

Small populations are mate-poor but pollinator-rich in a rare, self-incompatible plant, *Hymenoxys herbacea* (Asteraceae)

Lesley G. Campbell^{1,2} and Brian C. Husband¹

¹Department of Integrative Biology, University of Guelph, Guelph, Ontario, N1G 2W1, Canada; ²Present address: Department of Evolution, Ecology and Organismal Biology, Ohio State University, 318 W 12th Avenue, Columbus, OH 43210, USA

Summary

Author for correspondence:
Lesley G. Campbell
Tel: +1 614 292 8433
Fax: +1 614 292 2030
Email: campbell.633@osu.edu

Received: 10 December 2006
Accepted: 23 January 2007

- If pollinators or compatible mates are scarce, plants in small populations may be pollen-limited and consequently produce fewer offspring. However, determining the relative importance of the genetic vs ecological mechanisms limiting successful pollination is challenging.
- We explored the relationships among population size, population connectivity, pollinator visitation, allozyme diversity, mate availability (measured as percent compatible crosses among plants within a population), and pollen limitation in 12 populations ($N = 39\text{--}885274$) of the self-incompatible plant *Hymenoxys herbacea* in Ontario, Canada.
- Unexpectedly, small populations had more insect flower visitors per capitulum than large populations. Consistent with the effects of genetic drift, both allozyme polymorphism and mate availability decreased with decreasing population size. Pollen limitation was low and significant in only one population and could not be predicted based on knowledge of population size, connectivity, compatible mate availability, or pollinator visitation.
- Population size had detectable effects on both pollinator activity and mate availability. However, because the effect of population size was complementary on these two potentially limiting mechanisms, this threatened plant was rarely pollen-limited.

Key words: clonal, genetic diversity, *Hymenoxys herbacea*, insect pollinators, mate diversity, pollen limitation, population connectivity, population size.

New Phytologist (2007) **174**: 915–925

© The Authors (2007). Journal compilation © *New Phytologist* (2007)

doi: 10.1111/j.1469-8137.2007.02045.x

Introduction

Pollen limitation occurs when seed set is restricted by the availability of compatible pollen and results in the fertilization of fewer ovules than are available. It is traditionally measured as the difference in fecundity between open-pollinated flowers and those receiving a pollen supplement (Larson & Barrett, 2000). Surveys of the literature reveal that the majority of angiosperm species studied exhibit pollen-limited fecundity, but that the magnitude often varies spatially and temporally within a single species (reviewed in Burd, 1994; Larson & Barrett, 2000; Ashman *et al.*, 2004). Given that pollen limitation affects a diversity of taxa, its specific underlying ecological and

genetic mechanisms are likely diverse (Knight *et al.*, 2005). However, our understanding of the relative importance of factors affecting pollen limitation (Ashman *et al.*, 2004; Ghazoul, 2005) and the demographic consequences of pollen limitation is incomplete (Bond, 1994). Specifically, most pollen limitation studies reviewed by Ghazoul (2005) and Ashman *et al.* (2004) attempted to isolate single causal factors and rarely weighed the magnitude of ecological and genetic factors in a comprehensive manner (but see Waites & Ågren, 2004), yielding a less integrated understanding of the causes of pollen limitation.

Pollen limitation in self-incompatible plants may occur in small populations as a result of at least two mechanisms, one

ecological and one genetic. First, pollen may simply be transferred to flowers in insufficient quantities. In insect-pollinated plants, this shortage may be caused by reduced pollinator visitation, since small populations have smaller, more diffuse, and therefore less attractive floral displays (Groom, 1998; Liu & Koptur, 2003; Amarasekare, 2004) or lower plant densities and hence higher within-plant movement of pollinators (Mustajärvi *et al.*, 2001; Iwazumi & Sakai, 2004). All else being equal, having fewer pollinators will result in fewer insect visits per flower, less opportunity for pollen transfer among plants, increased geitonogamy, and ultimately lower seed production (Larson & Barrett, 2000). Second, pollen limitation in small populations may arise as a result of the stochastic loss of cross-compatible genotypes. In particular, the reproductive success of self-incompatible plant species is contingent upon the availability of compatible mating types. In small populations, genetic drift can cause mating types to become skewed in frequency or lost altogether (Wright, 1969; Les *et al.*, 1991). Consequently, adequate or even excessive amounts of pollen may be transferred, but the corresponding dominance by one or a few mating types renders most pollination ineffectual (Ågren, 1996; Waites & Ågren, 2004).

These two mechanisms of pollen limitation, hereafter referred to as pollinator limitation and mate limitation, are rarely distinguished in empirical studies and are often correlated because of the methodology used to measure pollen limitation. For example, in populations in which self-incompatible mating types are diverse and equally frequent (i.e. large populations), most pollen received by a plant will be compatible and only a few pollen grains are required for maximal fertilization of ovules. Under these circumstances a population may be limited by the abundance of pollinators but not by the availability of mates. On the other hand, populations with skewed self-incompatible mating-type frequencies and low mating-type diversity (i.e. small populations) will require more visits from pollinators to ensure an equally high probability of encountering compatible mates. Small populations will likely have both lower compatible mate availability and lower pollinator visitation rates than large populations, which are more visible and diverse. In a review by Ghazoul (2005), 12 out of 16 studies involving self-incompatible taxa showed that small populations had lower fecundity than large populations. However, few studies have examined the magnitude of pollen limitation, as opposed to resource limitation, in relation to population size (Sih & Baltus, 1987; Ågren, 1996; Willi & Fischer, 2005). Further, most studies of pollen limitation are examined in relation to plant density, which is a more localized measure of population attractiveness to insects. Fewer still have disentangled the underlying causes of pollen limitation (but see Waites & Ågren, 2004). Separating the underlying causes of pollen limitation is important for predicting when pollen limitation will occur in plant populations.

Declines in reproductive success and the correlated increase in pollen limitation may also result when previously inter-

connected populations become isolated from each other (reviewed in Ghazoul, 2005). Landscape fragmentation has become an increasingly common phenomenon in recent history as humans develop wild and semiwild areas and thereby create distinct patch isolates. Large-scale habitat modification leading to geographic isolation may limit the movement of pollinators and reduce gene flow among populations (Jennersten, 1988; Aizen & Feinsinger, 1994; Cunningham, 2000). The modification of plant-pollinator interactions may have serious consequences for plant reproduction and pollen limitation, reducing the number of visits by insects (Jennersten, 1988), the size of pollen loads (Cunningham, 2000) or pollen quality (Severns, 2003). Of specific importance to self-incompatible plants, pollinators may be less likely to move pollen with diverse self-incompatible alleles among populations, thereby restricting self-incompatible allele diversity and hence mate availability within populations. Therefore, as populations become increasingly isolated, with fewer opportunities for pollinator migration and gene exchange, pollen limitation may increase.

Pollen limitation and its associated causal factors are challenging to detect because, in part, pollen supplementation does not generally drastically alter a plant's reproductive success. In a meta-analysis, Knight *et al.* (2006) found that pollen supplementation raised the median value of reproductive success only slightly and researchers were rarely able to detect extremely high pollen limitation. The large natural variation in ecological and evolutionary processes affecting plant reproduction may make it difficult to detect important limiting factors of seed set (Knight *et al.*, 2006). Therefore, pollen limitation will be most easily detected using either a meta-analysis of accumulating evidence (Knight *et al.*, 2006) or large-scale experimental studies of pollen supplementation and ecological monitoring within and among populations. In this study, we examined the relationship between population size (N) and three aspects of pollination (pollinator visitation, compatible mate availability, pollen limitation) in the self-incompatible, perennial, herbaceous plant, *Hymenoxys herbacea*. Specifically, we addressed the following questions:

- Is the rate of insect visitation to inflorescences related to population size?
- Are small populations affected by genetic drift and does this manifest itself as reduced availability of compatible mating types?
- Are populations pollen-limited? If so, is the magnitude of pollen limitation correlated with population size or two potential causal factors, pollinator visitation or compatible mate availability?

Materials and Methods

Study system

Hymenoxys herbacea (E. L. Greene) Cusick (Asteraceae) (Cusick, 1991) is endemic to the Great Lakes region of

Canada and the USA. It is currently known from only 40 locations, 13 of which are located in the Bruce Peninsula (Campbell *et al.*, 2001). Populations occur in open alvar and cliff habitats, which are variable in size and isolated from each other by forest habitat. Plants comprise one or more rosettes (up to 10 cm in height), each with a cluster of fleshy, strap-shaped leaves, and may reproduce sexually to produce seeds or expand clonally through rhizomatous growth to produce additional rosettes (De Mauro, 1993; Campbell & Husband, 2005). Each plant produces a single inflorescence (capitulum) of yellow ray (female) and disc (hermaphrodite) florets, which are insect-pollinated (De Mauro, 1993), in the spring (May through June). We use the capitulum as the independent unit of study; therefore, our estimates of pollen limitation are at the whole-plant level (Knight *et al.*, 2006).

Mating in this species is governed by a sporophytic self-incompatibility system, which is a genetic mechanism that ensures successful fertilization only between genotypes carrying different self-incompatibility alleles (de Nettancourt, 1977; De Mauro, 1993). Achenes are produced approx. 3 wk after pollination and are dispersed primarily by gravity (De Mauro, 1993).

Experimental approach

To investigate the ecological and genetic factors that influence pollen limitation, and consequently seed production, we performed four related experiments using 12 populations of *Hymenoxys herbacea* of various sizes from the Bruce Peninsula, Ontario, Canada. Specifically, we related population size and interconnectivity of these populations with insect visitation rates, neutral allele diversity, compatible mate availability, and pollen limitation.

Estimating population size and connectivity

Population size (N) was estimated in each population as the total number of reproductive ramets during a single visit to each population in the first week of June. If the population had less than approx. 2000 inflorescences, all scapes were counted. For populations > 2000 plants, N was estimated by counting the scapes found within 1-m-wide belt transects, which spanned the width of the habitat and were placed every 20 m. From this survey, we estimated the number of floral scapes per m^2 and then multiplied this by the total area of the population for an estimate of the total number of scapes. The total area of each population was measured from infrared aerial photographs using the computer program Northern Eclipse vs 5.0 (Empix Imaging, Inc., Mississauga, ON, Canada). This measure best reflects the maximum number of potential mates and the amount of pollen available for fertilization in any single year. However, because *H. herbacea* clonally reproduces, the number of compatible, genetically unique mates will be lower. In fact, through a demographic analysis of effective

population size in *H. herbacea*, we estimated that effective population size was only one-third of the number of ramets ($N_e/N = 0.34$, Campbell & Husband, 2005). A preliminary analysis showed that population size was correlated with habitat area ($r = 0.58$, $P = 0.025$, d.f. = 11) but not with plant density ($r = 0.15$, $P = 0.65$, d.f. = 11). Habitat area may be a better reflection of the variation in the availability of soil in an environment largely covered by limestone.

Since the magnitude of pollen limitation may also be influenced by proximity to other populations (Ghazoul, 2005), we measured population connectivity using a modified version of Hanski's (1994) incidence function model (IFM). This model accounts for distances from the focal population to all possible neighboring populations and the size of those neighboring populations. Population connectivity or S_i was calculated as:

$$S_i = \sum_{j \neq i} \exp(-\alpha d_{ij}) A_j^b$$

(S_i , connectivity of patch i ; α , a scaling parameter for the effect of distance to migration ($1/\alpha$ is the average migration distance); d_{ij} , distance between patches i and j ; A_j , area of patch j ; b , scaling parameter of immigration as a function of the area of patch j). Population connectivity increases as S_i increases. Population connectivity of two populations' ranges between 0 and 1 and the maximum potential connectivity value increases additively as additional populations are considered. We used patch center-to-center distances for all connectivity measures in this study. Since small native bees generally move pollen of *H. herbacea* and seeds are gravity-dispersed (De Mauro, 1993), we estimated that the average migration distance could be as high as 500 m (Loveless & Hamrick, 1984; Hamrick *et al.*, 1993). This likely overestimates migration distance and therefore population connectivity. In a review of several common measures of population connectivity, Moilanen & Nieminen (2002) found that Hanski's (1994) approach had the best and most consistent performance for both highly fragmented habitats and for small data sets, whereas, for instance, the nearest neighbor measure failed repeatedly to find any significant effect of connectivity.

Insect visitation

To account for local variation in plant density within populations we randomly positioned four observation quadrats (2 m × 2 m) in each population. Insect visits were monitored in each quadrat for 30 min intervals between 10:30 and 15:00 h on any given day and for a minimum of four 30 min periods haphazardly distributed throughout the flowering season. We observed pollinator activity for 114 of these 30 min intervals, a total of 57 h. During that time, 2013 insect visits were recorded.

A visit was recorded each time an insect landed on a capitulum. The type of insect was noted and representative individuals were collected for identification to family (Borror

Table 1 Models of insect visitation, allozyme diversity, mate availability, and pollen limitation, with *Hymenoxys herbacea* population size and connectivity as predictors (for clarity α , ϵ are listed when included in the models)

Dependent variable	Model	ΔAIC_C	w	r^2
Insect visitation	$\alpha + \text{size} + \epsilon$	0 ^a	0.673	0.239
	$\alpha + \text{size} + \text{connect} + \epsilon$	3.981	0.092	0.281
	$\alpha + \text{density} + \epsilon$	4.278	0.079	0.027
	$\alpha + \text{size} + \text{density} + \epsilon$	4.280	0.079	0.296
	$\alpha + \text{connect} + \epsilon$	4.340	0.077	0.178
	$\alpha + \text{size} + \text{connect} + \text{density} + \epsilon$	9.852	0.005	0.342
% polymorphic loci	Size + connect + ϵ	0	0.673	0.920
	Size + ϵ	1.428	0.329	0.877
	Connect + ϵ	17.533	< 0.001	0.529
Mate availability	Size + ϵ	0	0.721	0.893
	Size + connect + ϵ	1.904	0.278	0.908
	Connect + ϵ	19.891	< 0.001	0.440
Pollen limitation	$\alpha + \text{mate} + \epsilon$	0	0.721	0.011
	$\alpha + \text{connect} + \epsilon$	0	0.721	0.041
	$\alpha + \text{size} + \epsilon$	0.051	0.703	0.033
	$\alpha + \text{insect} + \epsilon$	0.202	0.652	0.021
	$\alpha + \text{size} + \text{connect} + \text{size} \times \text{connect} + \text{mate} + \epsilon$	3.094	0.154	0.760

All models considered are ordered from best to worst according to Akaike information criterion corrected for small sample size (AIC_C). ΔAIC_C weight w , and r^2 are shown.

^aItalics indicate 'good' models (i.e. $\Delta AIC_C < 2$).

et al., 1981). Visitation frequency was low enough to allow all visits to be recorded. From these data, we estimated the number of visits per capitulum per observation period (= visitation rate). We estimated the relationship between population size (N), population connectivity (S_j), local plant density within quadrats, and total insect visitation rate using a multiple regression. For this and the following analyses, N was \log_{10} -transformed, S_j was arcsine-square-root-transformed, and insect visitation was natural log-transformed to normalize the data.

For this and all following linear regression models, we used an information-theoretic approach to model selection (Burnham & Anderson, 2002). For each full and all subset models, we calculated the Akaike information criterion, correcting for small sample size:

$$AIC_C = n(\ln[RSS/n]) + 2K + 2K(K + 1)/(n - K - 1)$$

(n , sample size (number of populations); RSS, residual sum of squares; K , number of parameters (including α and ϵ)). The first term in the equation represents the fit to data, the second term represents the penalty for including K parameters and the third term represents the correction for small sample size. We ranked models from best to worst based on the ascending AIC_C values. ΔAIC_C was calculated as the difference between each AIC_C value and the smallest value (i.e. for the best model $\Delta AIC_C = 0$). We calculated the relative likelihood of each model as $\exp[(-0.5)(\Delta AIC_C)]$. Finally, we calculated the information content or weight of each model, w , by dividing each model's relative likelihood by the sum of relative likelihoods for all models tested. The ratio of weights represents relative support (i.e. w_i/w_j represents support for model i vs j). We

report ΔAIC_C and w , as well as r^2 . All models considered are listed in Table 1.

Allozyme diversity

To determine whether genetic drift is a significant force in *H. herbacea* populations, we compared allozyme (putatively neutral markers) diversity among populations. From each population, a minimum of 15 seeds from 15 randomly selected mothers were collected and grown in the Botany Department's glasshouse. One leaf was harvested from each plant and ground in 45 ml 'Decodon' extraction buffer (Eckert & Barrett, 1994) after freezing in liquid nitrogen. The homogenate was centrifuged for 10 min at 4°C and the supernatant was applied to cellulose acetate gels. An electric current (200 V) was run across the gel for 45–60 min and a stain consisting of an enzymatic substrate and a colorimetric dye was added to visualize each enzyme.

Individuals were screened for seven enzymes: 6-phosphogluconate dehydrogenase (6-PGD, EC 1.1.1.44), acid phosphatase (α -ACP, EC 3.1.3.2), diaphorase – NADH (DIA-NADH, EC 1.8.1.4) and isocitrate dehydrogenase (IDH, EC 1.1.1.41) were resolved on morpholine citrate (pH 6.5) buffer. Malate dehydrogenase (MDH, EC 1.1.1.37), phosphoglucose isomerase (PGI, 5.3.1.9), and glucose-6-phosphate dehydrogenase (G6PDH, EC 1.1.1.49) were resolved on tris-citrate (pH 7.0) buffer systems (Hebert & Beaton, 1989; Manchenko, 1994). Only those loci that exhibited consistent activity and had clearly interpretable banding patterns were scored. In total, nine loci were scored.

Several population genetic metrics were estimated using the POPGENE computer program v.1.31 (Yeh *et al.*, 1999). First

we estimated population genetic diversity as: percentage polymorphic loci per population (P), mean number of alleles per locus (A), and Nei's (1978) measure of expected heterozygosity (H_N). To test for homogeneity of pattern among loci, we conducted the overall Ewens–Watterson test for neutrality for each locus (Manly, 1985). In the test of neutrality, the observed homozygosity (obs. F) fell within the 95% confidence intervals for expected homozygosity (exp. F). Therefore, for the remainder of the study, we considered all loci selectively neutral (results in Supplementary material, Table S1). We also estimated Nei's unbiased measures of genetic distance (Nei, 1978) for each population combination. Average expected heterozygosity (Nei, 1978), and fixation index values (Wright, 1978) were calculated for each population across the nine allozyme loci. Spatial genetic structure was also investigated by testing for isolation by distance (IBD). Geographic distances between pairs of populations were calculated from patch center-to-center distances. A Mantel test with 1000 random permutations was performed between the matrix of pairwise genetic differentiation between populations ($F_{ST}/(1 - F_{ST})$; Rousset, 1997), and the matrix of the natural logarithm of geographic distance. The analyses were performed with IBDWS (isolation by distance, web service; Jensen *et al.*, 2005).

As described above, we used multiple regression analysis with an AIC_C approach for model selection to examine the relationship between population size, population connectivity, and genetic diversity. If genetic drift affected neutral genetic diversity, we expected genetic diversity to increase as population size increased. If IBD affected genetic diversity, we would expect genetic diversity within populations to increase with increasing connectivity. The residuals associated with analyses including all genetic diversity variables were normally distributed and therefore genetic diversity variables were left untransformed.

Mating-type diversity

As a proxy for compatible mate availability, we estimated the probability that a random cross-pollination was cross-compatible. Seed families were collected randomly from 25 plants per population (except CCL ($N=20$) and CPS ($N=15$)). At least one representative from each seed family was grown in a glasshouse at the University of Guelph. Each plant served as both a maternal and a paternal parent within the experiment. Controlled cross-pollinations were conducted among pairs of plants within a population. A total of 285 plants were involved in the hand pollinations. Pollen from one randomly chosen paternal plant was applied to five florets per inflorescence such that all seeds produced on a given maternal plant were full-sibs. In addition, all plants were self-pollinated to confirm that *H. herbacea* was self-incompatible (average self-pollinated seed set = 0.78%).

After anthesis, the five florets were scored as filled (i.e. with mature seed) or unfilled (mature seed absent). Filled seeds were identified as being round, hard and dark achenes,

whereas unfilled achenes were flattened, soft and white. Germination tests showed that unfilled seeds were 100% nonviable ($N=30$ unfilled seeds from 30 different mothers; L. G. Campbell, unpublished). From this we estimated mean seed set for each cross and the percentage of crosses that were compatible (i.e. that had at least one filled seed). Since all plants were confirmed as self-incompatible (i.e. had \leq one seed per five pollinated florets; Hiscock, 2000), we estimated the effective mate availability per population as the percentage of compatible crosses from the random outcross treatment. This measure reflects the probability of encountering a compatible mate within the population while holding constant the resources available to produce seed.

We compared the percentage of compatible crosses among populations using a chi-squared test. We used multiple regression analysis with an AIC_C approach for model selection to examine the relationship between population size, population connectivity, and compatible mate availability. If genetic drift affected functional genetic diversity (i.e. mate availability or percentage of compatible crosses per population), we expected compatible mate availability to increase as population size increased. If population connectivity affected compatible mate availability, we would expect compatible mate availability to increase with increasing connectivity.

Pollen limitation

Twenty-five pairs of flowering rosettes were located within each population. To each pair, we randomly assigned each plant to one of two pollination treatments: open pollination and open pollination plus a pollen supplement. For each inflorescence, we marked a group of five receptive florets and removed the adjacent florets so the pollinated florets could be relocated. Open-pollinated inflorescences were naturally pollinated. In the second inflorescence of each pair, five florets were exposed to pollinators and also received bulk pollen from five randomly chosen donor plants. In small populations, pollen donor plants were sometimes used more than once within a bulk treatment because of the limited number of inflorescences available. Five weeks later, the florets were collected and scored as either filled or unfilled. Again, we estimated the proportion of seeds produced for supplemented and unsupplemented inflorescences to describe the probability of encountering compatible mates within the population regardless of resources available to produce the seed.

Mean percentage seed set for both pollination treatments was calculated for each population. A two-way ANOVA was used to test for variation in seed set between pollination treatments and among populations and for a pollination \times population interaction. To test for pollen limitation in each population, orthogonal contrasts were used to compare mean seed set in open-pollinated and pollen-supplemented inflorescences. If plants naturally received sufficient compatible pollen, then percentage seed set in open-pollinated flowers should have

Table 2 Summary of 12 *Hymenoxys herbacea* populations from the Bruce Peninsula, Ontario, Canada, surveyed in 2000 for population size (N = total number of inflorescences), population connectivity, and several genetic parameters including number of polymorphic loci, Nei's expected heterozygosity and Wright's fixation index

Population	Population size (N)	Connectivity (S_i) ^a	NP ^b	H_e ^c	F_{IS} ^d
1	39	0.02	2	0.11	-0.29
2	69	0.275	3	0.14	0.27
3	247	0.08	3	0.16	0.26
4	260	0.346	2	0.09	-0.15
5	522	0.519	3	0.16	0.17
6	574	0	3	0.15	0.13
7	592	0.497	4	0.12	0.09
8	783	0.356	2	0.08	-0.12
9	1 562	0.232	2	0.1	0.13
10	52 813	0	4	0.16	0.46
11	482 739	0.003	3	0.11	0.21
12	885 274	0.008	3	0.17	0.16

^a $S_i = \sum_{j \neq i} \exp(-\alpha d_{ij}) A_j^b$, where S_i is the connectivity of patch i , α is a scaling parameter for the effect of distance to migration ($1/\alpha$ is the average migration distance), d_{ij} is the distance between patches i and j , A_j is the area of patch j , and b is a scaling parameter of immigration as a function of the area of patch j . Population connectivity increases as S_i increases.

^bNumber of polymorphic markers of the nine allozyme loci sampled.

^cNei's (1978) expected heterozygosity calculated as a mean across nine allozyme loci per population.

^dWright's (1978) fixation index (F_{IS}) calculated across nine allozyme loci per population.

been equal to seed set in the pollen supplement treatment. Plants were considered pollen-limited when seed set in pollen-supplemented flowers significantly exceeded the open-pollinated treatment. A pollen limitation index (PLI) was calculated as:

$$(\text{seed set}_{\text{suppl}} - \text{seed set}_{\text{open}}) / \text{seed set}_{\text{suppl}}$$

which may range from -1 (no limitation) to 1 (complete). If $PLI < 0$, supplemented florets had lower seed set than open-pollinated florets and this suggests that supplemented florets were exposed to a more limited diversity of pollen donors than open-pollinated florets (Young & Young, 1992). Alternatively, reduced seed production with increased pollen load may also be the result of pollen removal or stigma damage by pollen consumers (Young & Young, 1992). To test the prediction that the magnitude of pollen limitation is related to population size, population connectivity, mate availability, and/or insect visitation rates, we used a linear multiple regression model with an AIC_C approach to model selection with PLI as the dependent variable and N , S_i , mate availability, and insect visitation as independent variables. Here we expected that small, isolated, mate- and insect-depauperate populations would exhibit high PLI, whereas the opposite characteristics would describe populations exhibiting low PLI.

Results

Population size and connectivity

Population size averaged 118 789.5 flowering plants (geometric mean = 1753.7) and ranged from 39 (population 1) to 885 274

flowering individuals (population 12) (Table 2). Population connectivity averaged 0.19 and ranged from 0 (populations 6, 10) to 0.52 (population 5). Population size and connectivity did not covary (Pearson's $r = -0.11$, $P = 0.37$, $n = 12$).

Insect visitation

Eight families of insects were represented in the pollinator survey, including Hymenoptera, Diptera, Lepidoptera, Neuroptera, Homoptera, Hemiptera, Coleoptera, and Orthoptera. The total number of insect visits per inflorescence per 30 min averaged 0.66, and ranged from 0 to 5.53 visits per plant per 30 min observation period. Given that populations varied in rates of insect visitation, we then evaluated whether population size, local inflorescence density, and connectivity could account for variability in insect visitation rates.

Population size was the single most important predictor of insect visitation rate (Table 1). Insect visitation rate decreased with increasing population size ($\beta = -0.468$, $P < 0.05$, Fig. 1). This model received approx. seven times more support than the next best model, which included population size and connectivity (i.e. based on the ratio of $w_i/w_j = 0.673/0.092$). Further, models predicting insect visitation that included plant density had even less support. Therefore, insect visitation was apparently most influenced by population size.

Isozyme diversity

Every population was variable for at least one isozyme locus, with an average of 1.33 alleles per locus. The percentage of polymorphic loci ranged from 11.1 to 44.4%, with a population mean of 30.8%. Nei's (1978) expected heterozygosity

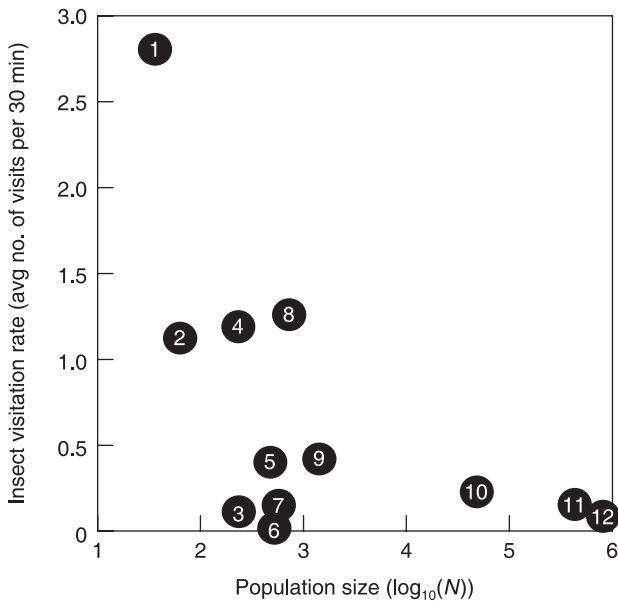


Fig. 1 The relationship between population size ($\log_{10}(N)$) and average insect visitation rate (number of visits by insects per 30 min period) for 12 populations of *Hymenoxys herbacea*. (Bee visitation rates = $-0.962 + 4.488 \log_{10}(N)^{-1}$, $P = 0.005$). Population identification numbers are as in Table 2.

(H_N) varied among populations from -0.29 (population 6) to $+0.46$ (population 4), with no value > 0.5 , and averaged 0.20 ($SE = 0.08$). A Mantel test revealed that geographic distances were not significantly correlated with genetic divergence among populations ($r = -0.0825$, $P = 0.68$) so IBD was not an appropriate explanation for the patterns of putatively neutral genetic diversity across populations. Further, these populations exhibited low rates of inbreeding ($F_{IS} = 0.11$), moderate genetic differentiation ($F_{ST} = 0.30$), and a moderate amount of gene flow among populations ($N_m = 0.5755$; Nei, 1978).

In our analysis of genetic diversity, both population size and connectivity were important predictors of the percentage of polymorphic loci (P , Fig. 2, Table 1). Allozyme diversity increased significantly with increasing population size ($\beta = 0.079$, $P < 0.001$) and connectivity ($\beta = 0.191$, $P < 0.05$, Fig. 3, Table 1). Because the patterns are consistent across metrics of genetic diversity, we present the graphical results associated with A , AP , and H_N in Fig. S1. The pattern of genetic diversity across populations is consistent with patterns expected with genetic drift. Further, an index of connectivity was a better predictor of patterns of allozyme variation than a simple IBD model. This is apparently another example where true population interconnectivity cannot be accurately predicted by simple nearest neighbor distance (Moilanen & Nieminen, 2002).

Compatible mate availability

Overall, the mean percentage of cross-pollinations that were compatible, and therefore mate availability within populations,

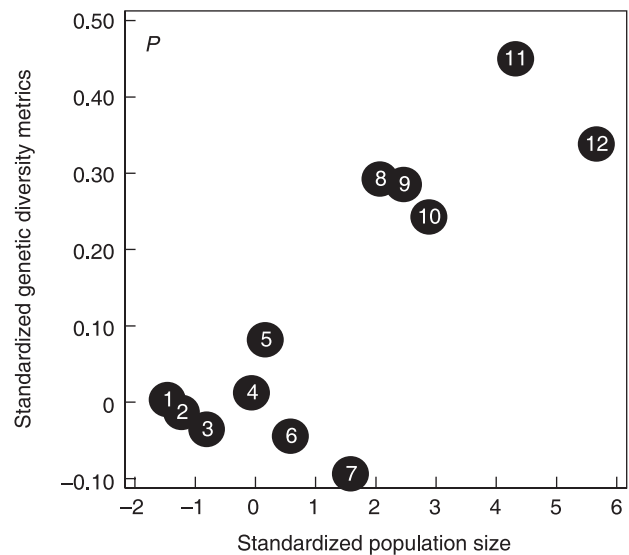


Fig. 2 Partial regression plots of genetic diversity (% polymorphism, P) and standardized population size for 12 populations of *Hymenoxys herbacea*. Connectivity was incorporated into the regression analyses but was not shown here for simplicity of presentation. Population identification numbers are as in Table 2; additional results are available in Supplementary Material (Fig. S1).

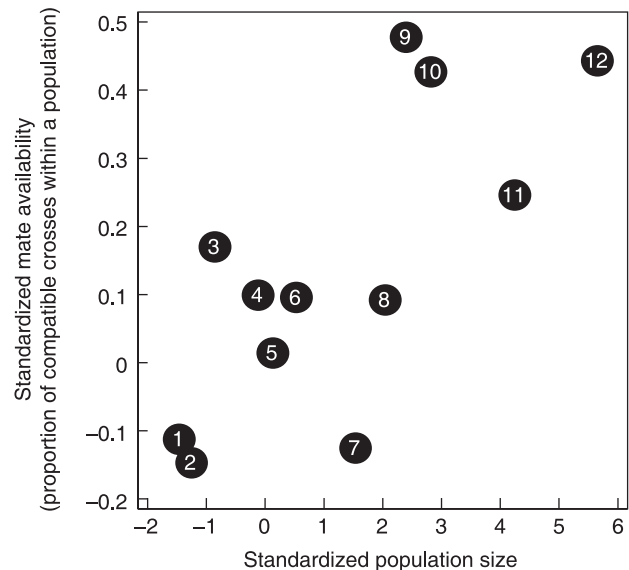


Fig. 3 The partial regression of compatible mate availability (see 'Mating-type diversity' for methods of estimation) and standardized population size for 12 populations of *Hymenoxys herbacea*. Connectivity was incorporated into the regression analyses but was not shown here for simplicity of presentation. Population identification numbers are as in Table 2.

was 33.5% ($SE = 0.04$). Population values ranged from 17% (population 8) to 58% (population 10) and differed significantly among populations ($\chi^2 = 23.7$, d.f. = 11, $P = 0.02$).

In the regression analysis of mate availability, population size alone was the best model evaluated (Table 1). Compatible

mate availability increased with increasing population size ($\beta = 0.172$, $P < 0.001$). This model received approx. 2.6 times more support than the next best model, which included both population size and connectivity. In this model, which is still considered 'good' ($\Delta AIC_C > 2$, Burnham & Anderson, 2002), compatible mate availability increased with increasing population size ($\beta = 0.172$, $P < 0.001$, Fig. 3) and with increasing connectivity ($\beta = 0.203$, $P = 0.24$). Thus, compatible mate availability was apparently influenced by population size and connectivity, with population size having a stronger influence.

Pollen limitation

Percentage seed set for pollen-supplemented plants averaged 47.5% (SE = 0.02) and ranged from 35% (population 1) to 59% (population 12), whereas seed set for open-pollinated plants averaged 47.4% (SE = 0.03) and ranged from 18% (population 2) to 65% (population 4). Averaged across all populations, there was no significant difference between the two pollination treatments (ANOVA, treatment effect: $F_{1,11} = 0.11$, $P = 0.75$). At the population level, one of the 12 populations had significantly greater seed set after pollen supplementation (41%) than after open pollination (18%) (population 2, orthogonal contrast: $F = 5.1$, $P = 0.02$). The PLI, averaged across all 12 populations, was 0.02 (SE = 0.06) and ranged from -0.30 to 0.54. Although not significant, open-pollinated florets had greater seed set than pollen-supplemented florets in four populations.

There were four 'good' models ($\Delta AIC_C < 2$) explaining variation among populations for the PLI, according to the information theoretic approach; each included one of the predictor variables (population size, connectivity, compatible mate availability and insect visitation). However, each of these models fitted the data extremely poorly (according to the r^2) and no model performed significantly better than random chance (i.e. $P > 0.05$). This may be a result of the low PLI of most populations.

Discussion

Reductions in seed set may arise under several ecological and genetic scenarios, including resource limitation (Zimmerman & Pyke, 1988), genic sterility (Horner & Palmer, 1995), and insufficient compatible pollen deposition on stigmas (Larson & Barrett, 2000; Knight *et al.*, 2006). The focus of this study was to test the role of pollination in the limitation of seed production in a rare plant, *Hymenoxys herbacea*. In cases of rare organisms, population size may be the dominant factor limiting plant fecundity by altering ecological and genetic processes that influence pollination. Seed production in small populations can be limited by insufficient pollinator visitation, a form of Allee effect (Groom, 1998; Ehlers *et al.*, 2002). If the plant is self-incompatible, the degree of pollen limitation

may also increase as a result of genetic drift, which causes the loss of self-incompatible alleles and hence compatible mating types (Waites & Ågren, 2004). These forces may collectively result in pollen limitation, although they are rarely considered simultaneously, and their relative contributions in natural populations are poorly understood. Our results revealed that population size was negatively correlated with insect visitation (Fig. 1) and positively correlated with compatible mate availability (Fig. 3). Perhaps because of these counteracting effects of population size, the PLI was negligible in most populations and not significantly correlated to the predictor variables measured in this study.

Rates of insect visitation increased with decreasing population size and were not correlated with population connectivity, and, ultimately, insect visitation was not a predictor of the PLI. This result was at odds with our expectation, that larger populations would attract more pollinators owing to their larger floral displays. In a recent review (Ghazoul, 2005), only one of nine studies measuring the association between population size and insect visitation rates in self-incompatible plants found a negative relationship. In the bee-pollinated orchid, *Calypto bulbosa*, pollen export increased with decreasing population size (Alexandersson & Ågren, 1996), suggesting that the frequency of visits per flower had increased. One explanation for such negative correlations is that pollinator populations are small and therefore spread their pollination services across more individuals or flowers in large plant populations.

Populations of *H. herbacea* varied widely in genetic diversity. The overall magnitude of diversity is similar to the means for other perennial herbaceous plants ($P = 39.3\%$) and outcrossing, animal-pollinated species ($P = 35.9\%$) but slightly higher than other endemic species ($P = 26.3\%$) (Hamrick, 1990; Hamrick & Godt, 1990). Furthermore, diversity was positively related to population size, suggesting that stochastic processes causing the loss of alleles are detectable within the range of population sizes we used. Since allozymes are believed to be selectively near neutral (but see Hanski & Saccheri, 2006), this effect should be readily observable with sufficient sampling. Several studies report a significant positive relationship between N and various measures of neutral diversity (Ellstrand & Elam, 1993; Prober & Brown, 1994), particularly in self-incompatible species (Severns, 2003; Willi & Fischer, 2005). The signature of genetic drift in *H. herbacea* suggests stochasticity in small populations may affect compatible mate availability and other genetic variants under selection.

Mate diversity, measured as the proportion of compatible crosses, ranged from 17 to 58% per population and averaged 33.5%. Given that most pollen dispersal will occur within limited distances, this likely overestimates the diversity of mates experienced by plants (Moran Palma & Snow, 1997). Even with that upward bias, the value is lower than compatible mate availability of other *H. herbacea* populations (De Mauro, 1993), where 58% of within-population crosses were

compatible. In addition, compatible mate availability was strongly correlated with population size, a result consistent with the prediction that, as N decreases, the loss of mating-type diversity through genetic drift should also increase (Byers & Meagher, 1992). While this pattern has been demonstrated in heteromorphic self-incompatible plants (Husband & Barrett, 1992; Eckert *et al.*, 1996; Barrett & Husband, 1997; Brys *et al.*, 2004; Waites & Ågren, 2004), to our knowledge, the negative effect of population size on self-incompatible allele diversity in homomorphic self-incompatible plants has not been detected (but see Byers (1995) and Paschke *et al.* (2002) for attempts to detect these effects). This explains why extremely small populations of *H. herbacea*, such as the Illinois population described by De Mauro (1993), lose all compatible mate availability and produce no seed. Descriptions of variation in self-incompatible diversity among populations are relatively scarce (but see Waites & Ågren, 2004) and our study provides valuable insight into mate limitation in endemic species such as *H. herbacea*.

The PLI, measured as the proportional increase in seed set after the addition of pollen, to open-pollinated florets was low (0.08) compared with other angiosperm species (Larson & Barrett, 2000: mean = 0.40; Knight *et al.*, 2006: mean = 0.21) and compared with self-incompatible plants, specifically (Knight *et al.*, 2006: mean = 0.59). There is likely a publication bias against low PLI values, a possible explanation of our relatively low estimate (Knight *et al.*, 2006). However, the low PLI estimates in our study are comparable to other PLI studies of *H. herbacea*. In one large US population of *H. herbacea* (Marblehead Quarry), pollen supplementation elevated seed set only 7% above open-pollinated values, a nonsignificant difference (t -test: $t_{df=73} = 0.23$, $P > 0.5$; De Mauro, 1993). In our study, open-pollinated florets tended to have higher seed set than pollen-supplemented florets; this may reflect heterogeneity in pollination conditions among plants within a population, perhaps through stochasticity in either insect visitation or sampling of mating types. These results reinforce the importance of high pollinator activity in small populations where compatible mate availability may be limited and suggest there are salient features of this species or its habitat associated with a low PLI.

We expected population size to influence the PLI through its effect on pollinator activity and stochastic loss of mating types. However, the populations we examined exhibited little variation in PLI and no detectable relationship with population size. Apparently, even in small populations, plants receive sufficient compatible pollen for maximum seed production (set by resources). High insect visitation in small populations may have compensated for the effects of lower self-incompatible allele diversity. Excess pollination would ensure that each plant receives some pollen from a compatible donor. This is consistent with the results of a recent study by Waites & Ågren (2004), who found that although the amount of incompatible pollen deposited on stigmas was high (73–98%), seed set increased with increasing pollen transfer. Second, our smallest

populations may not be small enough to be truly mate-limited. Mate diversity within a population was significantly related to population size. However, most populations are apparently not so limited in compatible mate availability that reproduction is reduced drastically. The population sizes of *H. herbacea* have, in general, not reached a threshold value, below which it is impossible to maintain self-incompatible allele diversity. Because we did not observe complete seed set in pollen-supplemented plants, resource limitation may be a more important factor, currently, than pollen limitation for ensuring reproductive success of *H. herbacea* in Canada (Zimmerman & Pyke, 1988).

Hymenoxys herbacea is a rare endemic of the Great Lakes and has been a target of North American conservation initiatives. For many plant species, habitat loss and fragmentation have led to greater population isolation and lowered pollination success within such isolates (Groom, 1998). Here, we found that most of the Bruce Peninsula populations were not pollen-limited. Under the current resources, populations have adequate pollinator activity and compatible mate availability to ensure seed set. However, should the size of these populations of *H. herbacea* become smaller, pollen limitation could increase. Our results suggest that mate limitation is more likely to limit fecundity in small populations of self-incompatible plants than pollinators.

Acknowledgements

The authors thank C. Hussell for field assistance, and J. Windus, T. Waite, and the Snow lab group for valuable discussions. Thanks are also due to the Cape Croker Band Council, the Federation of Ontario Naturalists, the Bruce Peninsula National Park and the Bruce Peninsula Provincial Parks for permission to conduct research on their land. Research was funded by an operating grant to BCH from the Natural Sciences and Engineering Research Council of Canada.

References

- Ågren J. 1996. Population size, pollinator limitation, and seed set in the self-incompatible herb *Lythrum salicaria*. *Ecology* 77: 1779–1790.
- Aizen MA, Feinsinger P. 1994. Forest fragmentation, pollination, and plant reproduction in a chaco dry forest, Argentina. *Ecology* 75: 330–351.
- Alexandersson R, Ågren J. 1996. Population size, pollinator visitation, and fruit production in the deceptive orchid *Calypso bulbosa*. *Oecologia* 107: 533–540.
- Amarasekare P. 2004. Spatial dynamics of mutualistic interactions. *Journal of Animal Ecology* 73: 128–142.
- Ashman TL, Knight TM, Steets JA, Amarasekare P, Burd M, Campbell DR, Dudash MR, Johnston MO, Mazer SJ, Mitchell RJ, Morgan MT, Wilson WG. 2004. Pollen limitation of plant reproduction: ecological and evolutionary causes and consequences. *Ecology* 85: 2408–2421.
- Barrett SCH, Husband BC. 1997. Ecology and genetics of ephemeral plant populations: *Eichhornia paniculata* (Pontederiaceae) in northeast Brazil. *Journal of Heredity* 88: 277–284.
- Bond WJ. 1994. Do mutualisms matter – assessing the impact of pollinator and disperser disruption on plant extinction. *Philosophical Transactions of the Royal Society of London Series B – Biology Sciences* 344: 83–90.

- Borror DJ, Delong DM, Triplehorn CA. 1981. *An introduction to the study of insects*. Philadelphia, PA, USA: Saunders Press.
- Brys R, Jacquemyn H, Endels P, Van Rossum F, Hermy M, Triest L, De Bruyn L, Blust GDE. 2004. Reduced reproductive success in small populations of the self-incompatible *Primula vulgaris*. *Journal of Ecology* 92: 5–14.
- Burd M. 1994. Bateman's principle and plant reproduction: the role of pollen limitation in fruit and seed set. *Botanical Review* 60: 83–139.
- Burnham KP, Anderson DR. 2002. *Model selection and multimodel inference: a practical information-theoretic approach, 2nd edn*. New York, NY, USA: Springer.
- Byers DL. 1995. Pollen quantity and quality as explanations for low seed set in small populations exemplified by *Eupatorium* (Asteraceae). *American Journal of Botany* 82: 1000–1006.
- Byers DL, Meagher TR. 1992. Mate availability in small populations of plant species with homomorphic sporophytic self-incompatibility. *Heredity* 68: 353–359.
- Campbell LG, Husband BC. 2005. Impact of clonal on effective population size in *Hymenoxys herbacea* (Asteraceae). *Heredity* 94: 526–532.
- Campbell LG, Husband BC, Oldham M. 2001. *Status report on the Lakeside Daisy (Hymenoxys herbacea)*. Report to the Committee on the Status of Endangered Status of Wildlife in Canada. Ottawa, Canada: Government of Canada.
- Cunningham, SA. 2000. Depressed pollination in habitat fragments causes low fruit set. *Proceedings of the Royal Society of London, Series B* 267: 1149–1152.
- Cusick AW. 1991. *Hymenoxys herbacea* (Asteraceae): an endemic species of the Great Lakes region. *Rhodora* 93: 238–241.
- De Mauro MM. 1993. Relationship of breeding system to rarity in the Lakeside Daisy (*Hymenoxys acaulis* var. *glabra*). *Conservation Biology* 7: 542–550.
- Eckert CG, Barrett SCH. 1994. Inbreeding depression in partially self-fertilizing *Decodon verticillatus* (Lythraceae): population-genetic and experimental analyses. *Evolution* 48: 952–964.
- Eckert CG, Manicacci D, Barrett SCH. 1996. Genetic drift and founder effect in native versus introduced populations of an invading plant, *Lythrum salicaria* (Lythraceae). *Evolution* 50: 1512–1519.
- Ehlers BK, Olesen JM, Ågren J. 2002. Floral morphology and reproductive success in the orchid *Epipactis helleborine*: regional and local across-habitat variation. *Plant Systematics and Evolution* 236: 19–32.
- Ellstrand NC, Elam DR. 1993. Population genetic consequences of small population size: implications for plant conservation. *Annual Review of Ecology and Systematics* 24: 217–242.
- Ghazoul J. 2005. Pollen and seed dispersal among dispersed plants. *Biology Reviews* 80: 413–443.
- Groom MJ. 1998. Allee effects limit population viability of an annual plant. *American Naturalist* 151: 487–496.
- Hamrick JL. 1990. Gene flow and distribution of genetic variation in plant populations. In: Urbanska KM, ed. *Differentiation patterns in higher plants*. London, UK: Academic Press, 53–67.
- Hamrick JL, Godt MJW. 1990. Allozyme diversity in plant species. In: Brown AHD, Clegg MT, Kahler AL, Weir BS, eds. *Plant population genetics, breeding and genetic resources*. Sunderland, MA, USA: Sinauer Associates Inc, 43–63.
- Hamrick JL, Murawski DA, Nason JD. 1993. The influence of seed dispersal mechanisms on the genetic structure of tropical tree populations. *Vegetatio* 108: 281–297.
- Hanski I. 1994. A practical model of metapopulation dynamics. *Journal of Animal Ecology* 63: 151–162.
- Hanski I, Saccheri I. 2006. Molecular-level variation affects population growth in a butterfly metapopulation. *PLOS Biology* 4: 719–726.
- Hebert PDN, Beaton MJ. 1989. *Methodologies for allozyme analysis using cellulose acetate electrophoresis. A practical handbook*. Windsor, Ontario, Canada: University of Windsor.
- Hiscock SJ. 2000. Genetic control of self-incompatibility in *Senecio squalidus* L. (Asteraceae): a successful colonizing species. *Heredity* 85: 10–19.
- Horner HT, Palmer RG. 1995. Mechanisms of genic male-sterility. *Crop Science* 35: 1527–1535.
- Husband BC, Barrett SCH. 1992. Genetic drift and the maintenance of the style length polymorphism in tristylous populations of *Eichhornia paniculata* (Pontederiaceae). *Heredity* 69: 440–449.
- Iwaizumi MG, Sakai S. 2004. Variation in flower biomass among nearby populations of *Impatiens textori* (Balsaminaceae): effects of population plant densities. *Canadian Journal of Botany* 82: 563–572.
- Jennersten O. 1988. Pollination in *Dianthus deltoides* (Caryophyllaceae): effects of habitat fragmentation on visitation and seed set. *Conservation Biology* 2: 359–366.
- Jensen JL, Bohonak AJ, Kelley ST. 2005. Isolation by distance, web service. *BMC Genetics* 6: 13.
- Knight TM, Steets JA, Ashman TL. 2006. A quantitative synthesis of pollen supplementation experiments highlights the contribution of resource reallocation to estimates of pollen limitation. *American Journal of Botany* 93: 271–277.
- Knight T, Steets JA, Vamasi JC, Mazer SJ, Burd M, Campbell DR, Dudash M, Johnston MO, Mitchell RJ, Ashman T. 2005. Pollen limitation of plant reproduction: Pattern and Process. *Annual Review of Ecology, Evolution and Systematics* 36: 467–497.
- Larson BMH, Barrett SCH. 2000. A comparative analysis of pollen limitation in flowering plants. *Biological Journal of the Linnean Society* 69: 503–520.
- Les DH, Reinhartz JA, Esselman EJ. 1991. Genetic consequences of rarity in *Aster furcatus* (Asteraceae), a threatened, self-incompatible plant. *Evolution* 45: 1641–1650.
- Liu H, Koptur S. 2003. Breeding system and pollination of a narrowly endemic herb of the Lower Florida Keys: impacts of the urban–wildland interface. *American Journal of Botany* 90: 1180–1187.
- Loveless MD, Hamrick JL. 1984. Ecological determinants of genetic structure in plant populations. *Annual Review of Ecology and Systematics* 15: 65–95.
- Manchenko GP. 1994. *Handbook of detection of enzymes on electrophoretic gels*. London, UK: CRC Press.
- Manly BFJ. 1985. *The statistics of natural selection*. New York, NY, USA: Chapman & Hall, 272–282.
- Moilanen A, Nieminen M. 2002. Simple connectivity measures in spatial ecology. *Ecology* 83: 1131–1145.
- Moran Palma P, Snow AA. 1997. The effect of interplant distance on mating success in federally threatened, self-incompatible *Hymenoxys herbacea* = *H. acaulis* var. *glabra* (Asteraceae). *American Journal of Botany* 84: 233–238.
- Mustajärvi K, Siikamaki P, Rytönen S, Lammi A. 2001. Consequences of plant population size and density for plant–pollinator interactions and plant performance. *Journal of Ecology* 89: 80–87.
- Nei M. 1978. Estimation of heterozygosity and genetic distance from a small number of individuals. *Genetics* 89: 583–590.
- de Nettancourt D. 1977. Incompatibility in angiosperms. In: Frankel R, Gall GAE, Linskens HF, eds. *Monographs in Theoretical and Applied Genetics*. Berlin, Germany: Springer Verlag.
- Paschke M, Abstract C, Schmid B. 2002. Effects of population size and pollen diversity on reproductive success and offspring size in the narrow endemic *Cochlearia bavarica* (Brassicaceae). *American Journal of Botany* 89: 1250–1259.
- Prober SM, Brown AHD. 1994. Conservation of the grassy white box woodlands: Population genetics and fragmentation of *Eucalyptus albens*. *Conservation Biology* 8: 1003–1013.
- Rousset F. 1997. Genetic differentiation and estimation of gene flow from F-statistics under isolation by distance. *Genetics* 145: 1219–1228.
- Severns P. 2003. Inbreeding and small population size reduce seed set in a threatened and fragmented plant species, *Lupinus sulphureus* ssp. *kincaidii* (Fabaceae). *Biological Conservation* 110: 221–229.

- Sih A, Baltus MS. 1987. Patch size, pollinator behavior, and pollinator limitation in Catnip. *Ecology* 68: 1679–1690.
- Waites AR, Ågren J. 2004. Pollinator visitation, stigmatic pollen loads and among-population variation in seed set in *Lythrum salicaria*. *Journal of Ecology* 92: 512–526.
- Willi Y, Fischer M. 2005. Genetic rescue in interconnected populations of small and large size of the self-incompatible *Ranunculus reptans*. *Heredity* 95: 437–443.
- Wright S. 1969. *Evolution and the genetics of populations. The theory of gene frequencies*, Vol. II. Chicago, IL, USA: University of Chicago Press.
- Wright S. 1978. *Evolution and the genetics of populations. Variability within and among natural populations*, Vol. IV. Chicago, IL, USA: University of Chicago Press.
- Yeh FC, Yang RC, Boyle TBJeZ-H, Mao JX. 1999. *POPGENE, the user-friendly shareware for population genetic analysis*. Edmonton, Alberta, Canada: Molecular Biology and Biotechnology Centre, University of Alberta.
- Young HJ, Young TP. 1992. Alternative outcomes of natural and experimental high pollen loads. *Ecology* 73: 639–647.
- Zimmerman M, Pyke GH. 1988. Reproduction in *Polemonium* – assessing the factors limiting seed set. *American Naturalist* 131: 723–738.

Supplementary Material

The following supplementary material is available for this article online:

Fig. S1 Partial regression plots of additional measures of genetic diversity (A , AP , H_N) and standardized population size for 12 populations of *Hymenoxys herbacea*.

Table S1 Results of the Ewens–Watterson test for selective neutrality

This material is available as part of the online article from: <http://www.blackwell-synergy.com/doi/abs/10.1111/j.1469-8137.2007.02045.x>
(This link will take you to the article abstract).

Please note: Blackwell Publishing are not responsible for the content or functionality of any supplementary materials supplied by the authors. Any queries (other than missing material) should be directed to *New Phytologist* Central Office.



About New Phytologist

- *New Phytologist* is owned by a non-profit-making **charitable trust** dedicated to the promotion of plant science, facilitating projects from symposia to open access for our Tansley reviews. Complete information is available at www.newphytologist.org.
- Regular papers, Letters, Research reviews, Rapid reports and both Modelling/Theory and Methods papers are encouraged. We are committed to rapid processing, from online submission through to publication 'as-ready' via *OnlineEarly* – our average submission to decision time is just 30 days. Online-only colour is **free**, and essential print colour costs will be met if necessary. We also provide 25 offprints as well as a PDF for each article.
- For online summaries and ToC alerts, go to the website and click on 'Journal online'. You can take out a **personal subscription** to the journal for a fraction of the institutional price. Rates start at £131 in Europe/\$244 in the USA & Canada for the online edition (click on 'Subscribe' at the website).
- If you have any questions, do get in touch with Central Office (newphytol@lancaster.ac.uk; tel +44 1524 594691) or, for a local contact in North America, the US Office (newphytol@ornl.gov; tel +1 865 576 5261).

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.