different floral colours and/or structures indicating that pollinators are responding positively to the presence of different floral forms. Previous studies provide evidence for facilitation only among plant species that have similar floral forms (except in the special case of deceit-pollinated flowers, Laverty 1992; Johnson *et al.* 2003) and it is not possible to determine whether pollinators are actively seeking multispecies mixtures or whether they fail to distinguish between similar floral cues and respond instead to an apparently uniform collective display.

In this study facilitation was largely insensitive to floral diversity *per se* as the facilitative effect often saturated on the addition of only one treatment species. Floral richness may still enhance facilitation through a sampling effect, as some species (*S. canadensis* and *H. perforatum*) were more effective facilitators than others

(*C. arvense*) (Fig. 1). The fact that the most marked negative interaction occurred between the two yellow-flowered species, *S. canadensis* and *H. perforatum*, suggests that facilitation effects may saturate more quickly among similarly coloured floral combinations, but additional work is required to confirm this idea.

Positive effects on pollinator attraction may be counteracted by pollen transfer between species within a patch. Alternatively, generalist pollinators attracted to a diverse stand may show preference for particular species within the stand. Research is therefore needed to determine whether there is mutual facilitation among species rather than the solely unidirectional effect demonstrated here. No information on the impact of interspecific pollen transfer is available within the context of this study, but it seems unlikely to be sufficiently large to overcome the positive benefit *R. raphanistrum* obtains from association with the additional species.

In *Cirsium–Raphanus* mixtures the balance between facilitation and competition was dependent on relative density of *C. arvense* inflorescences. Insect visits to *R. raphanistrum* were facilitated by *C. arvense* up to a peak of about 32 inflorescences per plot (2.5 inflorescences m^{-2}). Thereafter, a continued increase in the density of *C. arvense* inflorescences resulted in a disproportionate share of insects visiting *C. arvense* to the detriment of *R. raphanistrum* (Fig. 5). This confirms the conceptual model of the balance between facilitation and competition proposed by Rathcke (1983), where facilitation is expected to be observed only in a constrained set of circumstances based on a relatively equitable distribution of floral types at low population densities. These results echo Moeller's conclusion that small populations are expected to derive greater benefit from shared pollinators than large populations (Moeller 2004). This becomes particularly relevant for small populations that are susceptible to Allee effects, now widely reported among plant-pollinator systems (Ghazoul 2005), as facilitation by heterospecifics may mitigate the negative effects of low density. Species coexistence maintained by facilitative interactions in low-density floral mixtures is theoretically feasible provided pollinator visitation responds as an accelerating function of plant density (Feldman *et al.* 2004). In most circumstances, system stability is likely to be undermined as plant frequencies change and pollinators respond in a frequency-dependent manner (as for *C. arvense*) unless other frequency-dependent factors constrain the population size of the favoured species. Facilitation may therefore, at best, temporarily prolong the persistence of rare plants in degraded or fragmented populations by acting against Allee effects, and provide increased opportunities for population recovery.

Fruit set of *R. raphanistrum* among plots is likely to reflect weather conditions that determine pollinator activity regardless of treatment effects. Thus, on cool days, when pollinator activity was almost non-existent, few if any flowers were likely to be pollinated. As the flowers of *R. raphanistrum* last only a single day, fruit

set is determined by gross environmental conditions that affect all plants equally across treatments. In contrast, the number of seeds per fruit is determined by the quality of the pollinator service received by each flower during the relatively short time during which it is receptive. This pollinator service is a function of the number of visits per flower and the quality (i.e. compatibility) of the pollen transferred (Wilcock & Neiland 2002). This study has shown that the number of pollinators is subject to facilitation by local floral community structure, and its effects are clearly reflected in seed set.

MECHANISMS OF FACILITATION

The most frequently quoted mechanism for facilitation of pollination proposes convergent floral syndromes that attract a largely non-discriminating pollinator community to a collective floral display (Schemske 1981; Rathcke 1983; Roy 1994). Niche differentiation permits the coexistence of several flowering plant species at higher densities, which, if their flowers are similar, may provide combined floral displays greater than that achieved by single species stands. In this study, collective attraction was eliminated as an explanation because floral densities were kept constant and treatment species were selected because of their divergent floral cues. Pollinators such as bees and hoverflies would almost certainly be able to discriminate among these flowers (Sutherland *et al*. 1999; Giurfa & Lehrer 2001; Weiss 2001), and so enhanced attraction to diverse displays is more likely to be a function of display diversity than of apparent display size.

A second proposed mechanism, which is at present hypothetical, is that the availability of floral refuges or 'competitor-free space' provided by alternative flower types may allow the persistence of greater numbers of pollinator species and individuals within a patch. Pollinators displaced from preferred flowers by aggressive competitors may return to pollinate these preferred flowers if they temporarily relocate to alternative species not visited by the competitively dominant pollinator. Aggression and displacement of pollinators at flowers has been observed among insects (Johnson & Hubbell 1974; Nagamitsu & Inoue 1997; Goulson 2003) as well as birds (Smith-Ramirez & Armesto 2003), but its implications for pollination have as yet not been investigated. The 'competitor-free space' provided by less rewarding flowers within a diverse floral community may benefit the most rewarding flowers by maintaining a suite of pollinators 'in reserve' to continue the pollination service on the departure of the competitively dominant pollinator. The less attractive plants may in turn benefit from hosting displaced pollinators if the floral mixtures within which they occur attract disproportionately more pollinators than mono-specific stands of these species. Aggressive and mass-recruiting species such as honeybees may, for many flowers, be comparatively poor quality pollinators (Goulson 2003) and their displacement of other pollinators, such as solitary bees, from highly rewarding flowers may improve both the quantity and quality of the pollination service received by coflowering but less attractive plants.

A third mechanism is complementary attraction, which predicts differential attraction by plants to different generalist pollinating species such that the combined display maximally attracts a broad spectrum of pollinators (Rathcke 1988; Moeller 2004). This provides benefits to all plants that share generalist pollinators by damping variability in pollinator dynamics. This small-scale mechanism differs from the similar but broad-scale mechanism proposed by Moeller (2004) where diverse floral communities stabilize pollinator dynamics by providing a more temporally consistent supply of floral resources. Both the floral refuge and complementary attraction hypotheses predict increased species richness of pollinators with floral diversity, which, after correcting for sample size, was not observed by this study.

A fourth mechanism is complementarity of resource provision. If plants vary in their provision of different floral rewards, such as nectar and pollen, then pollinators seeking these resources may specialize on different plant species for different resources. In this experiment, bees (particularly bumblebees) visited *C. arvense* and *H. perforatum* primarily for nectar, but collected pollen from the nectar-deficient *R. raphanistrum* (J. Ghazoul, personal observations; see also Galen & Plowright 1985). Such pollinators may therefore prefer sites where both resources are readily available, and resource complementarity among plant species may be mutually beneficial for pollinator attraction as a result. Saturation of facilitative effects at only two species mixtures is not unexpected under this hypothesis if only two resources (pollen and nectar) are sought. Similarly, this mechanism predicts that facilitative effects should not be observed where preferred pollen and nectar resources are found within the same flowers, and is not likely to be relevant to pollinators that collect only pollen or only nectar (although nectar composition varies among species and may be the basis for pollinator discrimination: Galetto & Bernardello 2003; Pacini *et al*. 2003; Bluthgen & Fiedler 2004; Mevi-Schutz & Erhardt 2005). In this study it was not possible to track visitation patterns by individual foragers but this will be the focus of future work.

Conclusions

Müllerian convergence of floral characters has been suggested to facilitate pollination among low-density species by raising the apparent floral density (Schemske 1981; Feldman *et al*. 2004). Likewise, non-rewarding orchids benefit most from being located close to rewarding flowers that are most similar in flower colour and shape (Johnson *et al*. 2003). The stability of such systems is based on the assumption that pollinators do not distinguish between plant species when deciding upon which patch, and which plants within a patch, to visit.

For bees, at least, this assumption is known to be unrealistic (Thomson & Chittka 2001) and, in any case, contrasts with the results of this study, which show that facilitation occurs among species bearing morphologically highly distinctive flowers subject to discrimination by pollinators. I conjecture that resource complementarity allows optimal procurement of multiple floral resources to be achieved by pollinators at diverse floral arrays and that pollinators shape their foraging decisions on this basis.

By eliminating density and display size effects I have shown that facilitation can be affected by floral variety alone. However, when floral density of the facilitating species is allowed to increase, the benefits of facilitation give way to the costs of competition as the most rewarding or most abundant plants monopolize pollinator visits (Fig. 5). Facilitation of pollination may also explain floral colour change (Weiss & Lamont 1997; Oberrath & Bohning-Gaese 1999) which, by diversifying the floral displays of monospecific stands, may provide opportunities for intraspecific facilitation by attracting more pollinators from more species, thereby increasing both the number of pollinator visits and the stability of the pollination service. Finally, facilitation should be particularly important for specialist plants that depend exclusively on one or a few generalist pollinators, and the proclivity of asymmetric plant-pollinator networks (Vazquez & Aizen 2004) implies that many species are likely to fall within this category.

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