Relative density and dispersion pattern of two southern African Asteraceae affect fecundity through heterospecific interference and mate availability, not pollinator visitation rate

Caroli de Waal*, Bruce Anderson and Allan G. Ellis*

Department of Botany and Zoology, University of Stellenbosch, Private Bag X1, Matieland 7602, South Africa

Summary

1. Since co-flowering plants often share pollinators, their fecundity is likely affected by pollen transfer within and among plant species. Changes in pollinator visitation rates (e.g. through facilitation or competition for pollinators) are usually thought to exert the predominant influence on plant fecundity. However, the transfer of conspecific relative to heterospecific pollen between individuals may also play an important role. Indeed, the relative importance of these determinants of fecundity is expected to depend on the density, relative abundance and spatial arrangement of con- and heterospecifics.

2. We investigated the effects of con- and heterospecific density and spatial distribution pattern on pollinator visitation and plant fecundity within and between two annual, self-incompatible co-flowering species [*Dimorphotheca pinnata* and *Ursinia cakilefolia* (Asteraceae)] by manipulating their relative abundance, overall patch density and dispersion patterns in experimental arrays in Namaqualand, South Africa. We quantified pollinator visitation rates and fruit set in arrays of varying density and aggregation. This enabled us to determine which mechanism(s) were driving variation of fecundity, particularly through their influence on visitation rates, mate availability and heterospecific pollen interference. To test whether autogamy offers reproductive assurance when individuals are scattered among a dense population of heterospecifics, we included an autogamous species (*U. anthemoides*) in a separate experiment.

3. We found that increased fecundity with increasing conspecific density was not the result of higher visitation rates, but rather increased mate availability. Furthermore, increased spatial aggregation of conspecifics at low density significantly increased fecundity through reduced heterospecific interference.

4. In contrast to results for self-incompatible species, fruit set in *U. anthemoides* was consistently high and unaffected by scattered distribution patterns. This suggests that autogamy may offer reproductive assurance when mates are limited and the potential for interspecific pollen transfer (IPT) is high.

5. *Synthesis.* In this study of annual daisies, variation in fruit set is primarily driven by factors that affect the transfer of conspecific relative to heterospecific pollen, independent of pollinator visitation rate. Our findings demonstrate that mate limitation and IPT negatively affect fruit set and that these effects can be mitigated by intraspecific aggregation and the ability to autonomously self-pollinate.

Key-words: allee effects, competition for pollinators, density dependence, facilitation, plant–plant interactions, pollen limitation, self-fertilization

Introduction

An estimated 87.5% of flowering plants rely on pollinators to reproduce (Ollerton, Winfree & Tarrant 2011), and pollinators are often shared among co-flowering plants (e.g. Rathcke

*Correspondence authors: E-mails: agellis@sun.ac.za and dewaalc@gmail.com

1983; Mitchell *et al.* 2009). Consequently, the identity, morphology and spatial arrangement of neighbouring plants may influence the fecundity of co-flowering individuals. The effects of both conspecific and heterospecific plants on the fecundity of their neighbours are determined by factors such as pollinator foraging patterns (e.g. Morgan, Wilson & Knight 2005; Lázaro & Totland 2010) and the relative attractiveness of flowering species to pollinators (Hanoteaux, Tielbörger & Seifan 2013). In turn, these pollinator-mediated interactions may affect the evolution of plant mating systems (Fishman & Wyatt 1999; Kariyat, Sinclair & Golenberg 2013).

Pollinator-mediated interactions between plant species can affect fecundity of focal species positively or negatively through mechanisms dependent on their density, relative abundance and spatial dispersion patterns (i.e. the arrangement of individuals within the community, e.g. clumped or uniform). The predicted effects of these mechanisms on fruit set are traditionally thought to reflect changes in pollinator visitation rates to individuals. For example, aggregations of con- and heterospecific co-flowering plants may facilitate increased pollinator visitation to all individuals by increasing the floral display (Rathcke 1983; Moeller 2004). Facilitation may also occur within species, where pollen removal and deposition increase with density of neighbouring conspecifics (e.g. Duffy & Stout 2011). However, the latter mechanism may also act independently of pollinator visitation rate, that is increased mate availability (conspecific density) may result in higher seed set because of a higher probability of conspecific pollen transfer, even if visitation rates or pollinator abundance do not increase (Kunin 1993; Moeller 2004).

Increasing plant density (the number of flowering individuals per unit area) is predicted to facilitate an increase in pollinator visitation rate per flower until competition for pollinators occurs when pollinator visits become saturated (e.g. Rathcke 1983; Essenberg 2012). Consequently, reduced intraspecific competition for pollinators may increase fecundity at lower conspecific density (Wirth *et al.* 2011). On the other hand, individuals of less attractive species (in terms of morphology and/or reward) or species at low relative abundance may be unable to compete with co-flowering species for pollinator attraction (e.g. Feinsinger, Tiebout & Young 1991; Hanoteaux, Tielbörger & Seifan 2013). Such interspecific competition for pollination may drive divergence in floral traits between species and even drive shifts between specialist and generalist pollination strategies (Sargent & Otto 2006). Alternatively, traits associated with self-pollination may be selected in weaker competitors (Wyatt 1986).

Reduced conspecific density may give rise to component Allee effects, defined as a reduction in any component of individual fitness (e.g. number of matings, fecundity, etc.) with decreasing density or population size (Stephens, Sutherland & Freckleton 1999). Mate finding, a common mechanism that may generate component Allee effects (Gascoigne *et al.* 2009), is hampered in self-incompatible plants by inadequate pollen receipt at low densities (pollen limitation; Davis *et al.* 2004). For example, animal pollinators are less likely to discover, or forage from more isolated, sparser or smaller patches of plants (e.g. Ågren 1996; Groom 1998). Furthermore, when individuals occur at very low density, or are isolated from a source of conspecific pollen, pollinators may carry insufficient amounts of suitable pollen, even if visitation per plant does not decline (Duncan *et al.* 2004; Brys, Jacquemyn & Hermy 2007; Rodger, van Kleunen & Johnson 2013).

Fecundity of an individual may also be affected by negative interactions with its neighbours via interspecific pollen transfer (IPT), that is heterospecific pollen interference. The presence of heterospecific pollen may reduce the fertilization success of conspecific pollen, and pollen loss to heterospecific flowers reduces the amount of pollen transferred between conspecific flowers (reviewed in Morales & Traveset 2008; Ashman & Arceo-Gómez 2013). The negative effects of IPT may be particularly important for individuals at low density and low relative abundance. Visits to rare flowers are likely to be followed by visits to heterospecifics, and pollinators arriving at rare flowers are likely to carry more heterospecific pollen grains (Palmer, Stanton & Young 2003). Consequently, pollination success can be reduced at low conspecific density due to IPT, giving rise to a pattern akin to a mate-finding Allee effect (Gascoigne *et al.* 2009). In plant species, several studies have demonstrated how the presence of co-flowering heterospecifics can lower fecundity through IPT (e.g. Feinsinger, Tiebout & Young 1991; Jakobsson, Lazáro & Totland 2009; Matsumoto, Takakura & Nishida 2010), but to our knowledge, a study does not exist that explicitly explores the possibility that IPT may contribute to component Allee effects generated under conditions of low density or low relative abundance.

The strength of these interactions will, to a large extent, depend on the abundance of con- and heterospecific co-flowering plants (Rathcke 1983) and on the spatial arrangement, or dispersion, of individuals within the community (Stoll & Prati 2001). For example, competitive interactions can increase with a decline in absolute density of both conspecifics and heterospecifics, because there are not sufficient numbers of plants to attract enough pollinators (e.g. Caruso 2002). Alternatively, competition can increase at high plant density, because there are too few pollinators available to pollinate individuals in dense aggregations (Essenberg 2012; Ward, Johnson & Zalucki 2013). The frequency of IPT should decrease with a decrease in relative density of heterospecifics (e.g. Feinsinger, Tiebout & Young 1991; Stout, Allen & Goulson 1998). Similarly, aggregated (clumped) dispersion patterns reduce the extent of heterospecific pollen movement (e.g. Campbell 1986; Feinsinger *et al.* 1986) while retaining the benefits of joint attraction of pollinators (Moeller 2004). While several studies have demonstrated that density and dispersion affect pollinator visitation rates and/or fecundity of co-flowering individuals (e.g. Duffy & Stout 2011) as well as the intensity of interspecific competition for pollinators (Hanoteaux, Tielbörger & Seifan 2013), few have attempted to tease apart the confounding mechanisms (i.e. pollinator visitation, mate availability and heterospecific pollen transfer) which underlie these effects (but see Feinsinger, Tiebout & Young 1991; Kunin 1993; Rodger, van Kleunen & Johnson

2013). Here, we report results of arrays set up to experimentally determine the mechanisms responsible for density- (relative and absolute) and dispersion-dependent fecundity of two Namaqualand daisies in South Africa.

The Namaqualand region of southern Africa's Succulent Karoo biome is renowned for its spectacular displays of spring annuals. Following the winter rains, multiple species germinate and flower in the relatively short growing season, often forming dense co-flowering communities, particularly in disturbed sites (Cowling, Esler & Rundel 1999). These communities consist predominantly of various native Asteraceae species and are often dominated by a single species (usually *Ursinia* or *Dimorphotheca* species) with other species scattered in between at lower densities. It is therefore possible that individuals of these relatively sparsely distributed species may suffer greater fecundity costs through interspecific competition, low mate availability and/or IPT than individuals of relatively densely distributed species.

Here, we explore positive and negative interactions within and between co-flowering species. We aimed to tease apart the mechanisms by which density and dispersion affect fecundity, that is, through their influence on (i) pollinator visitation rates (intra- and interspecific facilitation and competition for visits), (ii) mate availability and (iii) heterospecific interference. To do this, we manipulated relative abundance, overall patch density and dispersion patterns in experimental arrays with two self-incompatible, annual Asteraceae species from Namaqualand, South Africa (Figs 1 and 2). An autogamous species was included in the final array. Specifically, we ask the following: (i) What are the effects of absolute density, relative abundance and dispersion pattern on pollinator visitation rates to each co-flowering species?; (ii) What are the effects of these factors on plant fecundity (fruit set)?; (iii) Do changes in pollinator visitation rates, if present, explain variation in fruit set in relation to density and dispersion patterns, according to predictions derived from the aforementioned mechanisms (outlined in Fig. 3)?; and (iv) Does self-compatibility potentially offer reproductive assurance to scattered individuals at low density?

Materials and methods

STUDY SPECIES

Three spring-flowering annual Asteraceae species native to the Namaqualand region of South Africa were used in our experimental arrays: *Dimorphotheca pinnata* (Thunb.) Harv., *Ursinia cakilefolia* DC. and *U. anthemoides* (L.) Poir. (Fig. 2). *Dimorphotheca pinnata* and *U. cakilefolia* frequently dominate spring displays in communities across Namaqualand (Van Rooyen 1999), and populations are often dense, with 39.9 ± 45.7 SD (maximum = 155) inflorescences per m^2 for *D. pinnata* and 17.7 ± 14.4 (maximum = 98) for *U. cakilefolia* (A.G. Ellis, unpubl. data). In our study area both species produce inflorescences with bright orange rays and offer nectar and pollen as rewards to visiting pollinators. In addition, both species tend to exhibit aggregated dispersion patterns resulting in a patchwork of local clumps with high density and relative abundance of a particular species. Although all three species coexist in the study area,

U. anthemoides individuals are frequently scattered among other species in these communities, and produce much smaller inflorescences with salmon-coloured rays (Fig. 2; C. de Waal, pers. obs.). The pollinator community in the region is dominated by a diverse assortment of insects with generalist visitation tendencies, predominated by bees, bee flies and monkey beetles (Struck 1994; Ellis & Johnson 2009; De Jager & Ellis 2014). These insects visit a wide range of plant species characterized by open flowers and inflorescences (e.g. Asteraceae and Mesembryanthemaceae), which make their rewards accessible to a wide range of insects. *Megapalpus capensis* (Bombyliidae), a key pollinator of orange daisies, does not exhibit floral constancy (Ellis & Johnson 2012). Despite the rich supply of floral reward during the flowering season, some authors have suggested that the abundance of flower-visiting insects is relatively low (Struck 1994; Esler 1999).

To determine the breeding system of *D. pinnata*, we conducted controlled pollination experiments, following the methods described in De Waal *et al.* (2014). We conducted these experiments on individuals from the same *D. pinnata* population used in our experimental arrays (Kamieskroon; 30° 12′ 19.96″S 17° 56′ 10.59″E, 757 m). An index of self-incompatibility (ISI), calculated as 1 – hand self-pollination/hand cross-pollination (Zapata & Arroyo 1978), indicated that this population is self-incompatible (ISI = 0.97). Sample sizes were 16 inflorescences for the hand self-pollination treatment and 13 inflorescences for the hand cross-pollination treatment. The breeding systems of *U. cakilefolia* (self-incompatible; ISI ≈ 0.96) and *U. anthemoides* (self-compatible and autogamous; ISI ≈ 0.03) were previously determined through controlled pollination experiments (De Waal *et al.* 2014).

EXPERIMENTAL ARRAYS

Experimental arrays were set up on the premises of the South African National Parks offices in Kamieskroon, Northern Cape Province. Plants with buds were collected from populations of our study species in the vicinity of Kamieskroon. Plants were transplanted into 18-cm-diameter pots – three plants of the same species per pot – with soil from the same site where they were collected.

We experimentally manipulated various naturally occurring plant dispersion/density scenarios in arrays of potted plants, approx. 1.5 m × 1.5 m in extent (Fig. 2). Each experimental block (five in total) consisted of six different treatment arrays. Arrays consisted of either 64 or eight pots depending on the treatment (see below; Figs 1 and 2), with 3 conspecific plants per pot. The experimental design consisted of the following: (i) a high-density background of *D. pinnata* (filled circles in Fig. 1) with *U. cakilefolia* (open circles in Fig. 1) widely dispersed among the background pots. This represented the high-density (HD) treatment for *D. pinnata* and low-density dispersed (LDD) treatment for *U. cakilefolia*; (ii) a high-density background of *D. pinnata* with *U. cakilefolia* arranged in an aggregated/clumped pattern in the centre of the array, representing the HD treatment for *D. pinnata* and low-density clumped (LDC) treatment for *U. cakilefolia*; (iii) the reciprocal arrangement of treatment 1, that is HD treatment for *U. cakilefolia* and LDD treatment for *D. pinnata*; (iv) the reciprocal arrangement of treatment 2, that is HD treatment for *U. cakilefolia* and LDC treatment for *D. pinnata*; (v) an array where both self-incompatible species occur at low density (LD treatment for both species); and (vi) a high-density background of *D. pinnata* with *U. anthemoides* (the autogamous species; triangles in Fig. 1) at low density and arranged in a dispersed pattern (LDD treatment for *U. anthemoides*). Focal plants in the HD treatments were adjacent to heterospecific plants in

| Experimental design	Treatment: Focal species (filled circles) = *D. pinnata*	Treatment: Focal species (open circles) = *U. cakilefolia*	Treatment: Focal species (open triangles) = *U. anthemoides*
	High density (HD)	Low density, dispersed (LDD)	-
	High density (HD)	Low density, clumped (LDC)	-
	Low density, dispersed (LDD)	High density (HD)	-
	Low density, clumped (LDC)	High density (HD)	-
	Low density (LD)	Low density (LD)	-
	NA	-	Low density, dispersed (LDD)
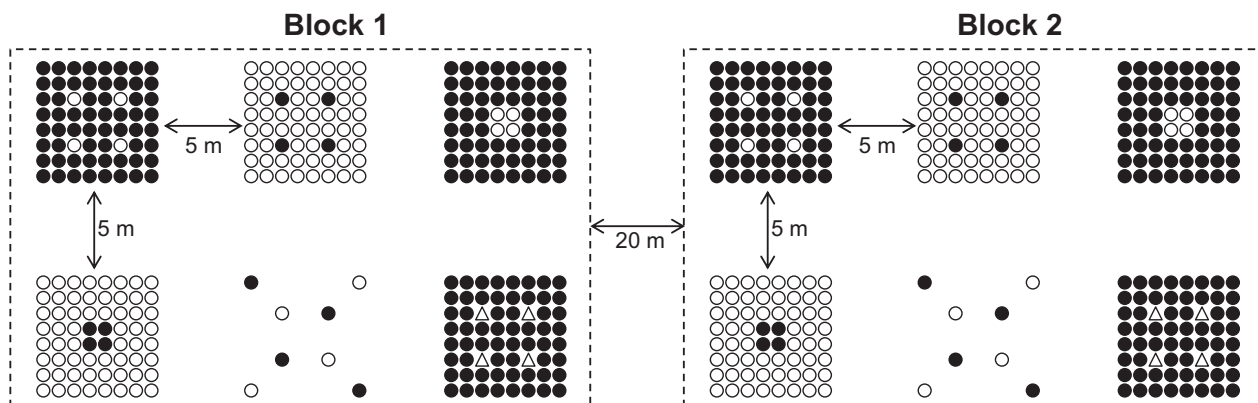
Fig. 1. Design and layout of experimental arrays with three annual Asteraceae species: the self-incompatible *Dimorphotheca pinnata* (filled circles) and *Ursinia cakilefolia* (open circles) and the autogamous *U. anthemoides* (triangles). Every experimental unit (array) represents a different arrangement of two species varying in relative abundance, dispersion and overall density. Each symbol represents a pot containing three plants. Pots with focal plants in the high-density (HD) treatments are indicated in grey. To maintain consistent abundance of inflorescences in the low-density treatments (LDD, LDC and LD), only one inflorescence per pot was allowed to flower. Fruit set of all focal inflorescences was determined for each treatment. Dashes indicate absence of the species in the array. NA indicates that individuals of the species were not used in analyses. The six arrays formed one block, and blocks were replicated five times. The entire experiment was repeated twice (Experiments 1 and 2).

the LDD and LDC treatments (Fig. 1) to avoid edge effects in the arrays and to ensure that these treatments differed only in conspecific abundance and not proximity to heterospecific inflorescences.

The six arrays within each experimental block were spaced 5 m apart, and the five experimental blocks were separated from one another by at least 20 m. Although *D. sinuata*, with inflorescences morphologically similar to *D. pinnata*, occurred on and around the

Fig. 2. Inflorescences of (a) *Ursinia cakilefolia* (×1.7), (b) *Dimorphotheca pinnata* (×1.4) and (c) *Ursinia anthemoides* (×0.9) used as study species in experimental arrays. Experimental arrays (see Fig. 1) include: (d) *D. pinnata* in the high-density (HD) treatment and *U. cakilefolia* in the low-density, clumped (LDC) treatment; (e) *U. cakilefolia* in the HD treatment and *D. pinnata* in the low-density, dispersed (LDD) treatment; and (f) *U. cakilefolia* and *D. pinnata* in the low-density (LD) treatment.

premises where the experiment was performed, we ensured that co-flowering individuals did not interfere with our experiment by removing inflorescences in close proximity to our experimental plants. All experiments were performed within a 100 × 100 m area. We repeated the full experiment twice during the spring-flowering season. The first run of the experiment (Experiment 1) was conducted at the end of August 2013, and the second run (Experiment 2) was conducted during mid-September 2013. To increase the magnitude of an interference effect (if present) in Experiment 2, pots in the LDD treatment were spaced even further apart towards the corners of the array, with four pots of the background species in between pairs of focal pots (instead of two pots as in Experiment 1).

To maintain an even density of plants in the low-density treatments (LDD, LDC and LD), only one of the three plants in each of the four focal pots was allowed to flower and to produce one inflorescence. This developing focal inflorescence on each of these plants was tagged with coloured string. Similarly, one developing focal inflorescence in each of four pots of the high-density background species (HD treatment) adjacent to the LDD/LDC pots was tagged (grey circles in Fig. 1). Focal inflorescences were left to mature on the plants at the end of each experiment, and infructescences were subsequently collected, that is eight infructescences per array (four per species). Following the approach of De Jager & Ellis (2014) and De Waal *et al.* (2014), the number of fruits per infructescence was counted, with clearly swollen ovaries regarded as fruit. In addition, we counted the number of unfertilized female-fertile florets (florets with small, unenlarged ovules) per infructescence under a dissecting microscope.

POLLINATOR OBSERVATIONS

To determine whether inflorescence density and/or dispersion in experimental arrays affected pollinator visitation rates, we conducted observations of pollinator visits to each species. Each array was observed for a five-minute interval once every day for four to six days for Experiment 1 (26 August–5 September 2013) and over two days for Experiment 2 (16 and 17 September 2013). We opted for short (five-minute) observation intervals so that all arrays could be observed through the course of one day. In total, observations occurred over 155 separate five-minute periods. Pollinator observations were performed only when all four focal inflorescences in the low-density treatment of a particular array were flowering. Each day, observations were conducted when inflorescences were fully open (as inflorescences open and close each day) and pollinators were active, approximately between 10:45 am and 4:15 pm. Before each observation interval, the number of open inflorescences for the background species in high-density arrays (HD treatment) was counted. The number of visits per inflorescence per five-minute observation period was used as a measure of visitation rate. All insects that made contact with the plants' reproductive organs, whether they were stationary during the observation period, moving between plants or entering/leaving the array, were recorded as visitors and identified to morphospecies level.

TREATMENT CONTRASTS FOR ELUCIDATING UNDERLYING MECHANISMS

Our experimental design allowed us to determine which mechanisms drive variation in fecundity under different dispersion patterns and relative densities (illustrated in Fig. 3). If variation in fecundity reflects the effects of density and dispersion on pollinator attraction (i.e. facilitation or competition for visits), we would expect fruit set across array treatments to track patterns of pollinator visitation. However, if density and dispersion affect plant fecundity through their influence on the quality of pollen loads arriving on stigmas (i.e. mate availability or interference), we do not expect fruit set patterns to be coupled with patterns of pollinator visitation across treatments. The contrasts outlined in Fig. 3 reflect treatment comparisons that are least likely to be confounded by mechanisms other than the one under consideration, resulting in predictions for combined pollinator visitation and fruit set patterns unique to each underlying mechanism.

To test for intraspecific effects of competition, facilitation and mate availability, we used contrasts between HD and LD treatments because density of the focal species was the only parameter that varied between these treatments (although we acknowledge that

Interaction	Mechanism	Density effect	Dispersion effect	Prediction for fruit set	Prediction for visitation rate
Intraspecific					
	Facilitation: positive density effect; more conspecific flowers attract more pollinators	+	NA	HD* > LD	Same
	Mate availability: more conspecific flowers in proximity enhance pollen transfer	+	NA	HD* > LD	No prediction
	Competition for pollinators: not enough pollinators to pollinate all flowers	−	NA	LD > HD*	Same
Interspecific					
	Facilitation: magnet species attracts pollinators to rare species; total density of patch attracts more pollinators	+	NA	LDD and LDC > LD	Same
	Competition for pollinators: not enough pollinators to pollinate all flowers (pollinator limitation)	−	NA	LD > LDD and LDC	Same
	Competition for pollinators: common species attracts more pollinators than rare species (rarity disadvantage)	− (relative density)	NA	HD* > LDD and LDC	Same
	Interference: heterospecific pollen transfer or reduced conspecific pollen transfer efficiency reduces reproductive success	NA	−	LDC > LDD	No prediction

Fig. 3. Predicted inequalities between array treatments for pollinator visitation rates and fruit set derived from the various mechanisms by which intra- and interspecific interactions may affect the fecundity of a self-incompatible focal species (filled circles), co-flowering with another species (open circles) in a community. Effects (positive or negative) of density (low or high) and dispersion (clumped or scattered) on fruit set and pollinator visitation rate are indicated for each mechanism. Expected inequalities for fruit set and visitation rates between experimental array treatments are shown in each case, where filled circles represent the focal species and where HD = high-density treatment; LDD = low-density, dispersed treatment; LDC = low-density, clumped treatment; LD = low-density treatment (Fig. 1). We have extracted the treatment comparisons that are least likely to be confounded by mechanisms other than the one under consideration, resulting in predictions for combined pollinator visitation and fruit set patterns unique to each underlying mechanism. * Only one of the HD arrays are shown here, although fruit set and visitation rates of plants in the HD treatment were established from two different HD arrays where only the arrangement of the rare species differed (see Fig. 1).

plants in the LD treatment might have experienced some heterospecific interference). To test for interspecific effects of facilitation and pollinator limitation, we used contrasts between the LD and LDD/LDC treatments, because these treatments differ in the density of heterospecifics but not conspecifics. To examine whether plant species that occur at low density among dense aggregations of heterospecifics are at a disadvantage because pollinators are attracted to the common species, we compared visitation rates to plants in the HD treatment to those in the LDD and LDC treatments, because these differ in the abundance of the focal species but not overall array density. To test for the presence of an interference effect, we used contrasts between the LDC and LDD treatments because these treatments differed only in the dispersion pattern of the focal species, whereas array density and relative abundance of the two species remained the same.

STATISTICAL ANALYSES

Pollinator visitation

All analyses were performed in R version 3.0.2 (R Core Team 2013). Overlap in pollinators between *D. pinnata* and *U. cakilefolia* was explored by comparing visitation rates (number of visits per inflorescence per five-minute observation period) of the predominant insect visitors, *Megapalpus capensis* (Bombyliidae) and monkey beetles (Scarabaeidae), across daisy species using Mann–Whitney *U*-tests with the wilcox.test function. The observed and expected frequencies of intra- and interspecific transitions on our arrays were compared with a Pearson's chi-squared test using the chisq.test function.

To determine whether pollinator visitation varied between daisy species and the first and second run of the experiment, we compared

the number of pollinator visits per five-minute observation period to *D. pinnata* and *U. cakilefolia* in the high-density (HD) treatments using a generalized linear model. This was done in the glm.nb function in the MASS package (Venables & Ripley 2002) using a negative binomial distribution and a log link function. Plant species, experiment and the species × experiment interaction were included as explanatory variables, with log (number of inflorescences) as an offset variable to account for variation in open inflorescences in the HD treatments. Significance of predictors was determined using likelihood ratio tests to compare the full model with reduced models after single-term deletion in the ANOVA function. To compare levels of the interaction effect between species and experiment, the model was run using an interaction variable (created with the function interaction) as the predictor, followed by Tukey's contrasts using the glht function in the package MULTCOMP (Hothorn, Bretz & Westfall 2008). In this analysis as well as other analyses of pollinator visitation rates and fruit set (see below), we pooled data from the two different HD treatments (Fig. 1) where only the dispersion pattern of the low-density species differed.

To examine the effects of our array treatments on total pollinator visitation rates, we used generalized linear mixed models (GLMMs) in the package LME4 (Bates *et al.* 2013) for each species-by-experiment combination. The response variable was the number of pollinator visits in each five-minute observation period, with log (number of inflorescences) as an offset variable. The models included treatment as a fixed factor (corresponding to the treatment factor used in fruit set analyses) and block as a random factor. To account for potential variation in visitation rates throughout the day, we incorporated the time of the start of each observation period as an additional fixed categorical factor with three categories: morning (observations conducted between 10:30 am and 12:30 pm), mid-day (between 12:31 pm and 2:30 pm) and afternoon (between 2:31 pm and 4:30 pm).

Models were selected following inspection of overdispersion parameters (ratio of residual scaled deviance to the residual degrees of freedom), visual inspection of residuals and, finally, comparisons of Akaike information criterion (AIC) values. Accounting for zero inflation did not improve models in any of the cases. The significance of fixed effects was examined by conducting likelihood ratio tests in which a model with only one of the two fixed effects was compared to the full model using the ANOVA function.

Based on model selection, visitation rates to *D. pinnata* (Experiment 1) and *U. cakilefolia* (Experiments 1 and 2) were analysed using a negative binomial GLMM and a log link function in the glmer.nb function. For *D. pinnata* in Experiment 2, two treatment categories (LD and LDC) as well as one time category (morning) had zero visitations which led to numerical problems in the analysis. Consequently, we randomly assigned a single visit to one observation period in each treatment category. Visitation rates in this case were analysed using a GLMM with a Poisson distribution and log link function with the glmer function. Tukey's contrasts were used to assess predicted array contrasts (Fig. 3), using the glht function in the package MULTCOMP (Hothorn, Bretz & Westfall 2008). Fitted values of the number of visits per five-minute interval were divided by the number of inflorescences to obtain the predicted estimates of visitation rate.

To test for an additional signal of an interference effect, we compared pollinator visitation rates to *D. pinnata* and *U. cakilefolia* plants in the LDD treatment in Experiment 1 with plants in Experiment 2, in which we increased the distance (and number of heterospecifics) between focal inflorescences. Visitation rates were analysed using the

same approach as before: a GLM with a negative binomial distribution and a log link function using the glm.nb function in the package MASS (Venables & Ripley 2002). Plant species, experiment and the species × experiment interaction were included as explanatory variables. However, the interaction effect was not significant, and therefore, the final model included only effects of species and experiment.

Fruit set

To assess the effect of our experimental array treatments on the fecundity of *D. pinnata* and *U. cakilefolia*, we again analysed each species × experiment combination separately, using GLMMs with a binomial distribution using glmer in the package LME4 (Bates *et al.* 2013), because the species × experiment interaction was significant in overall analyses. Fruit set was analysed as the total number of fruits out of the total number of female-fertile florets. The model included treatment as a fixed effect and block as a random (intercept-only) factor. Significance of the treatment effect was estimated by comparing a model in which treatment was dropped to the full model, using the function ANOVA. A Tukey's test for post hoc multiple contrasts was conducted using glht in the package MULTCOMP (Hothorn, Bretz & Westfall 2008) to establish the existence of predicted inequalities in fruit set (Fig. 3).

To assess whether the ability to self-fertilize autonomously ensures fecundity in conditions of low density and high heterospecific interference, we compared fruit set of the autogamous *U. anthemoides* and the self-incompatible *U. cakilefolia* and *D. pinnata* for focal plants in the LDD treatments in Experiment 1 *vs.* Experiment 2. This analysis was also performed with a GLMM and a binomial distribution using glmer (Bates *et al.* 2013). The fruit set response variable was calculated in the same way as described above. Species (*U. anthemoides*, *U. cakilefolia* or *D. pinnata*), experiment (1 or 2) and the species × experiment interaction were entered as fixed effects and block as random factor. Significance of the interaction effect and the two fixed effects was evaluated as described above. To compare levels of the interaction term, we created an interaction variable (using the function interaction), ran the full model with this as predictor variable and performed Tukey's contrasts using glht in the package MULTCOMP (Hothorn, Bretz & Westfall 2008).

Results

POLLINATOR OBSERVATIONS

Seventeen different insect morphospecies visited plants in our arrays during the observation periods across both experiments. The majority of recorded pollinator visits were by the bee fly *Megapalpus capensis* (Bombyliidae; 25.8%) and various species of monkey beetles (Scarabaeidae; 58.9%). Other visitors included horse flies (Tabanidae), blister beetles (Meloidae), *Corsomyza* (Bombyliidae), wasps (Hymenoptera) and biting midges (Ceratopogonidae). In total, we observed 516 visits by 316 pollinators during a total of 775 min of observation time. There was some overlap in pollinators between our study species: 8 of the 17 morphospecies (47% of recorded pollinator species) visited both *U. cakilefolia* and *D. pinnata* inflorescences in our arrays. In addition, we recorded 195 transitions between inflorescences, of which 10 (5.1%) were interspecific. The observed number of intra- and interspecific

transitions was too low for meaningful analyses. However, under random visitation (i.e. no preference of pollinators for either species), the expected frequencies of intraspecific (92.4%) and interspecific (7.6%) transitions on our arrays did not differ significantly from the observed intraspecific (94.9%) and interspecific (5.1%) transitions ($X^2 = 1.70$, d.f. = 1, $P = 0.193$). *Dimorphotheca pinnata* was visited more frequently by *Megapalpus capensis* (Mann–Whitney *U*-test; $W = 12891.5$, $P = 0.028$) and *U. cakilefolia* by monkey beetles ($W = 7046.5$, $P < 0.001$).

The mean number ($\pm$ SE) of inflorescences flowering per array in the HD treatments was 106.88 ± 3.19 and 70.5 ± 3.19 for *D. pinnata* in Experiments 1 and 2, respectively, and 93.2 ± 2.55 and 121.05 ± 4.02 for *U. cakilefolia* in Experiments 1 and 2, respectively. Total pollinator visitation rates for all taxa did not differ significantly between Experiments 1 and 2 (Fig. 4; likelihood ratio test, $X^2 = 0.47$, d.f. = 1, $P = 0.494$). Instead, we found a significant effect of plant species ($X^2 = 6.690$, d.f. = 1, $P = 0.010$) and a significant interaction between species and experiment ($X^2 = 8.46$, d.f. = 1, $P = 0.004$), where visitation rates were significantly higher in Experiment 1 than Experiment 2 for *D. pinnata* ($z = -2.77$, $P = 0.027$), but not for *U. cakilefolia* ($z = 1.17$, $P = 0.640$). In addition, visitation rates to *U. cakilefolia* were significantly higher than to *D. pinnata* in Experiment 2 ($z = 3.87$, $P < 0.001$), but not in Experiment 1 ($z = 0.83$, $P = 0.836$).

For *D. pinnata* in Experiment 1 (Fig. 4a), visitation rates did not differ significantly between time categories (likelihood ratio test, $X^2 = 1.38$, d.f. = 2, $P = 0.619$) or treatments ($X^2 = 1.38$, d.f. = 3, $P = 0.711$). In contrast, in Experiment 2 (Fig. 4b), treatment ($X^2 = 20.33$, d.f. = 3, $P < 0.001$) and time category ($X^2 = 13.18$, d.f. = 2, $P = 0.001$) significantly affected total pollinator visitation rate. For *U. cakilefolia* in Experiment 1 and Experiment 2 (Fig. 4c,d), treatment (Exp. 1: $X^2 = 38.82$, d.f. = 3, $P < 0.001$; Exp. 2: $X^2 = 11.56$, d.f. = 3, $P = 0.009$) and time category (Exp. 1: $X^2 = 15.54$, d.f. = 2, $P < 0.001$; Exp. 2: $X^2 = 10.24$, d.f. = 2, $P = 0.006$) significantly affected visitation rates. In *D. pinnata* (Experiment 2) and *U. cakilefolia* (Experiments 1 and 2), visitation rates to inflorescences in the HD treatment were significantly lower than to inflorescences in the LD treatment (Tukey's contrasts, $P < 0.05$), supporting our prediction for intraspecific competition for pollinators (Table 1). In *D. pinnata* (Experiment 2), visitation rates were higher around mid-day than in the morning ($P = 0.032$), but not different from the afternoon ($P > 0.05$). In *U. cakilefolia* (Experiments 1 and 2), visitation rates were higher in the afternoon compared to the morning ($P < 0.001$) and mid-day periods ($P \leq 0.007$).

No pollinator visits were observed to *U. anthemoides* inflorescences in either experiment. For *U. cakilefolia* and *D. pinnata* inflorescences in the same low density – high dispersion arrangement (i.e. LDD treatment), species identity
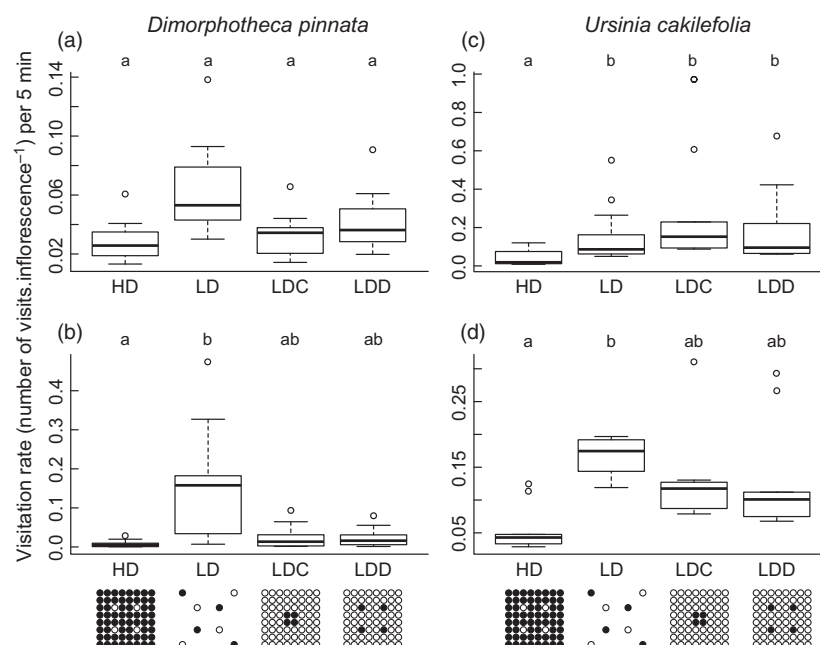

Fig. 4. Fitted estimates of pollinator visitation rates (number of visits. inflorescence^{-1}) per five-minute observation period in experimental arrays of *Dimorphotheca pinnata* in (a) Experiment 1 and (b) Experiment 2, and *Ursinia cakilefolia* in (c) Experiment 1 and (d) Experiment 2. Experimental arrays are indicated as in Fig. 1 where filled circles represent *D. pinnata* and open circles represent *U. cakilefolia*. Treatments are as follows: HD = high density; LD = low density; LDC = low density, clumped; and LDD = low density, dispersed. Only one of the HD arrays are shown here, although visitation rate of plants in the HD treatment was established from pooled data from two different HD arrays where only the arrangement of the rare species differed (see Fig. 1). Means that share the same letter are not significantly different ($P > 0.05$, Tukey's contrasts for multiple comparisons of means). Note that scale differs between graphs. Bold lines indicate the medians, boxes the interquartile range, whiskers the ranges and points are outliers.

Table 1. Summary of hypothesized mechanisms, predicted pollinator visitation rate and fruit set contrasts and visitation rate and fruit set results of experimental arrays with two self-incompatible annual Asteraceae species, *Dimorphotheca pinnata* and *Ursinia cakilefolia*, in two experimental runs (Experiments 1 and 2). Predicted visitation rate contrasts that differ from those for fruit set are indicated in square brackets. Bold type indicates likely mechanisms operating in each species-experiment combination, that is instances where combined visitation rate and fruit set data support the predicted contrasts

| Hypothesis	Contrast [contrast for visitation rate, if different]	*D. pinnata* Exp. 1		*D. pinnata* Exp. 2		*U. cakilefolia* Exp. 1		*U. cakilefolia* Exp. 2	
		Visitation rate	Fruit set	Visitation rate	Fruit set	Visitation rate	Fruit set	Visitation rate	Fruit set
Intraspecific facilitation	HD > LD	No	Yes	No	Yes	No	No	No	Yes
Mate availability	HD > LD [No prediction]	–	**Yes**	–	**Yes**	–	No	–	**Yes**
Intraspecific competition for pollinators	LD > HD	No	No	Yes	No	Yes	No	Yes	No
Interspecific facilitation	LDD and LDC > LD	No	No	No	No	No	No	No	No
Interspecific competition for pollinators (pollinator limitation)	LD > LDD and LDC	No	No	No	No	No	No	No	No
Interspecific competition for pollinators (rarity disadvantage)	HD > LDD and LDC	No	Yes	No	No	No	No	No	No
Interference	LDC > LDD [No prediction]	–	No	–	**Yes**	–	**Yes**	–	**Yes**
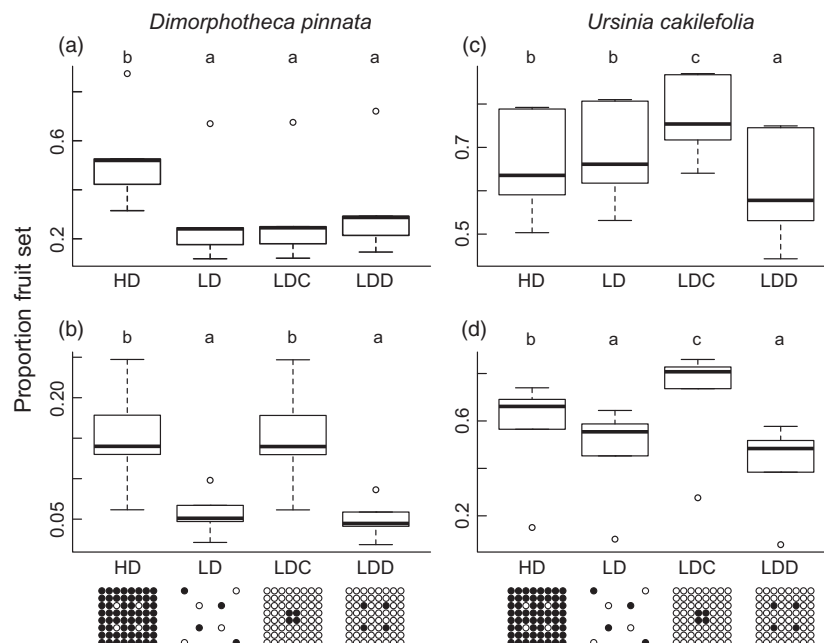
Fig. 5. Fitted estimates of proportion fruit set of *Dimorphotheca pinnata* in four experimental treatments in (a) Experiment 1 and (b) Experiment 2, and *Ursinia cakilefolia* in (c) Experiment 1 and (d) Experiment 2. Treatments are as follows: HD = high density; LD = low density; LDC = low density, clumped; and LDD = low density, dispersed. Experimental arrays are indicated as in Fig. 1 where filled circles represent *D. pinnata* and open circles represent *U. cakilefolia*. Only one of the HD arrays are shown here, although fruit set of plants in the HD treatment was established from pooled data from two different HD arrays where only the arrangement of the rare species differed (see Fig. 1). Means that share the same letter are not significantly different (P < 0.05; Tukey's contrasts for multiple comparisons of means). Note the different scale in b and c. Bold lines indicate the medians, boxes the interquartile range, whiskers the ranges and points are outliers.

significantly affected visitation rate ($X^2 = 4.45$, d.f. = 1, $P = 0.035$) with *U. cakilefolia* receiving significantly more visits than *D. pinnata* ($z = 2.18$, $P = 0.029$). However, visitation rates did not vary between Experiments 1 and 2 which differed in the level of dispersion ($X^2 = 0.47$, d.f. = 1, $P = 0.494$).

FRUIT SET

Plant density and/or dispersion patterns had significant effects on fruit set for both *D. pinnata* (likelihood ratio tests, Exp. 1: $X^2 = 89.94$, d.f. = 3, $P < 0.001$, Fig. 5a; Exp. 2: $X^2 = 35.53$, d.f. = 3, $P < 0.001$; Fig. 5b) and *U. cakilefolia* (Exp. 1:

$X^2 = 60.97$, d.f. = 3, $P < 0.001$, Fig. 5c; Exp. 2: $X^2 = 171.86$, d.f. = 3, $P < 0.001$, Fig. 5d).

Fruit set patterns did not reflect variation in visitation rates among treatments (Fig. 4 vs. Fig. 5; Table 1). In direct contrast to the visitation rate results, fruit set of inflorescences at high density (HD treatment) was significantly higher than at low density (LD treatment) in three of the four species-experiment combinations (*D. pinnata*, Exp. 1 and 2; *U. cakilefolia*, Exp. 2; Tukey's contrasts, $P < 0.05$), a pattern consistent with the presence of an intraspecific mate availability effect (Fig. 5). In three cases (*D. pinnata*, Exp.2; *U. cakilefolia*, Exp. 1 and 2), plants in the LDC treatment set more fruit than in the LDD treatment (Tukey's contrasts, $P < 0.05$). This pattern was also not evident in the visitation rate results (Fig. 4), consistent with the predictions for an effect of heterospecific interference on fruit set (Table 1).

When comparing fruit set of plants at low density and high probability of heterospecific interference (LDD treatment) in Experiment 1, proportion fruit set of *U. anthemoides* (0.61 ± 0.03) was similar to *U. cakilefolia* (0.61 ± 0.08; $z = -0.53$, $P = 0.995$), whereas fruit set of *D. pinnata* was significantly lower at 0.33 ± 0.08 ($z = 8.59$, $P < 0.001$). In Experiment 2, where the potential for an interference effect in the LDD treatment was increased, fruit set of *U. cakilefolia* (0.37 ± 0.08; $z = -8.31$, $P < 0.001$) and *D. pinnata* (0.05 ± 0.04; $z = -7.67$, $P < 0.001$) was significantly reduced compared to Experiment 1. In contrast, fruit set of *U. anthemoides* (0.74 ± 0.03) actually increased slightly ($z = 3.60$, $P = 0.004$), as was evident from a significant species × experiment effect ($X^2 = 107.97$, d.f. = 2, $P < 0.001$), suggesting that the ability to self-fertilize autonomously can ensure fecundity in low density-/high-dispersion scenarios.

Discussion

Pollinator visitation data suggest that intraspecific competition for pollinators had a negative effect on fecundity (Table 1). In contrast, visitation data provide no support for four of the other density-/dispersion-dependent mechanisms (intraspecific facilitation, interspecific facilitation, interspecific pollinator limitation and rarity disadvantage) which could potentially affect variation in fecundity. If we were to consider fruit set independently of the pollinator visitation rate data, fruit set patterns would suggest the influence of several density-/dispersion-related mechanisms (Table 1). But, since pollinator visitation rates and fruit set patterns need to be considered simultaneously to unravel the mechanisms involved, we can reject intraspecific facilitation, interspecific facilitation, interspecific pollinator limitation and rarity disadvantage as mechanisms affecting fecundity in our system, leaving mate availability and heterospecific interference as the most likely determinants of fecundity.

Plants in low-density patches (LD treatment) as well as plants at low relative abundance scattered among heterospecifics (LDD treatment) consistently performed poorly in terms of fruit set (Fig. 5). This reduction in fecundity was not the result of a significant reduction in pollinator visitation to inflorescences in these treatments. Instead, mechanisms affecting the transfer of conspecific vs. heterospecific pollen most likely resulted in the observed fruit set patterns. Fecundity was high when species were at high density (HD treatments), this despite negative density-dependent effects on visitation rates through intraspecific competition for pollinators. The negative effects of intraspecific competition were outweighed by the positive effects of increased mate availability and/or decreased interference at high conspecific densities. Fecundity was also high when individuals were aggregated despite being at low density (LDC treatments), a pattern that can only be attributed to reduced heterospecific interference.

Thus, at the community-level scale of our study (within a 100×100 m area), localized changes in pollinator visitation rates associated with our experimental treatments did not strongly affect fecundity. However, we cannot rule out the possibility that differences in visitation rates at broader spatial scales (e.g. due to landscape-level changes in pollinator abundance) may have stronger effects on plant fecundity.

EFFECTS OF CONSPECIFIC DENSITY ON POLLINATION AND FECUNDITY

Pollinator-mediated Allee effects in plant populations may arise when populations are too small, too isolated or too sparse to receive sufficient pollinator visitation (e.g. Groom 1998; Forsyth 2003). However, recent studies also emphasize the importance of mate finding in generating component Allee effects (reviewed in Gascoigne *et al.* 2009). To tease apart the mechanisms behind Allee effects, it is necessary to examine both pollinator activity (e.g. pollen deposition or pollinator visitation rates) and fruit/seed set consequences of density or spatial aggregation. While we did not explicitly study Allee effects (positive associations between population size and fecundity), our results may have important implications concerning the mechanisms behind the Allee effect. Where other studies suggest that low fecundity in small populations is the result of lower pollinator visitation rates, we show that low fecundity in small or relatively low-density populations may result from increased frequencies of IPT and decreased mate availability. Similar to our study, Moeller (2004) documented that seed set was more limited by pollen availability in small populations of *Clarkia xantiana* ssp. *xantiana* than in large populations. He suggested that low mate availability was the mechanism behind his observations, because pollinator visitation rates were not affected by population size. However, our results suggest that increased IPT with increasing heterospecific abundance can contribute towards a reduction in fecundity for individuals at low relative abundance.

EFFECTS OF DISPERSION ON POLLINATION AND FECUNDITY

Our results demonstrate that spatial aggregation of plants (clumping) enhances fecundity at low relative abundance.

A potential explanation for high fruit set in the LDC treatment compared to the LDD treatment in our arrays (Fig. 5) is that monospecific patches may be more attractive to pollinators or are more likely to retain pollinators within the patch (Hanoteaux, Tielbörger & Seifan 2013). Under this scenario, we would expect plants in the LDC treatment to receive more pollinator visits than plants in the LDD treatment; however, visitation rates did not differ between these treatments (Fig. 4). Based on this finding, and the fact that the dominant pollinators do not exhibit floral constancy (Ellis & Johnson 2012), we attribute the observed reduction in fecundity of scattered individuals to the negative effects of heterospecific interference.

Intraspecific aggregation of individuals results in more intraspecific interactions than would be expected from the species' overall abundance and may play an important role in the reduction of populations' vulnerability to pollinator-mediated Allee effects on fruit or seed production (Ghazoul 2005; Hanoteaux, Tielbörger & Seifan 2013). Clumped spatial distribution patterns therefore reduce the frequency of interspecific interactions, and also reduce the frequency of IPT. IPT can affect female fitness by interfering with conspecific pollen adhesion and germination (Galen & Gregory 1989) or by inhibiting ovule fertilization and seed development (Thomson, Andrews & Plowright 1981), while with male fitness, reduction occurs because pollen is lost to heterospecific flowers, reducing the amount of pollen transferred between conspecific flowers (pollen discounting; Lloyd 1992). Several studies highlight the significant influence of dispersion patterns on pollinator-mediated interactions (e.g. Duncan *et al.* 2004; Brys, Jacquemyn & Hermy 2007). In one such study, plant aggregation was associated with increased fecundity in *Kniphofia linearifolia* (Duffy, Patrick & Johnson 2013). In their system, the reduction in seed set resulted from reduced bird visitation rates in response to decreased conspecific aggregation, although no co-flowering heterospecifics were available to pollinators. In our study plant, aggregation was also associated with increased fecundity; however, this was not the result of increased visitation rates but rather a decline in heterospecific interference.

IMPLICATIONS FOR THE EVOLUTION OF SELF-FERTILIZATION

The evolution of autogamous selfing can alleviate the requirements for both pollinators and mates, thereby providing reproductive assurance under unpredictable or insufficient pollinator environments (Kalisz & Vogler 2003; Kalisz, Vogler & Hanley 2004; Eckert, Samis & Dart 2006). In addition, self-fertilization can mitigate the negative effects of low density on fecundity caused by low mate availability (Rodger, van Kleunen & Johnson 2013), pollinator competition (Rathcke 1988) and hybridization through IPT (Goodwillie & Ness 2013).

The ability of autogamy to mitigate the negative effects of low density on fecundity in this system was observed by the inclusion of the autogamous species *U. anthemoides* in our experimental arrays. Fecundity of *U. anthemoides* was consistently high, and in contrast to the self-incompatible species, it was unaffected when individuals were at low density and scattered among heterospecifics. Since no pollinators were observed visiting this species, we attribute its consistently high fruit set (even in low-density treatments) to its ability to reproduce autogamously. These findings suggest that the ability to autonomously self not only offers reproductive assurance under suboptimal pollination conditions, but also under conditions where the probability of heterospecific interference is high. Our results raise the interesting possibility that autogamy may evolve as a reproductive assurance response to highly dispersive seeds, if high dispersal causes individuals to be scattered among a background of heterospecific individuals. De Waal *et al.* (2014) found some evidence in support of this when they documented an association between dispersal ability and breeding system in annual Asteraceae in Namaqualand. They identified two distinct syndromes: species that are highly dispersive and self-compatible (including *U. anthemoides*), and those that are less dispersive and self-incompatible (including *U. cakilefolia*). From another perspective, a selfing strategy may be optimal for such highly dispersive, scattered individuals, because traits associated with the 'selfing syndrome' (e.g. reduced flower size and showiness; e.g. Armbruster *et al.* 2002) may cause pollinators to avoid them altogether and consequently minimize the probability of heterospecific interference.

Conclusions

Spatial dispersion patterns of plants and conspecific density can have a major effect on the fecundity of individuals in multispecies co-flowering communities. Our study emphasizes the importance of heterospecific interference and mate availability on fecundity. Both of these mechanisms are affected by plant density and dispersion, and operate independently of quantitative variation in visitation rates. We also highlight the importance of community structure: at low abundance and scattered dispersion patterns, individuals in our experimental arrays exhibited very low fruit set. Self-compatibility, however, ensured consistent fruit set and may provide a mechanism to enhance fecundity for species with scattered distributions in a community, such as species with highly dispersive propagules.

Acknowledgements

Data accessibility

Fruit set and visitation rate data: DRYAD entry doi:10.5061/dryad.jq966

References

Ågren, J. (1996) Population size, pollinator limitation, and seed set in the self-incompatible herb *Lythrum salicaria*. *Ecology*, **77**, 1779–1790.

Armbruster, W.S., Mulder, C.P.H., Baldwin, B.G., Kalisz, S., Wessa, B. & Nute, H. (2002) Comparative analysis of late floral development and mating-system evolution in tribe Collinsieae (Scrophulariaceae s.l.). *American Journal of Botany*, **89**, 37–49.

Ashman, T.-L. & Arceo-Gómez, G. (2013) Toward a predictive understanding of the fitness costs of heterospecific pollen receipt and its importance in co-flowering communities. *American Journal of Botany*, **100**, 1061–1070.

Bates, D., Maechler, M., Bolker, B. & Walker, S. (2013) lme4: Linear mixed-effects models using Eigen and S4. R package version 1.0-5. Available at http://CRAN.R-project.org/package=lme4

Brys, R., Jacquemyn, H. & Hermy, M. (2007) Impact of mate availability, population size, and spatial aggregation of morphs on sexual reproduction in a distylous, aquatic plant. *American Journal of Botany*, **94**, 119–127.

Campbell, D.R. (1986) Predicting plant reproductive success from models of competition for pollination. *Oikos*, **47**, 257–266.

Caruso, C.M. (2002) Influence of plant abundance on pollination and selection on floral traits of *Ipomopsis aggregata*. *Ecology*, **83**, 241–254.

Cowling, R.M., Esler, K.J. & Rundel, P.W. (1999) Namaqualand, South Africa – an overview of a unique winter-rainfall desert ecosystem. *Plant Ecology*, **142**, 3–21.

Davis, H.G., Taylor, C.M., Lambrinos, J.G., Strong, D.R. & Mooney, H.A. (2004) Pollen limitation causes an Allee effect in a wind-pollinated invasive grass (*Spartina alterniflora*). *Proceedings of the National Academy of Sciences*, **101**, 13804–13807.

De Jager, M.L. & Ellis, A.G. (2014) Floral polymorphism and the fitness implications of attracting pollinating and florivorous insects. *Annals of Botany*, **113**, 213–222.

De Waal, C., Rodger, J.G., Anderson, B. & Ellis, A.G. (2014) Selfing ability and dispersal are positively related, but not affected by range position: a multispecies study on southern African Asteraceae. *Journal of Evolutionary Biology*, **27**, 950–959.

Duffy, K.J., Patrick, K.L. & Johnson, S.D. (2013) Does the likelihood of an Allee effect on plant fecundity depend on the type of pollinator? *Journal of Ecology*, **101**, 953–962.

Duffy, K.J. & Stout, J.C. (2011) Effects of conspecific and heterospecific floral density on the pollination of two related rewarding orchids. *Plant Ecology*, **212**, 1397–1406.

Duncan, D.H., Nicotra, A.B., Wood, J.T. & Cunningham, S.A. (2004) Plant isolation reduces outcross pollen receipt in a partially self-compatible herb. *Journal of Ecology*, **92**, 977–985.

Eckert, C.G., Samis, K.E. & Dart, S. (2006) Reproductive assurance and the evolution of uniparental reproduction in flowering plants. *The Ecology and Evolution of Flowers* (eds L.D. Harder & S.C.H. Barrett), pp. 183–203. Oxford University Press, Oxford, UK.

Ellis, A.G. & Johnson, S.D. (2009) The evolution of floral variation without pollinator shifts in *Gorteria diffusa* (Asteraceae). *American Journal of Botany*, **96**, 793–801.

Ellis, A.G. & Johnson, S.D. (2012) Lack of floral constancy by bee fly pollinators: implications for ethological isolation in an African daisy. *Behavioral Ecology*, **23**, 729–734.

Esler, K.J. (1999) Plant reproductive ecology. *The Karoo: Ecological Patterns and Processes* (eds W.R.J. Dean & S.J. Milton), pp. 123–144. Cambridge University Press, Cambridge, UK.

Essenberg, C.J. (2012) Explaining variation in the effect of floral density on pollinator visitation. *American Naturalist*, **180**, 153–166.

Feinsinger, P., Tiebout, H.M. & Young, B.E. (1991) Do tropical bird-pollinated plants exhibit density-dependent interactions? Field experiments. *Ecology*, **72**, 1953–1963.

Feinsinger, P., Murray, K.G., Kinsman, S. & Busby, W.H. (1986) Floral neighbourhood and pollination success in four hummingbird-pollinated cloud forest plant species. *Ecology*, **67**, 449–464.

Fishman, L. & Wyatt, R. (1999) Pollinator-mediated competition, reproductive character displacement, and the evolution of selfing in *Arenaria uniflora* (Caryophyllaceae). *Evolution*, **53**, 1723–1733.

Forsyth, S.A. (2003) Density-dependent seed set in the Haleakala silversword: evidence for an Allee effect. *Oecologia*, **136**, 551–557.

Galen, C. & Gregory, T. (1989) Interspecific pollen transfer as a mechanism of competition: consequences of foreign pollen contamination for seed set in the alpine wildflower, *Polemonium viscosum*. *Oecologia*, **81**, 120–123.

Gascoigne, J., Berec, L., Gregory, S. & Courchamp, F. (2009) Dangerously few liaisons: a review of mate-finding Allee effects. *Population Ecology*, **51**, 355–372.

Ghazoul, J. (2005) Pollen and seed dispersal among dispersed plants. *Biological Reviews*, **80**, 413–443.

Goodwillie, C. & Ness, J.M. (2013) Interactions of hybridization and mating systems: a case study in *Leptosiphon* (Polemoniaceae). *American Journal of Botany*, **100**, 1002–1013.

Groom, M.J. (1998) Allee effects limit population viability of an annual plant. *American Naturalist*, **151**, 487–496.

Hanoteaux, S., Tielbörger, K. & Seifan, M. (2013) Effects of spatial patterns on the pollination success of a less attractive species. *Oikos*, **122**, 867–880.

Hothorn, T., Bretz, F. & Westfall, P. (2008) Simultaneous inference in general parametric models. *Biometrical Journal*, **50**, 346–363.

Jakobsson, L., Lazáro, A. & Totland, O. (2009) Relationships between the floral neighbourhood and individual pollen limitation in two self-incompatible herbs. *Oecologia*, **160**, 707–719.

Kalisz, S. & Vogler, D.W. (2003) Benefits of autonomous selfing under unpredictable pollinator environments. *Ecology*, **84**, 2928–2942.

Kalisz, S., Vogler, D.W. & Hanley, K.M. (2004) Context-dependent autonomous self-fertilization yields reproductive assurance and mixed mating. *Nature*, **430**, 884–887.

Kariyat, R.R., Sinclair, J.P. & Golenberg, E.M. (2013) Following Darwin's trail: interactions affecting the evolution of plant mating systems. *American Journal of Botany*, **100**, 999–1001.

Kunin, W.E. (1993) Sex and the single mustard: population density and pollinator behavior effects on seed-set. *Ecology*, **74**, 2145–2160.

Lázaro, A. & Totland, Ø. (2010) Local floral composition and the behaviour of pollinators: attraction to and foraging within experimental patches. *Ecological Entomology*, **35**, 652–661.

Lloyd, D.G. (1992) Self- and cross-fertilization in plants. II. The selection of self-fertilization. *International Journal of Plant Sciences*, **153**, 370–380.

Matsumoto, T., Takakura, K. & Nishida, T. (2010) Alien pollen grains interfere with the reproductive success of native congener. *Biological Invasions*, **12**, 1617–1626.

Mitchell, R.J., Flanagan, R.J., Brown, B.J., Waser, N.M. & Karron, J.D. (2009) New frontiers in competition for pollination. *Annals of Botany*, **103**, 1403–1413.

Moeller, D.A. (2004) Facilitative interactions among plants via shared pollinators. *Ecology*, **85**, 3289–3301.

Morales, C.L. & Traveset, A. (2008) Interspecific pollen transfer: magnitude, prevalence and consequences for plant fitness. *Critical Reviews in Plant Sciences*, **27**, 221–238.

Morgan, M.T., Wilson, W.G. & Knight, T.M. (2005) Plant population dynamics, pollinator foraging, and the selection of self-fertilization. *American Naturalist*, **166**, 169–183.

Ollerton, J., Winfree, R. & Tarrant, S. (2011) How many flowering plants are pollinated by animals? *Oikos*, **120**, 321–326.

Palmer, T.M., Stanton, M.L. & Young, T.P. (2003) Competition and coexistence: exploring mechanisms that restrict and maintain diversity within mutualist guilds. *American Naturalist*, **162**, S63–S79.

R Core Team. (2013) R: A language and environment for statistical computing.

Rathcke, B. (1983) Competition and facilitation among plants for pollination. *Pollination Biology* (ed. L.A. Real), pp. 305–329. Academic Press, Orlando.

Rathcke, B. (1988) Interactions for pollination among coflowering shrubs. *Ecology*, **69**, 446–457.

Rodger, J.G., van Kleunen, M. & Johnson, S.D. (2013) Pollinators, mates and Allee effects: the importance of self-pollination for fecundity in an invasive lily (ed D. Campbell). *Functional Ecology*, **27**, 1023–1033.

Sargent, R.D. & Otto, S.P. (2006) The role of local species abundance in the evolution of pollinator attraction in flowering plants. *American Naturalist*, **167**, 67–80.

Stephens, P.A., Sutherland, W.J. & Freckleton, R.P. (1999) What is the Allee effect? *Oikos*, **87**, 185–190.

Stoll, P. & Prati, D. (2001) Intraspecific aggregation alters competitive interactions in experimental plant communities. *Ecology*, **82**, 319–327.

Stout, J.C., Allen, J.A. & Goulson, D. (1998) The influence of relative plant density and floral morphological complexity on the behaviour of bumblebees. *Oecologica*, **117**, 543–550.

Struck, M. (1994) Flowers and their insect visitors in the arid winter rainfall region of southern Africa: observations on permanent plots. Insect visitation behaviour. *Journal of Arid Environments*, **28**, 51–74.

Thomson, J.D., Andrews, B.J. & Plowright, R.C. (1981) The effect of a foreign pollen on ovule development in *Diervilla lonicera* (Caprifoliaceae). *New Phytologist*, **90**, 777–783.

Van Rooyen, M.W. (1999) Functional aspects of short-lived plants. *The Karoo: Ecological Patterns and Processes* (eds W.R.J. Dean & S.J. Milton), pp. 107–122. Cambridge University Press, Cambridge, UK.

Venables, W.N. & Ripley, B.D. (2002) *Modern Applied Statistics with S*, 4th edn. Springer, New York.

Ward, M., Johnson, S.D. & Zalucki, M.P. (2013) When bigger is not better: intraspecific competition for pollination increases with population size in invasive milkweeds. *Oecologia*, **171**, 883–891.

Wirth, L.R., Waser, N.M., Graf, R., Gugerli, F., Landergott, U., Erhardt, A., Linder, H.P. & Holderegger, R. (2011) Effects of floral neighborhood on seed set and degree of outbreeding in a high-alpine cushion plant. *Oecologia*, **167**, 427–434.

Wyatt, R. (1986) Ecology and evolution of self-pollination in *Arenaria uniflora* (Caryophyllaceae). *Journal of Ecology*, **74**, 403–418.

Zapata, T.R. & Arroyo, M.T.K. (1978) Plant reproductive ecology of a secondary deciduous tropical forest in Venezuela. *Biotropica*, **10**, 221–230.