

Reduced reproductive success in small populations of the self-incompatible *Primula vulgaris*

REIN BRY^{s*}†‡, HANS JACQUEMYN^{*}, PATRICK ENDELS^{*}, FABIENNE VAN ROSSUM^{†§}, MARTIN HERMY^{*}, LUDWIG TRIEST[†], LUC DE BRUYN^{‡§} and GEERT D. E. BLUST[‡]

^{*}Laboratory for Forest, Nature and Landscape Research, Catholic University of Leuven, Vital Decosterstraat 102, B-3000 Leuven, Belgium, [†]Laboratory for Plant Science and Nature Management, Free University of Brussels, Pleinlaan 2, B-1050 Brussels, Belgium, [‡]Institute of Nature Conservation, Kliniekstraat 25, B-1070 Brussels, Belgium, and [§]Department of Biology, University of Antwerp, Groenenbrogerlaan 171, 2020 Antwerp, Belgium

Summary

1 Habitat fragmentation and the resulting decline in population size can affect biotic interactions and reproductive success of plant species. We investigated the impact of habitat type, population size, morph type and frequency, plant density, floral display and predation on different reproductive components in 16 populations of the distylous self-incompatible perennial herb, *Primula vulgaris*, a rare, declining species in Belgium.

2 Although habitat type accounted for significant variation in population size, we did not find any relation between habitat type and either reproductive and vegetative characteristics. Population size, however, strongly affected reproductive success, such that plants in small populations produced significantly fewer fruits per plant and seeds per fruit, and therefore fewer seeds per plant.

3 No significant difference was found between morph types for any reproductive characteristic, nor an interaction with population size. However, when morph frequency was strongly biased ($\geq 1 : 3$), the proportion of flowers setting fruit and the number of seeds per fruit were significantly lower in individuals of the common morph type.

4 Within populations, individual plants varied tremendously in size and floral display. Total number of fruits per plant significantly increased with floral display, but the highest fruit set per flower was found at intermediate flower number.

5 The proportion of fruit suffering pre-dispersal predation per plant significantly increased with floral display, but this did not offset the potential fitness gains of producing a large display. Furthermore, the absolute number of predated fruits per plant was significantly and positively affected by the interaction of the total number of fruits per plant and the density of the population.

Key-words: distyly, floral display, fruit predation, morph bias, pollinator limitation, reproduction

Journal of Ecology (2004) **92**, 5–14

Introduction

Habitat destruction and fragmentation are processes that threaten terrestrial plant ecosystems around the globe (Burgess 1988; Saunders *et al.* 1991). In extreme

cases, previously widespread plant communities now persist only in a vestigial state, with populations of their constituent species restricted to small and geographically isolated habitat remnants, e.g. *Primula veris* and *Gentiana lutea* (Kéry *et al.* 2000) and *Arnica montana* (Luijten *et al.* 2000). These processes, caused by natural disturbance regimes and, especially, increasing anthropogenic land use changes, have detrimental impacts on the number and size of many plant populations, factors believed to increase their local extinction risk (Ouborg 1993; Lynch *et al.* 1995; Lienert & Fischer 2003). The perennial herb *Primula vulgaris*

Correspondence: Rein Brys, Institute of Nature Conservation, Kliniekstraat 25, B-1070 Brussels, Belgium (tel. + 32 2558 18 40; fax + 32 2558 18 05; e-mail Rein.Brys@instnat.be).

§Present address: Laboratoire de Génétique et Evolution des Populations Végétales, UMR CNRS 8016, Université de Lille1, F-59655 Villeneuve d'Ascq Cedex, France.

Huds. (Endels *et al.* 2002a) shows such a dramatic decline in Belgium, and may illustrate the processes that lead towards extinction.

Besides their decrease in area, small habitat patches are subject to deteriorating environmental conditions (edge effects) such as nutrient enrichment, changes in moisture conditions and herbicide drift, which may affect vegetation structure and composition, and consequently may result in a decrease of species diversity (Kleijn & Verbeek 2000). In *Primula vulgaris*, Whale (1984) also showed that increasing competition and decreasing light penetration through the vegetation canopy reduced flower production. The resulting decline in total diversity of insect-pollinated plant species and/or reduced flowering, may result in decreased pollinator attractiveness at many sites (Kwak *et al.* 1998).

Population demographic traits, such as population size and plant density may have a great impact on plant–animal interactions, reproduction and population dynamics (e.g. Oostermeijer *et al.* 1994; Fischer & Matthies 1998; Kéry *et al.* 2000; Jacquemyn *et al.* 2003). Small and sparse floral patches may fail to attract sufficient pollinators (Sih & Baltus 1987; Jennersten 1988; Ågren 1996; Kwak *et al.* 1998), leading to pollen limitation and reduced seed set (Bosch & Waser 1999). This process may be the cause of reduced fecundity in small populations of several insect-pollinated species, e.g. *Silene regia* (Menges 1991), *Senecio integrifolius* (Widén 1993), *Gentianella germanica* (Fischer & Matthies 1998) and *Primula elatior* (Jacquemyn *et al.* 2002). Plant density may also affect visitation rates and foraging behaviour of pollinators and, consequently, seed output (Sih & Baltus 1987; Van Treuren *et al.* 1994; Roll *et al.* 1997).

Specific traits can influence the fecundity of an individual plant. Floral display may increase pollinator approaches and prolong visiting times (Klinkhamer & de Jong 1990; Cresswell 1997), such that many-flowered plants attract more pollinators (Ohashi & Yahara 1998) and have longer visitation times, as pollinators make more flights between flowers on the same plant (Klinkhamer & de Jong 1990). However, in self-incompatible species this can be a disadvantage because of the decrease in the overall rate of cross-pollination (Andersson 1988; Klinkhamer & de Jong 1990; Cresswell 1997). It is well known that genetic traits may lower reproductive success and vigour because of increased inbreeding and genetic load, increased abortion and reduced fecundity (Barrett & Kohn 1991; Ellstrand & Elam 1993; Oostermeijer *et al.* 1996; Fischer & Matthies 1998).

Even if seed is set, the reproductive success of an individual and/or population will depend on fruit predation (García *et al.* 2001), which can dramatically decrease reproductive success and therefore may affect demographic processes (Englund 1993; Hulme 1997; García *et al.* 2001).

All these determining factors can influence the viability of plant species in small populations, particularly if growing in suboptimal, more stressful conditions.

Moreover, sustainability is even more affected for plant species showing a self-incompatible breeding system (Byers & Meagher 1992; but see Aizen *et al.* 2002). In heterostylous plants with intramorph-incompatibility, morph frequencies have frequently been found to deviate from equality in small natural populations, as in the primrose (Endels *et al.* 2002a,b). This can be due to genetic drift, founder effects or population bottlenecks (Byers & Meagher 1992; Eckert & Barrett 1992; Byers 1995; Mal & Lovett-Doust 1997; Jacquemyn *et al.* 2002). The dominant morph did not depend on population size, indicating that morph bias in small populations is the result of demographic stochasticity (Endels *et al.* 2002b).

Although several authors (e.g. Jennersten 1988; Jennersten & Nilsson 1993; Fischer & Matthies 1998; Kéry *et al.* 2000) studied the relationship between abundance of flowering plants and its effect on plant fitness, little is known about the impact of limited mate availability on the reproductive success of populations (Byers & Meagher 1992). Moreover, biased morph frequencies may interact with the limited pollinator attraction observed in small populations to further decrease seed set (Byers 1995).

We investigated reproductive and vegetative components at the population as well as the individual plant level, in relation to population size, mate availability (morph bias), morph type, plant density and predation in populations of *P. vulgaris*. Only 89 populations remain in Belgium, and the species is almost completely restricted to small landscape elements (Endels *et al.* 2002a). Data from a long-term study showed that both the number and size of primrose populations has decreased dramatically during the last 14 years as a result of intensive agricultural land-use, in particular the conversion of permanent pastures to arable fields and the destruction of the ecological network of ditches, verges and small forest patches (Endels *et al.* 2002a). This self-incompatible, distylous species therefore provides great opportunities to study reproductive success and plant performance in remnant natural populations, as well as to test the hypothesis that the reproductive output of small populations with a biased morph frequency will be particularly sensitive.

More specifically, we address the following questions:

- Does adjacent land use influence vegetative and reproductive characteristics of *Primula vulgaris*, as well as demographic traits (Endels *et al.* 2002a)?
- Does reproductive success increase with (i) increasing population size and (ii) increasing plant density?
- Are there differences in reproductive characteristics between the two morph types, and if so, can these be related to population size?
- Do biased morph frequencies affect fruit and seed set?
- How do plant traits (plant size, floral display) affect reproductive success?
- Does fruit density influence pre-dispersal fruit predation at plant and population level, and does predation in turn affect reproductive success?

Methods

STUDY SPECIES

P. vulgaris (Primulaceae), primrose, is a perennial herb of moist, shaded habitats, with a North Atlantic and Mediterranean distribution (Hegi 1975; Whale 1984; Hultén & Fries 1986). In England, the species mostly grows in woodland (Whale 1984; Valverde & Silvertown 1995), but it can also be found in hedges and old grasslands that are protected from drought (Valentine 1948; Rackham 1980). In Flanders (Belgium), primrose is mainly confined to moist ditch banks, road verges, hedgerows, forest edges and woodland (Endels *et al.* 2002a).

Flowers appear in early spring (February to April); they are pale yellow, borne on separate stalks. The species is distylous, the genes controlling distyly being linked to a sporophytic incompatibility system as a super-gene (Dowrick 1956). Pin plants are the recessive (ss) and thrum plants the heterozygous (Ss) genotype (Richards 1986). Because the incompatibility mechanism works on the principle of self-rejection, seed set in *P. vulgaris* results from between-morph (legitimate) crosses (thrum \times pin and pin \times thrum) (Richards 1986). Disassortive mating is promoted by the different position of anthers and stigmas in the two morphs (pin plants are long-styled and their anthers are near the base of the corolla, whereas in thrum plants the anthers are positioned above the short style). The pollinators of *P. vulgaris* are mainly long-probed insects (bumbees, *Bombylus* species and moths), but also pollen-gathering bees (Woodell 1960) and its relatively early flowering period means that there are few other food plants available for nectar- and pollen-feeding insects. *P. vulgaris* is

myrmecochorous, because of the presence of an elaiosome, but occasionally seeds can be dispersed further by small mammals, mainly rodents (Valverde & Silvertown 1995). However, dispersal remains quite restricted and colonization of new habitats is a rare event. As a consequence, primrose clearly shows a local, clumped distribution pattern. The plant overwinters as a green rosette and vegetative spread occurs, albeit within very short distances, through the production of lateral rosettes. Although individual rosettes can die off, individual plants are relatively long-lived (10–30 years) (Boyd *et al.* 1990).

LOCATION OF THE STUDIED POPULATIONS
AND POPULATION CHARACTERISTICS
(HABITAT, SIZE, MORPH BIAS AND
PLANT DENSITY)

A total of 16 populations located in the vicinity of Bruges (northern Belgium) were studied. Groups of plants separated by 100 m or more from the next conspecifics were considered as separate populations. Most populations lay at least several hundred metres apart.

Following Endels *et al.* (2002a), who analysed the occurrence of *P. vulgaris* in Belgium, populations were divided into three groups, depending on whether they were found in the verges of pastures ($n = 6$), the verges of arable fields ($n = 5$) or forests ($n = 5$) (Table 1).

Population size was determined as the number of flowering individuals and ranged from 1 to 700 flowering individuals (Table 1).

The number of pin and thrum individuals was counted in 1999 for all populations. Morph bias was calculated for each population as the absolute value of the difference in number of individuals of the two morphs,

Table 1 Summary data for the 16 studied populations of *P. vulgaris*

Population	Habitat type	Population size*	Plant density†	Morph bias‡
1 Ten Torre Kasteel 1	Pasture	1		1.00 +
2 Ten Torre Kasteel 2	Forest	4	4.2	0.50 –
3 Vakeleiel	Arable field	13	7.8	0.15 +
4 Ten Torre Kasteel 3	Pasture	16	1.6	0.50 –
5 Donk West	Arable field	24	3.5	0.01 +
6 Noordermeers	Forest	29	17.1	0.10 –
7 Eyck ter Schans	Forest	35	8.1	0.17 –
8 Damme Golf	Arable field	48	2.2	0.67 +
9 Dale	Arable field	66	7.4	0.08 –
10 Ziltedreef 1	Arable field	97	11.8	0.03 –
11 Kerkhof Donk	Forest	104	17.8	0.07 –
12 Ziltedreef 2	Pasture	131	26.0	0.05 +
13 Zandberghoeve 1	Pasture	137	7.4	0.13 –
14 Vakeleie 2	Pasture	145	24.9	0.03 +
15 Zandberghoeve 2	Pasture	317	25.6	0.03 +
16 Merkenvelde	Forest	700	15.2	0.02 –

*Number of flowering individuals per population.

†Number of flowering plants per m².

‡| Number of pin individuals – number of thrum individuals |/total flowering individuals.

– = Pin individuals in minority.

+ = Pin individuals in majority.

divided by the total number of flowering plants (Table 1). It can vary from 0 (both morph types in equal frequency) to 1 (only one of the two morph types present). It was impossible to determine the morph type of some individuals, mostly due to damage by grazing, and the calculated morph bias of some populations was therefore slightly different from the true field value.

Population density ($N\ m^{-2}$) was calculated by dividing population size (number of flowering individuals) by area (m^2) (Table 1). Because almost all populations were growing in linear landscape elements (ditch banks and verges), population area was measured as the product of length and width of the population.

SAMPLING PROCEDURE AND DETERMINATION OF VEGETATIVE AND REPRODUCTIVE COMPONENTS

In each population 20 flowering rosettes were randomly chosen and individually marked, resulting in a total of 265 plants.

In April 2000, we measured plant size (circumference of all rosettes, in cm), morph type, the number of flowers (floral display) and leaves, and the length and width of the three longest leaves on each plant.

All locations were visited again at fruiting time (June), and the number of initiated fruits (capsules) and predated fruits was counted for each individual. Fruit capsules could generally be relocated quite accurately, because rodent predation leaves an empty damaged fruit capsule, still hanging on the flower stalk, and other predators leave a well-developed flower stalk. If plants were damaged by grazing and/or anthropogenic disturbance, they were omitted from further analysis.

From each plant, five mature, unopened fruits were collected; their seeds were counted and weighed to the nearest 0.001 g. Total number of seeds per plant was calculated as the average number of seeds per fruit multiplied by the total number of fruits per plant. Mean seed mass was calculated as the total seed mass of five collected fruits divided by the number of seeds. Total seed mass was calculated as the total number of seeds per plant (number of fruits per plant multiplied by the mean number of seeds per fruit) multiplied by mean seed mass. Components of reproductive success were determined as the percentage of flowers that developed into fruits (capsules), number of seeds per fruit, total seeds per plant, mean seed mass and total seed mass.

DATA ANALYSIS

The effects of habitat type (pasture, arable field and forest populations) on reproductive and vegetative characteristics were tested using a nested ANOVA model with habitat type as a fixed factor and population as a random factor nested in habitat type (Neter *et al.* 1996).

The relationship between population characteristics and reproductive output was assessed using mixed-model regression. The number of flowers and fruits per

plant, fruits per flower, number of seeds per fruit, seeds per plant, mean seed mass and total seed mass per plant were entered as dependent variables. Plant size, floral display and population characteristics (such as population size, morph bias and plant density) were entered as fixed effects, while population ID was entered as a random factor. Population size and morph bias turned out to be correlated but as this was only due to three small populations with a strong morph bias (≥ 0.5), the analysis was repeated with these populations omitted.

Differences between morph types for the number of flowers and fruits per plant, fruits per flower and seeds per fruit were analysed with a mixed-model ANCOVA. Morph type as a fixed effect was tested against the morph-by-population size interaction, while population ID was entered as a random factor.

We expect that large biased morph frequencies might reflect a lower proportion of flowers actually fertilized and lower seed set per fruit in individuals of the more common morph type, especially if populations are small. The three populations that had a morph bias ≥ 0.5 were used to investigate if mate availability affected reproductive success. A mixed-model ANCOVA was used to test whether the proportion of flowers setting fruit and the number of seeds per fruit depended on morph type abundance. Morph type (minority and majority) and the number of flowers (covariate) were entered as fixed factors, while population ID was entered as a random factor. The proportion of flowers setting fruit was based on total fruit number per plant divided by the number of flowers produced.

To analyse the influence of floral display (number of flowers per plant) on the proportion of flowers setting fruit and the total number of fruits per plant, we performed further mixed-model regressions. Population size and population ID were entered as covariate and a random factor, respectively.

Mixed-model regression was also applied to analyse the relationship between the number and proportion of predated fruits per plant on the one hand and plant density, the number of fruits per plant (independent variables) and their interaction (fixed effect) on the other. Population ID was again entered as a random factor.

Variables were transformed (log or square root) if necessary to achieve normality and homoscedasticity of residuals.

Mixed-model regressions and ANCOVA were analysed using proc MIXED in SAS 8.02 (Littell *et al.* 1996). The degrees of freedom of the fixed effects *F*-test were adjusted for statistical dependence using Satterthwaite formulas. Variance components were estimated by restricted maximum likelihood (REML). Even in the situation when both variables (X and Y) were subject to an error (measurement and/or biological error), Model I regression was used, because an unambiguous and optimal Model II strategy is still an open question (Sokal & Rohlf 1995). Univariate linear and non-linear regressions were analysed using Statistica (Version 6). The nested ANOVA model was fitted in SPSS (Version 10.0).

Table 2 Overall mean (\pm SE) of vegetative and reproductive characteristics in 16 populations of *P. vulgaris*

	<i>n</i>	Mean (\pm SE)
Vegetative variable		
Plant circumference (cm)	265	15.3 \pm 11.3
Number of leaves	265	31.1 \pm 25.6
Leaf length (cm)	265	14.9 \pm 4.2
Leaf width (cm)	265	4.6 \pm 1.0
Reproductive variable		
Flowers per plant	265	30.7 \pm 30.1
Flowers setting fruit	221	0.35 \pm 0.30
Fruits per plant	229	5.4 \pm 8.5
Seeds per fruit	181	31.8 \pm 18.1
Seeds per plant	221	194.5 \pm 424.1
Mean seed mass (mg)	175	1.5 \pm 0.6
Seed mass per fruit (mg)	175	46.4 \pm 28.0
Total seed mass per plant (mg)	174	346.8 \pm 671.1

Results

Although 50% of the studied populations was smaller than 50 individuals, main reproductive components (number of flowers, fruits and seeds per plant) were quite high (Table 2). Few of the reproductive and vegetative components differed between the different habitat types, but all were significantly different among populations within habitat types ($P < 0.05$). Only in the case of mean seed mass and mean leaf length was there a significant habitat effect (ANOVA; $F_{1,12} = 4.08$, $P = 0.040$; $F_{1,13} = 202.27$, $P = 0.021$). Seeds produced by individuals growing in arable field populations were significantly lighter (1.3 mg) compared with seeds from pasture and forest populations (1.5 and 1.6 mg, respectively). Plants growing in pastures produced longer leaves (16.3 cm) than those in forest and arable field populations (14.2 and 13.5 cm, respectively).

The number of flowers per plant was mainly determined by plant size (measured as circumference) and population size (Table 3). Large individuals and plants from large populations developed more flowers. There was, however, no relationship between plant size and population size ($F_{1,13.7} = 6.02$, $P = 0.124$). A much higher proportion of flowers developed into fruits in large than in small populations (Table 3, Fig. 1b), and consequently the number of fruits per plant was higher (Table 3, Fig. 1a). Both reproductive components were also significantly positively influenced by floral display (Table 3). Seed production per fruit significantly increased with population size (Table 3, Fig. 1c), floral display and leaf length (Table 3). As a result, the number of seeds per plant was also significantly and positively dependent on plant and population size (Table 3, Fig. 1d). Plants growing in large populations produced more seeds than individuals in small populations. Total seed mass per plant only increased with plant size, and mean leaf length had a positive impact on mean seed mass (Table 3). The impact of population size on fruit and seed set was still significant ($P < 0.05$),

Table 3 Mixed model regression analysis of population variables (population size^a, plant density^b, morph bias^c) and vegetative variables (plant size^d, leaf length^e) on seven parameters of reproductive success in *P. vulgaris*. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

Variable	Parameter estimate ^f	d.f.	<i>F</i> -value
Flowers per plant ($n = 265$)			
Plant size	1.124	12.3	1154.97***
Population size	0.078	13.7	5.14*
Fruits per flower ($n = 229$)			
Number of flowers	-0.336	9.45	9.24*
Population size	0.251	15	4.44*
Fruits per plant ($n = 229$)			
Number of flowers	0.411	10	17.08**
Population size	0.221	13.4	3.37*
Seeds per plant ($n = 185$)			
Plant size	0.002	12.5	9.58**
Population size	0.157	9.83	3.41*
Seeds per fruit ($n = 181$)			
Number of flowers	-0.007	13.2	3.50*
Population size [†]	0.179	7.14	5.85*
Mean leaf length	0.014	89.1	5.01*
Total seed mass ($n = 174$)			
Plant size	570.36	95.7	12.14***
Mean seed mass ($n = 175$)			
Mean leaf length	0.865	164	5.59**

^aLog(total flowering plants).

^bLog(flowering plants m⁻²).

^c[(number thrum plants - number pin plants) / total flowering plants].

^dLog(plant circumference).

^eLog(mean length of the three longest leaves).

^fParameter estimates were derived from the reduced model with only variables that had a significant effect.

[†]Not significant when three populations with the strongest morph bias were omitted.

except for the number of seeds per fruit, if three populations with a morph bias ≥ 0.5 were omitted from the analysis.

Of the 265 selected plants over the 16 populations, 122 individuals were pin and 143 were thrum plants. Pin plants did not differ significantly from thrum individuals in any of the four reproductive characteristics studied and no interaction was found with population size (Table 4).

In the three populations with a morph bias ≥ 0.5 , individuals of the minority morph type had a significantly higher proportion of flowers setting fruit and produced a significantly larger number of seeds per fruit than the majority morph type (Table 5). The number of seeds per fruit was significantly and positively affected by the number of flowers per plant (Table 5).

At the individual plant level, the proportion of flowers that developed successfully into fruits decreased significantly with floral display ($F_{1,6.56} = 15.08$, $P = 0.007$, Fig. 2a). However, net fruit production per plant significantly increased with floral display ($F_{1,13.1} =$

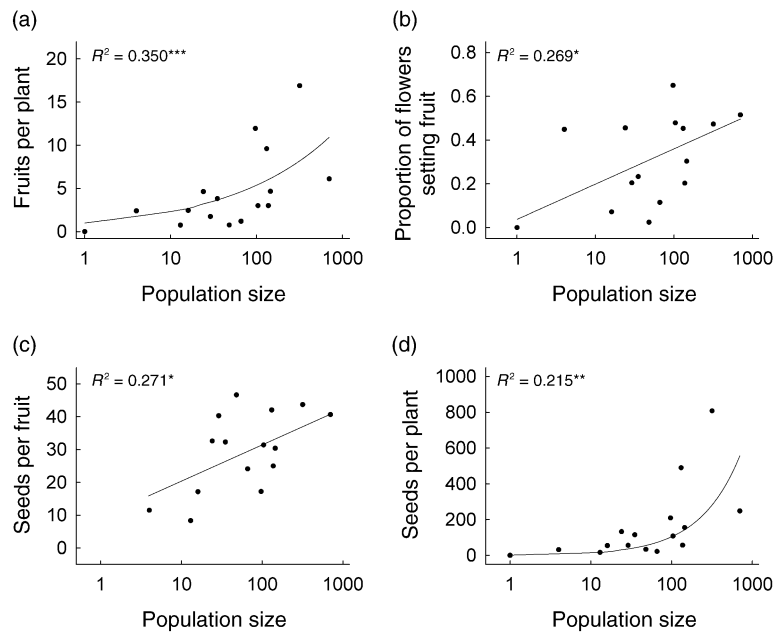


Fig. 1 Relationship between log population size and (a) mean number of fruits per plant ($Y = e^{(0.84 \times X)}$), (b) mean proportion of flowers setting fruit ($Y = 0.09 + 0.13 \times X$), (c) mean number of seeds per fruit ($Y = 10.59 + 10.58 \times X$) and (d) mean number of seeds per plant flower ($Y = 1.98 \times e^{(1.98 \times X)}$) in *P. vulgaris*. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Table 4 Mixed model ANCOVA of the effects of morph type and the interaction of morph type and population size on four parameters of reproductive success in *P. vulgaris*

Effect	F-value	d.f.	P
Flowers per plant ($n = 265$)			
Morph	2.64	1, 11.6	0.131
Morph \times Population size	3.36	2, 2.65	0.188
Fruits per flower ($n = 229$)			
Morph	0.24	1, 15.6	0.628
Morph \times Population size	0.84	2, 13.6	0.454
Fruits per plant ($n = 229$)			
Morph	0.12	1, 15.9	0.731
Morph \times Population size	3.20	2, 15.6	0.069
Seeds per fruit ($n = 181$)			
Morph	0.02	1, 179	0.891
Morph \times Population size	1.90	2, 26.6	0.169

Table 5 Mixed model ANCOVA of the effects of morph bias (morph type in minority and morph type in majority) and the number of flowers on the proportion of flowers setting fruit and the number of seeds per fruit in three populations with a morph bias equal to or larger than 1 : 3 in *P. vulgaris*

Effect	F-value	d.f.	P
Fruits per flower ($n = 31$)			
Morph type (minority vs. majority)	62.03	1, 25.7	< 0.001
Number of flowers	0.46	1, 1.94	0.568
Seeds per fruit ($n = 17$)			
Morph type (minority vs. majority)	5.67	1, 12.3	0.034
Number of flowers	10.60	1, 12.4	0.007

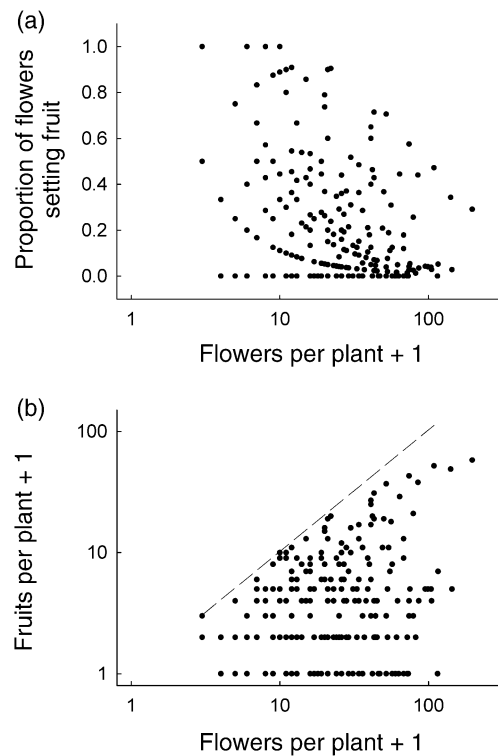


Fig. 2 The effect of floral display (log(number of flowers per plant + 1)) on (a) the number of fruits per flower ($Y = 0.57 - 0.24 \times X$) and (b) number of fruits per plant ($Y = 0.17 + 0.31 \times X$) in *P. vulgaris*. The dotted line represents a regression slope = 1.

16.17, $P = 0.001$, Fig. 2b). No significant interaction was found between floral display or population size and either reproduction component (respectively $F_{1,10.3} = 0.63$, $P = 0.445$ and $F_{1,29.4} = 1.61$, $P = 0.214$).

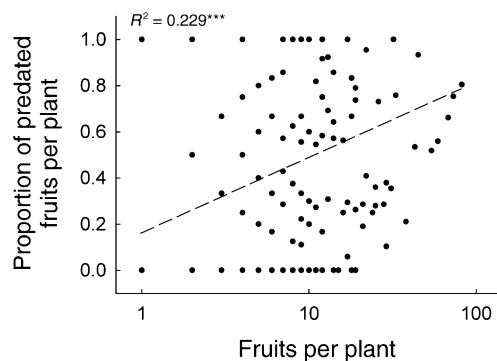


Fig. 3 Relationship between log fruits per plant and the proportion of predated fruits per plant ($Y = 0.02 + 0.39 \times X$) in *P. vulgaris*. *** $P < 0.0001$.

Although, net fruit predation was not related to either density ($F_{1,30.7} = 2.23$, $P = 0.146$) or fruits per plant ($F_{1,217} = 3.58$, $P = 0.059$), it was significantly affected by their interaction ($F_{1,217} = 13.58$, $P < 0.001$). Fruit predation thus increased significantly with the number of fruits per plant when the density of those plants increased. When the proportion of fruits predated per plant was considered, the total number of fruits had a significant impact ($F_{1,217} = 17.52$, $P < 0.001$, Fig. 3), but density ($F_{1,17.2} = 0.96$, $P = 0.340$) and interaction did not ($F_{1,217} = 0.22$, $P = 0.637$).

Discussion

Although habitat effects have been observed on population characteristics of this species (Endels *et al.* 2002a), they were not detected for most of the reproductive and vegetative components investigated in the present study. Furthermore, plants in Flanders produced twice as many flowers, fruits and seeds per plant, compared with plants growing in Buckinghamshire (Valverde & Silvertown 1995). Most populations in Flanders occur in small landscape elements (such as ditch banks, road verges and hedgerows) near pastures and arable fields. Pronounced differences in light and nutrient conditions at these locations compared with the environmental conditions in *Fraxinus excelsior*–*Acer campestre*–*Mercurialis perennis* woodland in England may therefore explain the observed difference in flower and seed production.

Population size effects appear to underlie the reduced reproductive success in the studied populations of *P. vulgaris*. Several lines of evidence suggest that this is driven by alterations in demographic factors and pollination success in small and sparse populations. Bumblebees, which are important pollinators for primrose (Woodell 1960), have been shown to fertilize higher proportions of flowers in larger and more dense populations (Sih & Baltus 1987; Jennersten 1988; Rathcke & Jules 1993). Insufficient quantity and quality of pollen may therefore reduce the number of fruits per plant and seeds per fruit (Klinkhamer & de Jong 1990; Cresswell

1997; Kwak *et al.* 1998; Mustajärvi *et al.* 2001). The fact that small populations often grow under deteriorating site conditions (Jacquemyn *et al.* 2003) may also contribute (Vergeer *et al.* 2003a,b).

In intramorph incompatible, distylous plants, populations with a biased morph frequency may experience reduced reproduction (Byers & Meagher 1992). Although heterostyly was thought to have evolved as a means of avoiding inbreeding and of ensuring the effective exchange of pollen between different mating types (Barrett 1992), it may result in a lower fruit and seed set when morph frequencies move away from 50% (Byers & Meagher 1992). Our results indicate reduced availability of compatible pollen for stigmas of the majority morph type in strongly biased populations. This is in agreement with findings of Kéry *et al.* (2003), that an increasing morph bias in the related *Primula veris* negatively affected reproductive success. Similar patterns of decreased seed set and of higher variance among plants in the proportion of flowers setting fruit in smaller populations were observed in *Eupatorium resinosum* (Byers & Meagher 1992) and *Primula elatior* (Jacquemyn *et al.* 2002). Furthermore, we found no differences in the reproductive characteristics of the two morph types and no interaction was observed with population size. This is consistent with studies on the related *P. elatior* (Jacquemyn *et al.* 2001) and *P. veris* (Kéry *et al.* 2003), and on other heterostylous plants (e.g. Husband & Barrett 1992; Mal & Lovett-Doust 1997). Hence, we may conclude that the negative effect of population size on the process of reproduction can be stronger if morph frequency is significantly skewed.

Finally, genetic factors, such as inbreeding, may also contribute to reduced reproduction in small populations (Soulé 1986). In contrast to results found for several self-compatible species (Oostermeijer *et al.* 1994; Van Treuren *et al.* 1994; Heschel & Paige 1995; Fischer & Matthies 1998), an increased frequency of close inbreeding is unlikely to cause lower fecundity in *P. vulgaris*, as primrose is an obligate out-crosser, and self-pollination cannot occur or is very restricted in pin morphs (Woodell 1960; Richards 1986). This most extreme form of inbreeding is probably a very rare event, and only biparental inbreeding is likely to occur in the field. A study of allozyme variation and structure of populations from the same region showed no relationship between Wright's inbreeding coefficient (F_{IS}) and population size or plant density (Van Rossum *et al.*, unpublished results).

Within populations, individual plants varied tremendously in size and floral display. Although one may expect that increasing floral display might result in increased reproductive success, it appeared that such investment had significant costs. The optimal floral display, in terms of successful development of fruits on a per-flower basis, decreased significantly with increasing floral display. Individual flowers may thus have a lower probability of being successfully pollinated on

large plants than on small ones, suggesting that differences in pollinator service may be the causal factor. Previous studies have shown that pollinator visitation rates and visit durations were positively related to floral display (Mitchell 1994; Brody & Mitchell 1997; Murren 2002). Although individual bumblebees usually visit a larger total number of flowers per plant and remain longer on large plants (Waser & Price 1984; Geber 1985; Ohashi & Yahara 1998), the proportion of flowers visited decreases with plant size (Andersson 1988; Klinkhamer *et al.* 1989). If a pollinator is assumed to deposit cross-pollen only on the first few flowers, the chances of cross-pollination will decrease with increasing floral display. As a consequence, the 'effective' visitation rate per flower decreases very rapidly with size and hence the optimal plant size is expected to be small (Andersson 1988).

Furthermore, large floral displays are likely to attract other visitors, such as pre-dispersal fruit predators (Zimmerman & Pyke 1988; Brody & Mitchell 1997; García *et al.* 2001), which might modulate the effects of floral display on pollination success. In *P. vulgaris*, Valverde & Silvertown (1995) showed that the number of fruits per plant affected pre-dispersal fruit predation by small rodents. Analogously, proportional fruit predation here was significantly larger in plants with large displays, while there appeared a significant interaction between plant density and the total number of fruits produced per plant on the absolute number of predated fruits per plant. Similar results were found by Englund (1993) and García *et al.* (2001) in *Viburnum opulus* and *Juniperus communis*, respectively.

However, the positive effects gained through increased fruit and seed set are not entirely counteracted by the negative effects of higher losses expressed on a per-flower and per-fruit basis. It can be misleading to express reproductive success on a per-flower or per-fruit basis, as selection responds to the total reproduction of a plant. During a 4-year study, Endels *et al.* (unpublished results) found that neither floral display nor total fruit production affected future flowering performance or survival in *P. vulgaris*. This suggests that the costs of reproduction due to high floral displays and increased seed production will not reduce future survival and flowering rates. Furthermore, initiating a large number of flowers may allow plants to selectively abort fruits and seeds and thereby increase the average quality of the remaining offspring, as has been shown in *Lotus corniculatus* (Stephenson & Winsor 1986) and *Cynoglossum officinale* (Melser & Klinkhamer 2001). Even if flowers fail to set fruit, they may still function as pollen donors and thereby increase the number of seeds that a plant sires (Stanton *et al.* 1986).

A better understanding of the relationship between population characteristics and reproductive components is essential for effective conservation and management of rare and threatened plant species. While most previous studies of small populations have focused on isolation, population size and genetics, the

breeding system of a plant may also have an impact on seed set and therefore influence minimal viable population size estimates and management strategies (Aspinwall & Christian 1992; DeMauro 1993; Husband & Barrett 1996; Luijten *et al.* 2000). However, further experimental studies are needed to separate the effects of population size and morph bias and their interaction on the reproductive output of a population.

Acknowledgements

The authors wish to thank Dr P. Klinkhamer, Dr L. Haddon and two anonymous referees for their well-founded and constructive comments on an earlier draft of the manuscript. We are grateful to Dirk Bauwens for useful comments and help with statistical problems. This study was carried out as part of a VLINA (Flemish Impulse Programme for Nature Development) project (98/03); R. Brys has been supported by a IWT (Institute for Science and Technology) PhD grant.

References

- Ågren, J. (1996) Population size, pollinator limitation, and seed set in the self-incompatible herb *Lythrum salicaria*. *Ecology*, **77**, 1779–1790.
- Aizen, M.A., Ashworth, L. & Galetto, L. (2002) Reproductive success in fragmented habitats: do compatibility systems and pollination specialization matter? *Journal of Vegetation Science*, **13**, 885–892.
- Andersson, S. (1988) Size-dependent pollination efficiency in *Anchusa officinalis* (Boraginaceae): causes and consequences. *Oecologia*, **76**, 125–150.
- Aspinwall, N. & Christian, T. (1992) Pollination biology, seed production, and population structure in queen-of-the-prairie, *Filipendula rubra* (Rosaceae) at Botkin Fen, Missouri. *American Journal of Botany*, **79**, 488–494.
- Barrett, S.C.H. (1992) Heterostylous genetic polymorphisms: model systems for evolutionary analysis. *Evolution and Function of Heterostyly* (ed. S.C.H. Barrett), pp. 1–29. Springer-Verlag, Berlin.
- Barrett, S.C.H. & Kohn, J.R. (1991) Genetic and evolutionary consequences of small population size in plants: implication for conservation. *Genetics and Conservation of Rare Plants* (eds D.A. Falk & K.E. Holsinger), pp. 3–30. Oxford University Press, New York.
- Bosch, M. & Waser, N.M. (1999) Effects of local density on pollination and reproduction in *Delphinium nuttallianum* and *Aconitum columbianum* (Ranunculaceae). *American Journal of Botany*, **86**, 871–879.
- Boyd, M., Silvertown, J. & Tucker, C. (1990) Population ecology of heterostyle and homostyle *Primula vulgaris*: growth, survival and reproduction in field populations. *Journal of Ecology*, **78**, 799–813.
- Brody, A.K. & Mitchell, R.J. (1997) Effects of experimental manipulation of inflorescence size on pollination and pre-dispersal fruit predation in the hummingbird-pollinated plant *Ipomopsis aggregata*. *Oecologia*, **110**, 86–93.
- Burgess, R.L. (1988) Community organisation: effects of landscape fragmentation. *Canadian Journal of Botany*, **66**, 2687–2690.
- Byers, D.L. (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, D.L. & Meagher, T.R. (1992) Mate availability in small populations of plant species with homomorphic sporophytic self-incompatibility. *Heredity*, **68**, 353–359.
- Cresswell, J.E. (1997) Spatial heterogeneity, pollinator behavior and pollinator-mediated gene flow: bumblebee movements in variously aggregated rows of oil-seed rape. *Oikos*, **78**, 546–556.
- DeMauro, M.M. (1993) Relationship of breeding system to rarity in the lakeside daisy (*Hymenoxys acaulis* var. *glabra*). *Conservation Biology*, **7**, 542–550.
- Dowrick, V.P.J. (1956) Heterostyly and homostyly in *Primula obconica*. *Heredity*, **10**, 219–236.
- Eckert, C.G. & Barrett, S.C.H. (1992) Stochastic loss of style morphs from populations of tristylous *Lythrum salicaria* and *Decodon verticillatus* (Lythraceae). *Evolution*, **46**, 1014–1029.
- Ellstrand, N.C. & Elam, D.R. (1993) Population genetic consequences of small population size: implications for plant conservation. *Annual Review of Ecology and Systematics*, **24**, 217–242.
- Endels, P., Jacquemyn, H., Brys, R. & Hermy, M. (2002b) Changes in pin-thrum ratios in populations of the heterostyle *Primula vulgaris* Huds. Does imbalance affect population persistence? *Flora*, **197**, 326–331.
- Endels, P., Jacquemyn, H., Brys, R., Hermy, M. & De Blust, G. (2002a) Temporal changes (1986–99) in populations of primrose (*Primula vulgaris* Huds.) in an agricultural landscape and implications for conservation. *Biological Conservation*, **105**, 11–25.
- Englund, R. (1993) Fruit removal in *Viburnum opulus*: copious seed predation and sporadic massive seed dispersal in a temperate shrub. *Oikos*, **67**, 503–510.
- Fischer, M. & Matthies, D. (1998) Effects of population size on performance in the rare plant *Gentianella germanica*. *Journal of Ecology*, **86**, 195–204.
- García, D., Zamora, R., Gómez, J.M. & Hódar, J.A. (2001) Frugivory at *Juniperus communis* depends more on population characteristics than on individual attributes. *Journal of Ecology*, **89**, 639–647.
- Geber, M.A. (1985) The relationship of plant size to self-pollination in *Mertensia ciliata*. *Ecology*, **66**, 762–772.
- Hegi, G. (1975) *Illustrierte Flora Von Mittel-Europa*. 5. Band: *Dicotyledones*. P. Parey, Berlin.
- Heschel, M.S. & Paige, K.N. (1995) Inbreeding depression, environmental stress, and population size variation in scarlet gilia (*Ipomopsis aggregata*). *Conservation Biology*, **9**, 126–133.
- Hulme, P.E. (1997) Post dispersal seed predation and the establishment of vertebrate dispersed plants in Mediterranean scrublands. *Oecologia*, **111**, 91–98.
- Hultén, E. & Fries, M. (1986) *Atlas of North European Vascular Plants of the Tropic of Cancer*. Koeltz Scientific Books, Königstein.
- Husband, B.C. & Barrett, S.C.H. (1992) Pollinator visitation in populations of tristylous *Eichhornia paniculata* in northern Brazil. *Oecologia*, **89**, 365–371.
- Husband, B.C. & Barrett, S.C.H. (1996) A metapopulation perspective in plant population biology. *Journal of Ecology*, **84**, 461–469.
- Jacquemyn, H., Brys, R. & Hermy, M. (2001) Within and between plant variation in seed number, seed mass and germinability of *Primula elatior*: effect of population size. *Plant Biology*, **3**, 561–568.
- Jacquemyn, H., Brys, R. & Hermy, M. (2002) Patch occupancy, population size and reproductive success of a forest herb (*Primula elatior*) in a fragmented landscape. *Oecologia*, **130**, 617–625.
- Jacquemyn, H., Van Rossum, F., Brys, R., Endels, P., Hermy, M., Triest, L. *et al.* (2003) Effects of agricultural land use and fragmentation on genetics, demography and population persistence of the rare *Primula vulgaris*, and implications for conservation. *Belgian Journal of Botany*, **136**, 5–22.
- Jennersten, O. (1988) Pollination in *Dianthus deltoides* (Caryophyllaceae): effects of habitat fragmentation on visitation and seed set. *Conservation Biology*, **2**, 359–366.
- Jennersten, O. & Nilsson, S.G. (1993) Insect flower visitation frequency and seed production in relation to patch size of *Viscaria vulgaris* (Caryophyllaceae). *Oikos*, **68**, 283–292.
- Kéry, M., Matthies, D. & Schmid, B. (2003) Demographic stochasticity in population fragments of the declining distylous perennial *Primula veris* (Primulaceae). *Basic and Applied Ecology*, **4**, 197–206.
- Kéry, M., Matthies, D. & Spillmann, H.H. (2000) Reduced fecundity and offspring performance in small populations of the declining grassland plants *Primula veris* and *Gentiana lutea*. *Journal of Ecology*, **88**, 17–30.
- Kleijn, D. & Verbeek, M. (2000) Factors affecting the species composition of arable field boundary vegetation. *Journal of Applied Ecology*, **37**, 256–266.
- Klinkhamer, P.G.L. & de Jong, T.J. (1990) Effects of plant size, plant density and sex differential nectar reward on pollinator visitation in the protandrous *Echium vulgare* (Boraginaceae). *Oikos*, **57**, 399–405.
- Klinkhamer, P.G.L., de Jong, T.J. & de Bruijn, G.J. (1989) Plant size and pollinator visitation in *Cynoglossum officinale*. *Oikos*, **54**, 201–204.
- Kwak, M.M., Velterop, O. & van Andel, J. (1998) Pollen and gene flow in fragmented habitats. *Applied Vegetation Science*, **1**, 37–54.
- Lienert, J. & Fischer, M. (2003) Habitat fragmentation affects the common wetland specialist *Primula farinosa* in NE Switzerland. *Journal of Ecology*, **91**, 587–599.
- Littell, R.C., Milliken, G.A., Stroup, W.W. & Wolfinger, R.D. (1996) *SAS System for Mixed Models*. SAS Institute, Cary, North Carolina.
- Luijten, S.H., Dierick, A., Oostermeijer, G.J.B., Raijmann, L.E.L. & Den Nijs, H.C. (2000) Population size, genetic variation, and reproductive success in a rapidly declining, self-incompatible perennial (*Arnica montana*) in The Netherlands. *Conservation Biology*, **14**, 1776–1787.
- Lynch, M., Corney, J. & Bürger, R. (1995) Mutation accumulation and the extinction of small populations. *American Naturalist*, **146**, 489–518.
- Mal, K.M. & Lovett-Doust, J. (1997) Morph frequencies and floral variation in a heterostylous colonizing weed, *Lythrum salicaria*. *Canadian Journal of Botany*, **75**, 1034–1045.
- Melser, C. & Klinkhamer, P.G.L. (2001) Selective seed abortion increases offspring survival in *Cynoglossum officinale* (Boraginaceae). *American Journal of Botany*, **88**, 1033–1040.
- Menges, E.S. (1991) Seed germination percentage increases with population size in a fragmented prairie species. *Conservation Biology*, **5**, 158–164.
- Mitchell, R.J. (1994) Effects of floral traits, pollinator visitation, and plant size on *Ipomopsis aggregata* fruit production. *American Naturalist*, **143**, 870–889.
- Murren, C.J. (2002) Effects of habitat fragmentation on pollination: pollinators, pollinia viability and reproductive success. *Journal of Ecology*, **90**, 100–107.
- Mustajärvi, K., Siikamäki, 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.
- Neter, J., Kunter, M.H., Nachtsheim, C.J. & Wasserman, W. (1996) *Applied Linear Statistical Models*, 4th edn. Irwin, Illinois.
- Ohashi, K. & Yahara, T. (1998) Effects of variation in flower number on pollinator visits in *Cirsium purpuratum* (Asteraceae). *American Journal of Botany*, **85**, 219–224.

- Oostermeijer, J.G.B., Berholz, A. & Poschlod, P. (1996) Genetical aspects of fragmented plant populations. *Species Survival in Fragmented Landscapes* (eds J. Settele, C.R. Margules, P. Poschlod & K. Henle), pp. 93–101. Kluwer Academic, Dordrecht, The Netherlands.
- Oostermeijer, J.G.B., van Eijck, M.W. & den Nijs, J.C.M. (1994) Offspring fitness in relation to population size and genetic variation in the rare perennial plant species *Gentiana pneumonanthe* (Gentianaceae). *Oecologia*, **97**, 289–296.
- Ouborg, N.J. (1993) Isolation, population size and extinction: the classical and metapopulation approaches applied to vascular plants along the Dutch Rhine-system. *Oikos*, **66**, 298–308.
- Rackham, O. (1980) *Ancient Woodland – its History, Vegetation and Uses in England*. Edward Arnold, London.
- Rathcke, B.J. & Jules, E.S. (1993) Habitat fragmentation and plant–pollinator interactions. *Current Science*, **65**, 273–277.
- Richards, A.J. (1986) *Plant Breeding Systems*. George Allen & Unwin, London.
- Roll, J., Mitchell, R.J., Cabin, R.J. & Marshall, D.L. (1997) Reproductive success increases with local density of conspecifics in a desert mustard (*Lesquerella fendleri*). *Conservation Biology*, **11**, 738–746.
- Saunders, D.A., Hobbs, R.J. & Margules, C.R. (1991) Biological consequences of ecosystem fragmentation. *Conservation Biology*, **5**, 18–32.
- Sih, A. & Baltus, M. (1987) Patch size, pollinator behavior, and pollinator limitation in catnip. *Ecology*, **68**, 1679–1690.
- Sokal, R. & Rohlf, F.J. (1995) *Biometry. The Principles and Practice of Statistics in Biological Research*. W.H. Freeman, New York.
- Soulé, M.E. (1986) *Conservation Biology: the Science of Scarcity and Diversity*. Sinauer Associates, Sunderland, Massachusetts.
- Stanton, M.L., Snow, A.A. & Handel, S.N. (1986) Floral evolution: attractiveness to pollinators increases male fitness. *Science*, **232**, 1625–1627.
- Stephenson, A.G. & Winsor, J.A. (1986) *Lotus corniculatus* regulates offspring quality through selective fruit abortion. *Evolution*, **40**, 453–458.
- Valentine, D.H. (1948) Studies in British *Primulas*. II. Ecology and taxonomy of primrose and oxlip (*Primula vulgaris* Huds. & *P. elatior* Schreb.). *New Phytologist*, **47**, 111–130.
- Valverde, T.H. & Silvertown, J. (1995) Spatial variation in the seed ecology of a woodland herb (*Primula vulgaris*) in relation to light environment. *Functional Ecology*, **9**, 942–950.
- Van Treuren, R., Bijlsma, R., Ouborg, N.J. & Kwak, M.M. (1994) Relationships between plant density, outcrossing rates and seed set in natural and experimental populations of *Scabiosa columbaria*. *Journal of Evolutionary Biology*, **7**, 287–302.
- Vergeer, P., Rengelink, R., Copal, A. & Ouborg, N.J. (2003a) The interacting effects of genetic variation, habitat quality and population size on performance of *Succisa pratensis*. *Journal of Ecology*, **91**, 18–26.
- Vergeer, P., Rengelink, R., Ouborg, N.J. & Roelofs, J.G.M. (2003b) Effects of population size and genetic variation on the response of *Succisa pratensis* to eutrophication and acidification. *Journal of Ecology*, **91**, 600–609.
- Waser, N.M. & Price, M.V. (1984) Experimental studies of pollen carry-over: effects of floral variability in *Ipomopsis aggregata*. *Oecologia*, **62**, 262–268.
- Whale, D.M. (1984) Habitat requirements in *Primula* species. *New Phytologist*, **97**, 665–679.
- Widén, B. (1993) Demographic and genetic effects on reproduction as related to population size in a rare, perennial herb, *Senecio integrifolius* (Asteraceae). *Biology Journal of the Linnean Society*, **50**, 179–195.
- Woodell, S.R.J. (1960) What pollinates *Primulas*? *New Scientist*, **8**, 568–571.
- Zimmerman, M. & Pyke, G.H. (1988) Reproduction in *Polemonium*: assessing the factors limiting seed set. *American Naturalist*, **131**, 723–738.

Received 5 March 2002

revision accepted 1 July 2003