COMMUNITY ECOLOGY - ORIGINAL PAPER

Do pollinators influence the assembly of flower colours within plant communities?

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Abstract The co-occurrence of plant species within a community is influenced by local deterministic or neutral processes as well as historical regional processes. Floral trait distributions of co-flowering species that share pollinators may reflect the impact of pollinator preference and constancy on their assembly within local communities. While pollinator sharing may lead to increased visitation rates for species with similar flowers, the receipt of foreign pollen via interspecific pollinator movements can decrease seed set. We investigated the pattern of community flower colour assembly as perceived by native honeybee pollinators within 24 local assemblages of co-flowering *Oxalis* species within the Greater Cape Floristic Region, South Africa. To explore the influence of pollinators on trait assembly, we assessed the impact of colour similarity on pollinator choices and the cost of heterospecific pollen receipt. We show that flower colour is significantly clustered within *Oxalis* communities and that this is not due to historical constraint, as flower colour is evolutionarily labile within *Oxalis* and communities are randomly structured with respect to phylogeny. Pollinator observations reveal that the likelihood of pollinators switching between co-flowering species is low and increases with flower colour similarity. Interspecific hand pollination significantly reduced seed set in the four *Oxalis* species we

investigated, and all were dependant on pollinators for reproduction. Together these results imply that flower colour similarity carries a potential fitness cost. However, pollinators were highly flower constant, and remained so despite the extreme similarity of flower colour as perceived by honeybees. This suggests that other floral traits facilitate discrimination between similarly coloured species, thereby likely resulting in a low incidence of interspecific pollen transfer (IPT). If colour similarity promotes pollinator attraction at the community level, the observed clustering of flower colour within communities might result from indirect facilitative interactions.

Introduction

Elucidating the rules that govern the assembly of species into communities is a central objective of ecological research. This field has received recent stimulus through the debate around whether communities are assembled randomly (Hubbell 2001) or through adaptive processes (Ackerly 2003) and through advances in the tools available to investigate the evolutionary history of community membership (Webb et al. 2002). Many recent studies have found nonrandom patterns of community membership with respect to phylogeny (reviewed by Vamosi et al. 2009) and putatively adaptive traits (e.g. Armbruster et al. 1994; Muchhala and Potts 2007). Studies of plant community structure have largely focussed on the assembly of physiologically relevant traits (e.g. Keddy 1992; Weither and Keddy 1995), thus emphasizing the role of abiotic factors

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and competition between plants for these resources (Tilman 2004) in governing species co-occurrence. The role that pollinators play in structuring plant communities has received less attention (reviewed by Sargent and Ackerly 2008).

Communities are thought to be assembled by either or both of two main non-neutral processes: (1) ecological sorting, whereby certain preadapted species can successfully invade and persist within a community while others cannot (Strong et al. 1979), and (2) the evolution of interacting species, which typically leads to the divergence of traits related to resource use (i.e. character displacement; Brown and Wilson 1956). Pollinators can potentially contribute to the assembly of communities of outcrossing plants through both processes. They can facilitate or impede the establishment of particular species within a community by either promoting or inhibiting their reproductive success. Alternatively, they may impose convergent or divergent selection on sympatric species through differential preferences, thus permitting coexistence.

Plant species using different pollinators can coexist without competing for pollination services. Many sympatric plant species, however, attract the same pollinators and thus compete directly for visits as well as interact indirectly through interspecific pollen transfer or IPT (reviewed by Morales and Traveset 2008). Studies have shown that IPT can have negative effects on female fitness (Brown and Mitchell 2001; Moragues and Traveset 2005) as well as male fitness through the loss of viable pollen to heterospecific stigmas (Feinsinger and Busby 1987; Brown and Mitchell 2001). Divergence in pollen placement positions likely reduces IPT and facilitates the coexistence of plant species sharing pollinators (Armbruster et al. 1994; Pauw 2006; Muchhala and Potts 2007). Alternatively, a divergence in traits promoting flower constancy in pollinators may reduce the proportion of heterospecific pollen they carry.

Flower colour plays a key role in pollinator attraction, affecting both pollinator preference (Bradshaw and Schemske 2003) and constancy on a particular flower type (Hill et al. 1997). The distribution of flower colours within flowering plant communities may therefore reflect the impact of pollinators on their assembly. If flower colour similarity reduces the ability of pollinators to discriminate between sympatric co-flowering species, the fitness costs of IPT may promote flower colour dissimilarity within communities through evolutionary divergence or ecological filtering. However, some communities of co-flowering congeners that share pollinators have converged on certain flower colours and floral morphologies (e.g. Brown and Kodric-Brown 1979). Moeller (2004) showed that increasing the number of ecologically similar congeners at a site leads to increased pollinator visitation, as well as

decreased pollen limitation. In this way, co-flowering plants that share pollinators can actually have a facilitative effect on each other, increasing fitness and potentially promoting similarity of flower colour within communities (Ghazoul 2006). Thus, pollinator-mediated interactions between plant species could result in either overdispersed (more dissimilar than expected by chance) or clustered (more similar than expected by chance) distributions of floral traits within communities (Armbruster et al. 1994; Muchhala and Potts 2007).

Here we determine whether and how pollinators contribute to the assembly of flower colour within communities of co-flowering *Oxalis* species in the Greater Cape Floristic Region of South Africa. *Oxalis* is a very species-rich genus within South Africa, with ca. 210 species (Dreyer and Makgakga 2003). All are geophytes with true bulbs that produce actinomorphic flowers in inflorescences, with some species exhibiting a reduced single-flowered inflorescence. Most species flower from May to September, and there is extensive variation in flower colour, with white, yellow, orange and pink being the predominant colours.

Oxalis in the Cape provides a useful system for investigating community flower colour assembly by pollinators for a number of reasons. First, preliminary observations suggest that all *Oxalis* species investigated share the native Cape honeybee (*Apis mellifera capensis*) as their predominant visitor. Second, most of the South African *Oxalis* species flower simultaneously during winter when few other plants are flowering and thus compete with one another for pollinators. Lastly, flower colour is highly variable, with more than a third of the species observed in this study exhibiting flower colour polymorphisms. We examined both the pattern of assembly of flower colour within *Oxalis* communities and the possible mechanisms by which pollinators could generate this pattern. Specifically, we asked the following questions. (1) Are *Oxalis* communities randomly assembled with respect to flower colour? (2) Is the assembly of flower colour a result of community-level phylogenetic relatedness and patterns of trait evolution? (3) Does flower colour influence pollen transfer among sympatric species? (4) Does the receipt of foreign pollen decrease maternal fitness?

Materials and methods

Flower colour distribution in *Oxalis* communities

Flower colour in *Oxalis*

We studied the distribution of flower colour within 24 *Oxalis* communities, each containing two or more

flowering *Oxalis* species. Communities were widely separated, spanning a geographic distance of 420 km in both the Succulent Karoo and Fynbos biomes of the Western Cape of South Africa. Very few other plants were flowering during the time of this study. We measured the adaxial petal reflectance of all co-flowering congeners of *Oxalis* with an Ocean Optics 4000 spectrophotometer (Ocean Optics, Dunedin, FL, USA), coupled to an Ocean Optics Mini–D2T light source. Three flowers of each species/colour morph were randomly picked from plants at least 1 m apart. The fiber-optic probe was kept at a constant distance (6 mm) and angle (45°) from the top centre of the petal. We only measured outer corolla colour as the central throat of the flower was yellow for all species investigated. The spectrophotometer was regularly recalibrated between measurements by using the Ocean Optics WS-1 diffuse reflectance standard.

To determine the appearance of these colours from a honeybee's perspective, we used the model proposed by Chittka (1992). Honeybees have three types of photoreceptors (UV, blue and green) and their differential stimulation relative to the background (leaves in this case) under ambient sunlight results in all the colours observed by the honeybee (Chittka 1996). The proportional stimulation of these photoreceptors was transformed to a single x and y coordinate and plotted onto a hexagon designed to represent bee perceptual space, which is from here on referred to as "beespace" (Chittka 1992). Using the centroid of the three replicate coordinates for each species or colour morph, we calculated Euclidian distances between sympatric species and colour morph pairs within beespace.

The assembly of flower colours in communities

We used the regional pool of all species and colour morphs observed in this study and their beespace coordinates to construct a null model. By shuffling species and colour morphs without replacement into random communities, we created 5,000 permutations of the observed regional co-occurrence pattern. This null model retained both the frequency of occurrence of each species/colour morph regionally, as well as the number of species within each community. Species or colour morphs occurring at more than one site were first independently shuffled without replacement to obtain a single beespace coordinate for each draw in order to avoid the same species/colour morph occurring more than once in a given random community.

We calculated the Euclidean colour distance in beespace between each species/colour morph and its most similar sympatric congener (minimum colour distance) across all communities for each random assemblage. As test statistics we used: (1) the median minimum colour distance across the assemblage, and (2) counts of species/colour morph pairs with minimum distances less than 0.05 and 0.2, respectively. We used minimum Euclidean colour distances because these best reflect the maximal similarity between species/colour morphs at a site, and the cut-off values of 0.05 and 0.2 because they are likely colour distances below which colours become indistinguishable to bees without differential conditioning (0.05; Dyer and Chittka 2004), or below which floral constancy was disrupted in experiments with model flowers (0.2; Chittka et al. 2001). The median was used because minimum colour distances were non-normally distributed across assemblages. We used two-tailed tests to determine whether the observed values of the test statistics were significantly different from the expected values generated under random community assembly.

Phylogeny and community flower colour assembly

To investigate the role of evolutionary history in structuring flower colour within *Oxalis* communities, we investigated: (1) the phylogenetic structure of *Oxalis* communities, and (2) the relationship between genetic distance and flower colour similarity within *Oxalis*. An estimate of evolutionary relationships between the investigated *Oxalis* species was generated by pruning the Bayesian phylogenetic tree with the highest log likelihood score from Oberlander (2008). As different colour morphs of the same species were not included in the original phylogeny, we assumed polytomies for all colour morphs within each species, and used branch length estimates between colour morphs of 5% of the original terminal branch length for the given species, which is probably a conservative approximation of intraspecific divergence in *Oxalis* (Zietsman et al. 2009).

We assessed the phylogenetic structure of *Oxalis* communities by comparing the observed phylogenetic distance within communities to random expectation under the independent swap null model implemented in Phylocom (Webb et al. 2004). This procedure generates random species assemblages by resampling from the regional species pool, retaining both the number of species per community and the frequency of occurrence of each species in the assemblage, which makes it directly comparable to our colour distance null model. We tested for phylogenetic signal (i.e. the tendency for closely related species to have similar flower colour) using a matrix correlation approach. A matrix of Euclidean beespace distances between species/colour morphs was compared to a genetic distance matrix (branch lengths) using a Mantel test. One thousand permutations were used for all randomization procedures.

Mechanisms through which pollinators could structure flower colour

Honeybees as Oxalis pollinators

To determine the identity and effectiveness of *Oxalis* pollinators, we observed pollinator activity within 7 of the 24 *Oxalis* communities used in this study. All flowers along random walk transects were inspected for potential insect pollinators. Observations were conducted between 9 a.m. and 3 p.m. over 5 days in June 2008 and another 5 days in August 2008 when temperatures were high enough for flowers to open. At each site, between one and four honeybees were captured and examined for the presence of *Oxalis* pollen. To further assess the effectiveness of honeybees as pollinators, we measured the amount of pollen deposited on stigmas of virgin flowers of *Oxalis pes-caprae* during single visits by honeybees.

Foraging behaviour of honeybees in response to flower colour

To determine what effect the flower colour of sympatric species had on pollinator transitions between them, we observed honeybee foraging patterns within the same seven *Oxalis* communities over the same time period. We followed individual honeybees on foraging trips for up to 20 sequential flower visits or until they were lost. We calculated the observed and expected probabilities of pollinator transition between species as well as between and within colour morphs of species that are polymorphic for flower colour. To calculate the observed probability of transition between a species pair [$P(T_{A \leftrightarrow B})$], we divided the number of pollinator transitions between them by the sum of within (n_{AA}, n_{BB}) and between (n_{AB}, n_{BA}) species transitions (Eq. 1):

$$P(T_{A \leftrightarrow B}) = \frac{n_{AB} + n_{BA}}{n_{AA} + n_{AB} + n_{BA} + n_{BB}}. \tag{1}$$

To determine expected transitions under random foraging, we recorded the identity and colour of 100 successive flowers along random walk transects through each site. This method was chosen as it accounts for both the density and degree of clumping of each species within a community. We then calculated an expected probability of transition between each species pair using Eq. 1. G-tests were used to compare the observed and expected transition probability matrices for each site.

The probability of pollinator transitions within or between colour morphs of the same species was calculated as the probability of a pollinator leaving the focal colour morph (A1) and landing on the same or a different colour morph:

$$P(T_{A1 \leftrightarrow Ax}) = \frac{n_{A1Ax}}{\left(\sum_{x=1}^{f} n_{A1Ax} + \sum_{y=1}^{m} n_{A1By} \right)}. \tag{2}$$

n_{A1Ax} represents the number of transitions from colour morph A1 to colour morph Ax; n_{A1By} represents the number of transitions from A1 to another species (By) in the community. Species A comprises f colour morphs, and the community has m *Oxalis* species.

Using the observed and expected probabilities of transition, we calculated an index of floral constancy for each pair of sympatric species or colour morphs which quantified the ability of pollinators to discriminate between species/colour morph pairs, controlling for their density and distribution within communities. We modified the constancy index of Gegear and Laverty (2005): $CI = (O - E)/(O + E - 2OE)$, where O is the observed probability of transition between species or colour morphs and E is the expected probability of transition. This index of constancy ranges from -1 (complete discrimination between species/colour morphs) through 0 (random visitation) to 1 (continuously switching between species/colour morphs).

To investigate the effects of flower colour on the probability of pollinator transitions between species/colour morphs, we regressed the constancy index (CI) against the Euclidian colour distance between species/colour morph pairs in bee visual space. We used the Euclidean distance between centroids for interspecies and intermorph comparisons, whereas we used the average Euclidean distance between individuals of the same colour morph for intramorph comparisons. Separate regressions were run for inter- and intraspecies transitions. Whereas the probability of interspecies transitions may be influenced by floral traits other than colour, intraspecific probabilities are less likely to be affected by other traits, as these are less likely to vary within species. Thus, the intraspecific pattern is likely to reflect a more accurate relationship between flower colour and the likelihood of pollen transfer.

Reliance of Oxalis species on their pollinators

To determine the dependence of *Oxalis* on pollinators for seed set, we investigated the compatibility status of four species within a single community in the Jan Marias Nature Reserve in Stellenbosch (Western Cape Province, South Africa—33° 55° 56′8S; 18° 51° 49′1E) between June and September 2008. Four *Oxalis* species co-flowered at more or less equal densities at our experimental site during this time, namely *O. purpurea* (29%), *O. glabra* (17%), *O. tenuifolia* (38%) and *O. pes-caprae* (16%). We hand-pollinated all open flowers on at least ten plants per treatment by applying freshly dehisced pollen to the exposed stigmatic branches of virgin long-styled morph flowers. We

removed the corollas of treated flowers to avoid future visitation by pollinators. Inspection of the stigmas of un-pollinated controls confirmed that pollinators did not visit flowers without corollas. Pollen application by hand delivered equivalent amounts of pollen to single visits by honeybees to virgin flowers with receptive stigmas (ANOVA $F = 1.71$; $df = 1.32$; $p = 0.200$).

First, we tested for selfing ability by comparing seed set in selfed versus outcrossed hand-pollination treatments for each species using Mann–Whitney U tests. Next, we investigated pollinator limitation of seed set by using t tests to compare open naturally pollinated controls with a sup-plemental pollination treatment where all open flowers received additional outcross pollen. Sample sizes are reported in the "Results" section throughout.

The effects of interspecific pollen transfer on reproductive fitness

Pollen transfer between species within communities can result in a reduction in female fitness through two mech-anisms: (1) increased production of potentially less fit hybrid seeds, and (2) decreased seed production due to interference by heterospecific pollen (e.g. stigma clogging). To distinguish between these mechanisms, we conducted two artificial pollination experiments simulating IPT within the same community mentioned above. We hand-pollinated all open flowers on at least ten plants per treatment as follows: (1) hybridization treatment, where we applied only heterospecific pollen in the following combinations: *O. purpurea–O. glabra* pollen, *O. pes-caprae–O. glabra* pol-len, *O. tenuifolia–O. pes-caprae* pollen and *O. glabra–O. purpurea* pollen, and (2) stigma clogging treatment, where we applied conspecific outcross pollen 24 h after hetero-specific pollen using the same species combinations. We marked outcross control treatments and applied conspecific outcross pollen 24 h later, but no heterospecific pollen. As flowers generally lasted 3 days or more, this delay was deemed a likely scenario when sharing pollinators. Mann–Whitney U tests were used to compare the seed sets of the hybridization and stigma clogging treatments to that of the outcrossed hand pollination control for the four species tested.

Results

Flower colour distribution in *Oxalis* communities

Flower colour in *Oxalis*

A total of 309 spectral reflectance measurements were made for 20 *Oxalis* species, seven of which were

polymorphic for flower colour. *Oxalis* flower colours were not evenly distributed in beespace, but tended to form several clusters of species/colour morphs with similar flower colours (Fig. S1 of the Electronic supplementary material, ESM). All but one community with four or more sympatric species or colour morphs had a sympatric spe-cies pair within the same colour cluster in beespace (Fig. 1). These were often so similar in beespace that it is unlikely that bees could distinguish between them on the basis of colour alone. Graphs of all communities with four or more species are presented in Fig. S2 of the ESM. Ninety-three percent of all observed communities had sympatric species pairs with Euclidian distances in bee-space below 0.2, which is the colour distance below which honeybees exhibit low flower constancy on model flowers (Chittka et al. 2001).

The assembly of flower colours in communities

The median minimum Euclidian distance between sym-patric species or colour morph pairs across all communities was significantly clustered when compared to random assemblages. Only 81 out of 5,000 model runs produced a lower median than the observed communities ($p = 0.016$), indicating that flower colours within communities are more similar than expected under random assembly. The observed communities contained significantly more species pairs with Euclidian distances below the values of 0.05 and 0.2 than random communities ($p = 0.035$ and $p = 0.024$ respectively, Fig. 2).

Phylogeny and community flower colour assembly

Only one of the 24 communities of *Oxalis* sampled was assembled nonrandomly with respect to phylogeny (Table S1 of the ESM). There was no significant relationship between genetic and flower colour distance across the species sampled (Mantel test $r = 0.029$, $p = 0.719$).

Mechanisms through which pollinators could structure flower colour

Honeybees as *Oxalis* pollinators

At all sites, honeybees were the predominant visitors on all *Oxalis* species and colour morphs (97.3% of all observed visits), and all captured honeybees carried *Oxalis* pollen. Honeybees deposited 165 ± 53 ($n = 8$) pollen grains per visit on *O. pes-caprae* stigmas, confirming their effec-tiveness as pollinators. A few other insect species (Hymenoptera, Coleoptera, and Diptera) were rare visitors on *Oxalis* flowers.

Pollinator activity in response to flower colour

A total of 1,910 flower-to-flower transitions were recorded
for 142 individual bees. Ninety-three percent of these
transitions were within the same species or colour morph
and the rest were between different species/colour morphs.
In all communities studied for pollinator activity, honey-
bees made significantly fewer transitions between species
or colour morphs than expected from random foraging, as
determined from flower sequences along random walk
transects through the sites (Table 1).

Sympatric species with more divergent flower colours
had a negative constancy index, indicating that pollinators
rarely switched between them while foraging (Fig. 3a).
Only 25% of the variation in the probability of pollinator
transitions between species was explained by the colour

Table 1 Flower constancy of honeybees within communities of co-flowering *Oxalis* species

Community	No. of species (*n*) and colour morphs	Total observed transitions	Proportion of interspecific transitions	*G* value
Turnoff flava	(3) 4	287	0.01	275.25*
Constantia neck	(3) 3	205	0.03	51.73*
Doornfontein	(4) 5	192	0.12	97.47*
Jan Marias1	(4) 4	717	0.04	407.65*
Pools	(2) 3	57	0	43.60*
KBK	(5) 6	260	0.03	241.22*
Before waterfall	(4) 5	192	0.03	444.16*

The number of *Oxalis* species/colour morphs per site, the total number of observed transitions by bees between flowers, and the proportion of these transitions that were between different species/colour morphs are indicated

G tests compared observed and expected transition frequencies between all species/colour morphs at each site and indicate that bees made significantly fewer interspecies/intermorph transitions than expected under random foraging

* $p < 0.0001$

distance between them, suggesting that other traits possibly also act as cues that influence pollinator choice ($R^2 = 0.253$, $p = 0.007$). The intraspecific analysis of colour morphs revealed a similar but stronger relationship ($R^2 = 0.684$, $p < 0.0001$; Fig. 3b). Pollinator transitions were more likely between colour morphs with more similar flower colours.

Reliance of Oxalis species on their pollinators

None of the four *Oxalis* species investigated produced any seeds in the selfing treatment (Table 2), and therefore are probably self-incompatible and hence obligate outcrossers. *O. purpurea* and *O. pes-caprae* were significantly pollen

Fig. 3 The relationship between Euclidean distance in bee perceptual space and flower constancy of bees on **a** sympatric species pairs and **b** colour morphs of the same species. Negative values of the constancy index indicate constancy on one species/colour morph, whereas positive values indicate inconsistent visitation (switching between species/colour morph). Statistically significant logarithmic *trend lines* are plotted
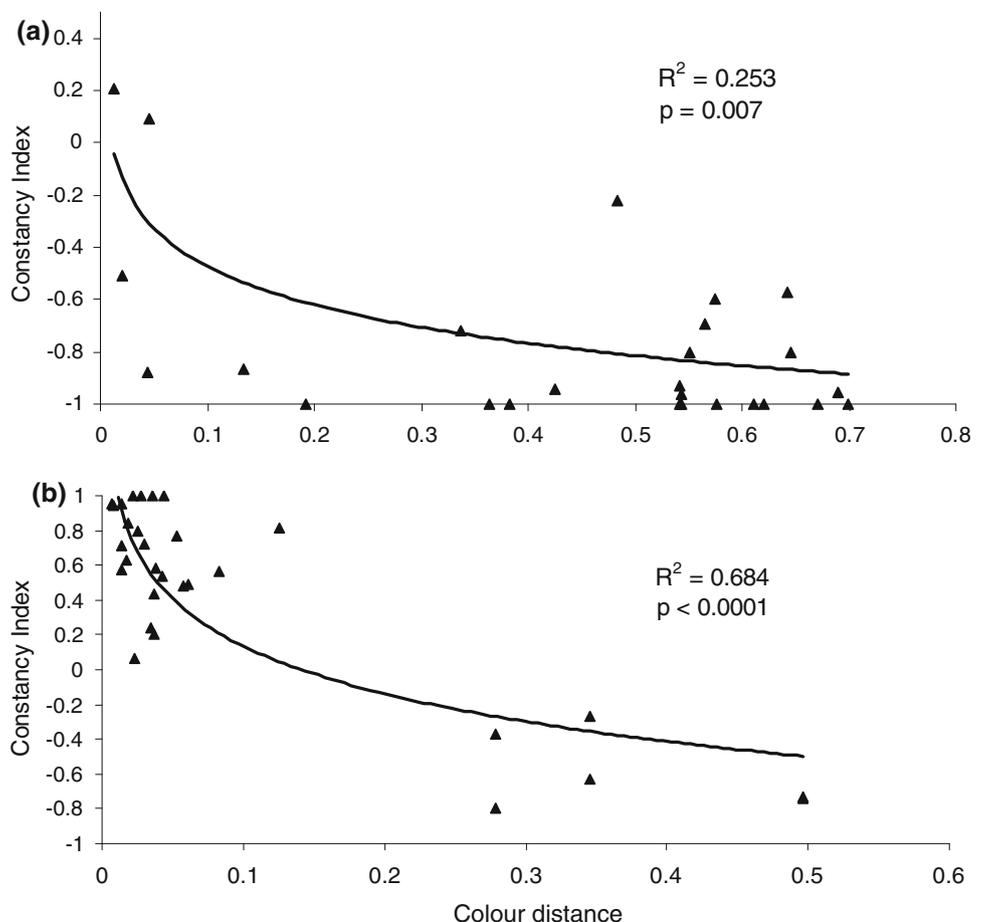
Table 2 Experimental results from the pollen limitation and breeding system analyses, showing the reliance of *Oxalis* on pollen transfer for maximal seed set

Species	Pollen limitation			Breeding system		
	Natural	Supplemental	*t*	Outcross	Selfing	*U*
O. purpurea	22.4 ± 10.5 (42)	39.5 ± 8.6 (24)	6.76*	35.3 ± 10.3 (16)	0.0 (10)	0.0*
O. pes-caprae	13.0 ± 3.9 (18)	–	5.14*	26.4 ± 10.3 (16)	0.0 (11)	0.0*
O. tenuifolia	3.1 ± 1.4 (17)	2.6 ± 1.4 (18)	−1.06	4.0 ± 1.1 (12)	0.0 (11)	0.0*
O. glabra	11.6 ± 5.7 (58)	10.6 ± 4.7 (34)	−0.80	8.1 ± 3.5 (12)	0.0 (14)	0.0*

Values are mean seed number (±1 SD), with sample size shown in brackets

Natural seed set (open pollination) served as the control in the pollen limitation analyses, and the outcross hand-pollination treatment served as control in the breeding system analyses. For *O. pes-caprae*, we compared natural seed set to the outcross hand-pollination treatment to determine pollen limitation because the supplemental pollination treatment was lost. Results of appropriate statistical tests are given for each experiment (*t* tests and Mann–Whitney *U* tests, respectively)

* $p < 0.0001$

limited, suggesting that selection through female function might be stronger for these species than for *O. glabra* and *O. tenuifolia*, which are already setting maximum seed in their natural environment.

The effects of interspecific pollen transfer on reproductive fitness

Seed set in the hybridization treatments was significantly lower than in outcrossed plants (*O. tenuifolia* $U = 0.0$, $df = 22$, $p < 0.0001$; *O. glabra* $U = 1.5$, $df = 21$, $p < 0.0001$; *O. purpurea* $U = 0.0$, $df = 26$, $p < 0.0001$; *O. pes-caprae* $U = 0.0$, $df = 26$, $p < 0.0001$). *O. glabra* (0.40 ± 1.26 seeds/capsule) and *O. tenuifolia* (0.09 ± 0.30) formed very low numbers of hybrid seed, whilst *O. purpurea* and *O. pes-caprae* formed none, making it unlikely that IPT affects reproductive fitness through hybridization. Application of foreign pollen 24 h prior to conspecific pollen in the stigma clogging experiment drastically reduced seed set across all four species (*O. tenuifolia* $U = 19.5$, $df = 25$, $p < 0.0001$; *O. glabra* $U = 21$, $df = 25$, $p < 0.005$; *O. purpurea* $U = 0.0$,

$df = 39$, $p < 0.0001$; *O. pes-caprae* $U = 0.0$, $df = 39$, $p < 0.001$; Fig. 4). The strongest response to stigma clogging was a 99.05% reduction in mean seed set in *O. pes-caprae*, followed by a 98.94% reduction in *O. purpurea*, a 70.84% reduction in *O. glabra* and a 60.75% reduction in *O. tenuifolia*.

Discussion

In this study we investigated the patterns of flower colour distribution within plant communities, as well as the underlying mechanisms by which pollinators could give rise to these patterns. Our null model based survey of flower colour assembly revealed that flower colour within *Oxalis* communities is significantly clustered. This result is not concordant with recent studies that found patterns of overdispersion in traits related to pollination (Armbruster et al. 1994; Muchhala and Potts 2007) or that found no difference in flower colour composition within communities from that expected under random assembly (Arnold et al. 2009). Flower colour similarity in *Oxalis*

Fig. 4 The effect of heterospecific pollen transfer and stigma clogging on the maternal fitness of *Oxalis* species. Bars are means + 1 SD. Sample sizes are displayed above each bar. *Asterisks* indicate significant differences between outcross and clogging treatments within each species ($p < 0.0001$)
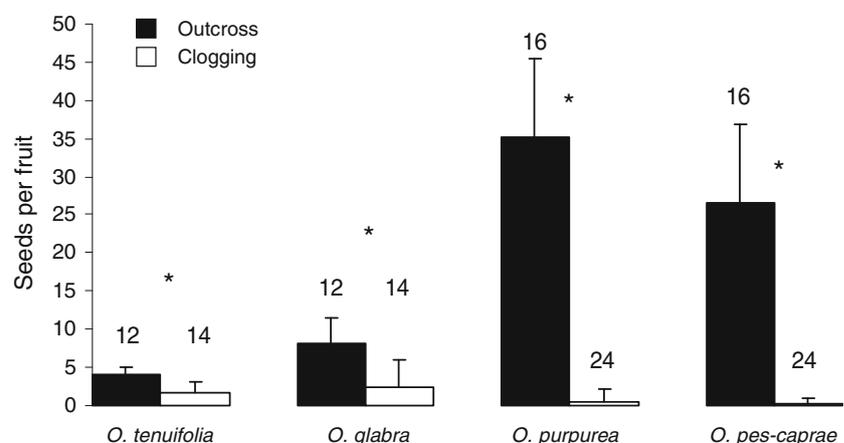
communities is not a by-product of the evolutionary history of sympatric species, as communities are randomly assembled with regard to phylogeny and there is no relationship between genetic and flower colour distances between species within a community.

Our pollination experiments revealed that the co-flowering sympatric *Oxalis* species we investigated are obligate outcrossers and were effectively pollinated and predominantly visited by native honeybees. Indirect interactions between *Oxalis* species, mediated through honeybee visitation, therefore have the potential to structure floral traits within these communities. Since the *Oxalis* species investigated are self-incompatible and often pollen limited, reproductive output in *Oxalis* is likely to be strongly dependent on the attraction of pollinators. If the floral traits of co-flowering sympatric species are perceived as similar by their shared pollinators this may produce an effect analogous to that of an increased density of a single species, which has been shown to increase pollen receipt and seedset (Groom 1998). Brown and Kodric-Brown (1979) argued that convergence of flower color in communities of hummingbird-pollinated plants can increase the attractiveness of a community to pollinators as a whole. Recent studies have also demonstrated that co-flowering species can increase visitation rates to neighbouring species if they are similar in flower colour (Johnson et al. 2003).

Kunin (1993) showed that focal plants at low densities suffered a reduction in visitation rate from pollinators except when sympatric co-flowering species were similar in floral colour. However, species at low densities experienced a reduction in seed set despite increased visitation in the presence of co-flowering species, presumably due to IPT. Our own IPT experiments showed that the receipt of foreign pollen significantly reduces reproductive fitness in *Oxalis*. Most previous studies have also reported a reduction in seed set due to foreign pollen receipt, but the magnitude is generally lower than the 61–99% reduction in seed set we report here (reviewed in Morales and Traveset 2008; see Kohn and Waser 1985 for an exception).

Our observations of pollinator foraging transitions showed that honeybees are more likely to switch between sympatric *Oxalis* species with similar flower colours. Corresponding results have recently been demonstrated for bumblebees as well (Raine and Chittka 1997; Internicola et al. 2007). This implies that although similarity in flower colour between co-flowering sympatric species might lead to an increased pollinator visitation rate, it does so at the expense of seed production due to the effects of IPT. The clustered flower colour pattern we observe in *Oxalis* communities is therefore counterintuitive, as a pattern of overdispersion is to be expected if IPT is an important factor structuring community assembly.

One reason for this discrepancy could be that flower colour functions in a variety of interactions other than pollinator attraction that may affect fitness, such as herbivory (Simms and Bucher 1996), pathogen damage (Frey 2004) and drought resistance (Warren and Mackenzie 2001). Colour variants of species polymorphic for flower colour have been shown to exhibit differences in fitness components that are not necessarily related to pollinator attraction, such as survivorship and flower production (Levin and Brack 1995) and vegetative size and seed size (Rausher and Fry 1993). Thus, selection on flower colour exerted by pollinators could be countered by these alternative selection pressures (Irwin et al. 2003). Although they were not investigated in this study, these alternative mechanisms could contribute to the clustered distribution of flower colours we observe within *Oxalis* communities, despite strong pollinator-mediated IPT favouring overdispersion of flower colour.

Another possibility is that our experimental approach overestimated the cost of IPT, as we applied pure loads of heterospecific and conspecific pollen 24 h apart, whereas a more realistic situation under indiscriminate foraging may be the receipt of mixed pollen loads. Honeybees also remained extremely flower constant during our observations, even when species had a very low flower colour distance separating them. Only 3% of 1,910 observed flower-to-flower transitions were between different species and had the potential to lead to IPT. If IPT has a less pronounced effect on reproduction than our experiments suggest, or is rare within *Oxalis* communities due to high pollinator constancy, the benefits of increased community-level pollinator visitation might indeed drive the clustered flower colour pattern we observe.

Flower colour is an important component of flower constancy (Hill et al. 1997; Keaser et al. 1997), but it can also be induced by other floral traits, such as scent (Anderson 2003) and handling times (Sanderson et al. 2006). Pollinators are also more flower constant when flowers differ in multiple traits, such as size, colour and complexity (Gegear and Laverty 2001) or size and odour (Gegear and Laverty 2005). Preliminary investigations revealed limited overlap in flower size between sympatric *Oxalis* species in two communities, especially when these species had very similar flower colours. The co-flowering species pairs *O. purpurea–O. glabra* and *O. versicolour–O. lanata*, for example, had flower colours which bees are unlikely to be able to distinguish (i.e. beespace colour distance <0.05), but differed greatly in flower size (ANOVA $F = 191.26$; $df = 1.68$; $p < 0.0001$, $F = 17.36$; $df = 1,58$; $p < 0.0005$). This divergence in floral traits other than colour may be of importance for maintaining flower constancy between sympatric species that are indistinguishable to pollinators by flower colour alone.

Pollinator observations in these communities revealed that honeybees made very few transitions between these species whilst foraging, despite their extreme similarity in flower colour (2.2 and 2% of all transitions in those communities, respectively).

Although we find that flower colour is significantly clustered within *Oxalis* communities, perhaps suggesting a role for pollinator-mediated facilitative interactions in the assembly of *Oxalis* communities, our experimental results are ambiguous as to the importance of competitive versus facilitative interactions. Whereas previous studies of floral traits involved in pollen placement suggest an important role for competition (Armbruster et al. 1994; Pauw 2006; Muchhala and Potts 2007), our results suggest that traits involved in pollinator attraction might also be structured by facilitative interactions. We suggest that floral traits involved in pollinator attraction are not assembled independently of one another, and thus future studies of pollinator-driven community assembly should employ more integrated measures of floral phenotype.

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