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PERFORMANCE OF *LYCHNIS FLOS-CUCULI* FROM FRAGMENTED POPULATIONS UNDER EXPERIMENTAL BIOTIC INTERACTIONS

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Abstract. To study genetic effects of habitat fragmentation on plant performance and plant response to biotic interactions, we performed a greenhouse study with plants from 27 populations of the common plant *Lychnis flos-cuculi* differing in size, isolation, and microsatellite heterozygosity. We germinated seeds of 449 plants and grew up to nine offspring per maternal plant in single pots assigned to a factorial competition-by-pathogen infection treatment. We applied competition by sowing seeds of the grass *Anthoxanthum odoratum* into half of the pots. Moreover, half of the plants were inoculated with infective sporidia of the anther smut *Microbotryum violaceum*. Significant variation among populations in most size measures indicated genetic differentiation between populations. Plants from smaller populations developed fewer flowers than plants from larger populations indicating a genetic Allee effect. A decrease in flower number was also observed for populations with decreased microsatellite heterozygosity, suggesting higher inbreeding depression. Competition and pathogen infection reduced plant size independently from one another and independent from the fragmentation of the habitats of plant origin. While pathogen infection increased the total number of flowers per plant, it decreased the number of uninfected flowers per plant. This study demonstrates that even common species are negatively affected by habitat fragmentation. At the same time, it suggests little effect of habitat fragmentation on plant response to experimental competition and pathogen infection.

Key words: *Allee effect; anther smut; biotic interactions; common garden experiment; competition; fitness; greenhouse experiment;* Lychnis flos-cuculi; Microbotryum violaceum; *population size;* Silene flos-cuculi.

INTRODUCTION

Habitat fragmentation is a major threat to biodiversity and the survival of populations and species (Clarke and Young 2000, Davies et al. 2001). Habitat fragmentation leads to smaller habitat area and therefore smaller population sizes and the remaining fragments are more isolated from one another (Saunders et al. 1991). In smaller populations, genetic erosion may lead to a loss of genetic variation (Van Treuren 1991, Fischer and Matthies 1998*a*, Hendrix and Kyhl 2000, Galeuchet et al. 2002). Small population size and isolation between remnant populations reduce gene flow, and therefore, the amount of inbreeding within populations will be increased (Slatkin 1987, Ellstrand 1992). Reduced heterozygosity due to inbreeding may lead to inbreeding depression, and therefore, plant fitness might be negatively affected (Aizen and Feinsinger 1994, Hauser and Loeschcke 1994, Fischer and Matthies 1997). Most previous studies of genetic variation and fitness in relation to habitat fragmentation focused on rare plants (e.g., Oostermeijer et al. 1994, Fischer and Matthies 1998*b*, Fischer et al. 2000*a*, *b*, Paschke et al. 2002), while only few rather common plants were studied (Hooftman 2001, Lienert et al. 2002*a*, *b*).

Plant fitness in field populations depends on the genetic constitution of plants and on external environmental factors. For wetland plants in a mountain range, differences in altitude and moisture in the habitat may be especially important. In a greenhouse, environmental conditions are the same for all plants, and therefore, differences in performance among plants of different populations will arise only because of genetic differences (Thompson et al. 1991). Reduced fitness of more inbred plants may become especially apparent under stress such as that exerted by biotic interactions (Dudash 1990, Cheptou et al. 2000). While it has been suggested that ecologists must go beyond the study of simple effects to do justice to the many abiotic and biotic factors in complex natural systems (Weiner 1993, Shabel and Peart 1994), so far biotic interactions have been neglected in the context of genetic effects of habitat fragmentation on plant fitness.

We investigated the genetic consequences of habitat fragmentation for the plant *Lychnis flos-cuculi* (syn. *Silene flos-cuculi*) while also considering plant response to biotic interactions. This species is a common plant in Central Europe and occurs mainly in wet meadows. In Switzerland, 90% of the wetlands were destroyed in the last century (Broggi and Schlegel 1989,

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TABLE 1. Effects of altitude, moisture indicator value, mean observed heterozygosity, population size, isolation, and population of origin on the germination percentage of *Lychnis flos-cuculi* seeds from 27 populations under common greenhouse conditions.

Source of variation	Germination rate	
	df	MS
Altitude	1	32 607.7***
Moisture indicator value	1	3506.8†
Heterozygosity	1	6228.9*
Population size	1	15.2
Isolation	1	850.7
Population size × isolation	1	687.6
Population of origin	21	985.6**
Residual	420	469.4

† $P < 0.1$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Keller 1996). Therefore, the local distribution of *L. flos-cuculi* declined, but the species is still widespread in northeastern Switzerland, the study region. In a previous microsatellite study larger populations of *L. flos-cuculi* had higher gene diversity than smaller ones (Galeuchet 2003). *L. flos-cuculi* generally grows in competition with grasses such as *Anthoxanthum odoratum*. Competition has strong effects on plant size (reviewed in Weiner [1988]). Moreover, some populations are infected by the anther smut fungus *Microbotryum violaceum*, which may systemically infect plants and sterilize flowers and thus may have profound effects on plant fitness. The *Silene–Microbotryum* plant–pathogen system is one of the best studied plant–pathogen systems in natural populations (e.g., Antonovics et al. 1994, Alexander and Antonovics 1995, Biere and Honders 1996, Shykoff and Kaltz 1997, Bucheli et al. 2001). In the field, larger populations of *Silene alba* were more likely to be diseased than smaller populations (Antonovics et al. 1994), and among our sampling populations we observed a similar trend (Galeuchet 2003). Because variation in resistance to the anther smut is partly heritable (Alexander and Antonovics 1995), this suggests the hypothesis that plants from larger populations could be better adapted and thus less susceptible to the anther smut than plants from smaller populations. An alternative explanation of the hypothesized poorer response of plants from smaller populations could be inbreeding depression.

We grew offspring of 27 populations differing in size, isolation, and microsatellite heterozygosity in a factorial experiment of competition and infection with *M. violaceum*. We addressed the following specific questions: (1) Do offspring plants from different populations differ in their performance and susceptibility to biotic interactions? (2) Are fitness traits from offspring plants of smaller and more isolated populations decreased in the greenhouse compared to offspring of larger and less isolated populations? (3) Are offspring from smaller and more isolated populations more strongly affected by competition, pathogen infection, and their combination than plants from larger and less isolated populations?

MATERIAL AND METHODS

Species

Lychnis flos-cuculi L. (= *Silene flos cuculi* (L.) Clairv., *Coronaria flos-cuculi* (L.) Braun) (Caryophyllaceae) is widespread and abundant throughout its distribution range, which comprises Europe with Iceland, but not the arctic region (Jäger 1977). *L. flos-cuculi* occurs in sunny and moist habitats, such as wet hay meadows and calcareous fens, from the plains to the montane level (Münch 1979). In Switzerland the loss of wet meadows in the last decades has led to a decrease in sizes and numbers of populations and an increase in the degree of isolation.

The species is a polycarpic perennial plant. It overwinters as a green rosette and forms secondary rosettes from axillary buds, which replace the primary rosette. Flowering stalks reach heights of 20–90 cm and bear dichasial inflorescences with 3–50 flowers. The protandrous flowers are self-compatible but predominantly outcrossed (Biere 1996). Flowers of *L. flos-cuculi* are visited by several potential pollinator species of the Lepidoptera, Diptera, and Hymenoptera (Vejsnæs and Høvsgaad 1990). Ripe fruit capsules open at the top and up to 200 seeds, each weighing ~0.15 mg, are dispersed by vibrations of the stiffened stalk. Seeds germinate immediately after dissemination in autumn or in the following spring (Biere 1991).

Lychnis flos-cuculi is one of the host species of the anther smut fungus *Microbotryum violaceum* (Pers.: Pers.) Deml & Oberwinkler (= *Ustilago violaceum* (Pers.) Roussel) (Microbotryaceae). Races of *M. violaceum* are host species specific (Biere and Honders 1996, Bucheli et al. 2001). Plant infection, which may become systemic, may occur in the seedling stage or later, at axillary buds or in the flowers by spores trans-

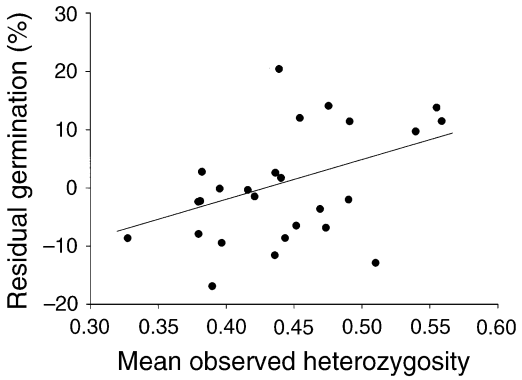

FIG. 1. Positive relationship of the residual of the germination percentage after regression on altitude and moisture indicator value and mean observed microsatellite heterozygosity measured on maternal plants ($P < 0.05$; Table 1). Study populations of *Lychnis flos-cuculi* were selected in calcareous fens in central and northeastern Switzerland.

TABLE 2. Effects of experimental competition and pathogen infection on fitness measures (means, with standard errors in parentheses) of *Lychnis flos-cuculi* in a greenhouse.

	All plants		Vegetative plants		Reproductive plants	
Treatment	*N*	Reproductive plants (%)	Plant diameter (cm)	Rosette diameter (cm)	Plant diameter (cm)	Rosette diameter (cm)
Control	742	76	14.50 (0.22)	7.94 (0.17)	14.03 (0.12)	8.20 (0.11)
Competition (C)	528	24	10.91 (0.16)	6.67 (0.11)	12.64 (0.22)	7.75 (0.21)
Pathogen (P)	525	75	14.01 (0.22)	7.66 (0.18)	14.12 (0.13)	8.09 (0.11)
C × P	513	22	10.64 (0.17)	6.47 (0.11)	12.31 (0.25)	7.49 (0.20)

ferred by pollinators (Vánky 1994). Flowers of infected plants bear fungal soris in their anthers, which, instead of pollen grains, release dusty, dark violet fungal spores. Spores germinate without a resting period and develop into three- to four-celled basidia. The basidia disarticulate into one-celled to three-celled sections, each of which produces basidiospores. Conjugation of two basidiospores of different mating type gives rise to infectious hyphae.

Population characteristics

In May 2000, we selected 27 study populations in calcareous fens in central and northeastern Switzerland at altitudes of 850–1350 m above sea level (Appendix). These populations represented a wide range of population sizes, which we estimated by counting the number of flowering individuals per population at peak of flowering, in June–July. Population sizes ranged from 40 to 51 000 flowering individuals in 2000 and from 149 to 57 000 in 2001. For further computations we always used the log of the harmonic mean of the number of flowering plants in 2000 and 2001 as population size.

In line with our impression in the field we classified 13 populations as ''isolated'' (Appendix) where the nearest population was more than 300 m away or isolated by at least 50 m of forest. For each population we obtained its longitude, latitude, and altitude from Swiss topographic maps. To describe abiotic factors for each population we performed vegetation surveys in two plots of 2 m^2 and estimated the cover of each species following Braun-Blanquet (1964). To estimate the ecologically very important moisture level for each vegetation, we used these data to calculate site means of Landolt's moisture indicator value (Landolt 1977), weighted by the cover of each species.

Plant material

In 15 larger populations we established two blocks of 50 × 50 m at distances of 5–135 m from each other. We established only one such block in the 12 smaller populations. In each block, we marked 24 randomly selected *L. flos-cuculi* individuals. In July and August 2000 we collected fruits of those 615 of the marked plants that had produced fruits (10–35 per population). In September 2000, we sowed 30 seeds per fruit of each sampled maternal plant (seed family) into each of two 7 × 7 cm pots filled with BF4 substrate (Tref de Baat, Coevorden, The Netherlands). We let seeds germinate at a day/night regime of 16/8 h and constant temperature of 25°C and scored germination at 5-d intervals. After 5 wk no further germination occurred.

In October 2000, we randomly selected 8–9 plantlets in the 4–8 leaf stage from 5–16 maternal plants per population (median = 12, mean = 10.4, total = 280 maternal plants) and transplanted each of the 2308 plantlets individually into 10 cm diameter pots filled with BF4 substrate. We kept the pots wet in a greenhouse where temperatures did not drop below 2°C. In April 2001, we added 5 g of a controlled-release fertilizer (Osmocote plus, Grace-Sierra International, Heerlen, The Netherlands) to each pot. From January to March 2002, we watered plants weekly with 0.4% liquid fertilizer (Vegesan N:P:K 1:0.87:1, Hauert, Grossaffoltern, Switzerland).

Competition and pathogen infection treatments

We applied a factorial competition-by-pathogen infection treatment to the offspring of each maternal plant. To create competition, in four pots per maternal seed family we sowed 20 seeds of the grass *Anthoxanthum odoratum* per pot after transplantation in October 2000, creating a grass density comparable to natural conditions.

To infect plants in four pots per maternal seed family (two with competition and two without) with a pathogen, we collected teliospores of *M. violaceum* from two field populations in August 2000. We stored the spores at 4°C in the dark until December 2000. Then, we germinated the spores in petri dishes on a sterilized solid 2% agar medium, containing 10% glucose and 0.5% yeast extract (Fluka, Buchs, Switzerland). We picked two fungal colonies of different populations to grow fungal strains. We propagated the colonies in 30 petri dishes per strain in the same substrate as mentioned above. Two days before we infected the plants, we collected the grown fungal mass with a razor blade, mixed the two fungal strains, and added it to 1 L of

TABLE 2. Extended.

Reproductive plants					
Plant height (cm)	No. reproductive branches	No. flowers	No. uninfected flowers	Infected flowers (%)	Biomass (mg)
40.61	3.14	23.13	23.10	0	1088
(0.48)	(0.09)	(0.75)	(0.77)	(0.00)	(32)
35.53	1.78	10.09	9.72	3	573
(0.84)	(0.12)	(0.75)	(0.83)	(0.02)	(28)
39.47	2.93	25.82	13.97	35	989
(0.50)	(0.10)	(0.98)	(0.78)	(0.02)	(31)
33.53	1.52	10.76	5.41	42	498
(0.95)	(0.08)	(0.84)	(0.58)	(0.05)	(27)

distilled water. To stimulate production of sporidia, the infective spores, we poured the solution into petri dishes to a height of 1 mm and stored them in the dark at 4°C. Then we mixed the solution again and injected 0.2 mL with a syringe into half of the plant rosettes in February and September 2001.

Performance measures

In April 2002 after 18 mo of growth, we classified plants as dead, vegetative, or reproductive. As vegetative fitness parameters we measured the largest diameter of the whole plant, the diameter of the largest rosette, the length of the longest stalk, and biomass. As reproductive traits, we measured the number of reproductive branches and flowers. To score systemic infection with *M. violaceum* we recorded the proportion of visibly infected reproductive branches and the number and proportion of visibly infected flowers.

Data analysis

We analyzed the noncontinuous variables survival, percentage of reproductive plants, and infected plants with logistic regression and all continuous variables with analysis of variance (ANOVA). We used a stepwise model simplification to remove nonsignificant covariates and interactions from the model. All models were implemented in the statistical software GENSTAT 5.3 (Payne et al. 1993). We analyzed mean values for each maternal seed family per treatment for all plants and for vegetative plants and reproductive plants separately, weighted by the number of replicates, which usually was three for control plants and two for treated plants.

First, to study effects of characteristics of populations of origin on offspring performance in benign conditions, we analyzed variation among control plants. We used altitude, moisture indicator value, mean microsatellite heterozygosity (from Galeuchet [2003]), and population size of the population of origin as covariates. Effects of covariates, isolation, and the interaction of population size with isolation were tested against residual variation among populations. Variation among populations was tested against residual variation among maternal families.

Second, we tested the effects of the treatments competition and pathogen infection and interactions of the treatments with population of origin against residual variation among maternal plants. We tested treatment interactions with covariates and isolation against treatment interactions with residual variance among populations of origin nested within isolation. To test the variables plant infected and percentage of infected flowers, only inoculated plants were considered.

Because of the mathematical, statistical, logical, and practical problems of sequential Bonferroni corrections pointed out by Moran (2003), we did not apply such corrections to our test. However, because of the high number of tests in our study we are very cautious in the biological interpretation of our results.

RESULTS

Germination

Mean germination percentage was 31.9% and population means ranged from 7.1% to 61.1%. Germination percentage decreased significantly with increasing altitude of the population of origin ($P < 0.001$) and increased significantly with increasing mean observed heterozygosity of the population ($P < 0.05$; Table 1; Fig. 1), indicating that plants from more inbred populations had a lower germination percentage.

Performance of control plants

All control plants survived during the experiment and 76% were reproductive after 18 mo of growth (Table 2). Total plant diameter ranged from 8.8 to 22.5 cm (mean = 14.5 cm) for vegetative plants and from 8.0 to 22.1 cm (mean = 14.1 cm) for reproductive plants. Diameters of the largest rosette ranged from 3.7 to 13.6 cm (mean = 7.9 cm) for vegetative plants and from 4.1 to 14.2 cm (mean = 8.2 cm) for reproductive plants. Flowering plants grew to heights between 23.8 and 65.3 cm (mean = 40.8 cm) and formed up to 9 flowering branches (mean = 3.1) and up to 64 flowers (mean = 23.3; Table 2).

With increasing altitude of the population of origin, there was a decrease in the percentage of reproductive plants ($P < 0.01$) and plant height ($P < 0.05$; Table

TABLE 3. Effects of altitude, moisture indicator value, mean observed heterozygosity, size, and isolation of the population of origin on stage, size, and reproductive parameters of offspring plants from 27 populations of *Lychnis flos-cuculi* in a greenhouse.

Source of variation	All plants		Vegetative plants		
	df	Percentage of reproductive plants	df	Plant diameter	Rosette diameter
Altitude	1	29.722**	1	0.956	15.845†
Moisture indicator value	1	10.970*	1	19.557	11.909
Heterozygosity	1	0.155	1	0.667	2.937
Population size	1	1.381	1	26.759†	18.220†
Isolation	1	0.316	1	7.875	4.461
Population size × isolation	1	0.045	1	0.850	0.002
Population of origin	20	2.506	19	8.140	4.379
Residual	231	1.992	68	5.754	3.158

Note: Values for ''All plants'' are MD (mean deviance changes); values for ''Vegetative plants'' and ''Reproductive plants'' are MS (mean squares).
† $P < 0.1$; * $P < 0.05$; ** $P < 0.01$.

3). The percentage of reproductive plants per population of origin ranged from 26% to 100% and was significantly smaller for plants from populations with a higher moisture indicator value ($P < 0.05$; Table 3). The number of flowers per plant was significantly higher for more heterozygous ($P < 0.05$; Fig. 2a) and for larger populations of origin ($P < 0.05$; Fig. 2b), indicating a genetic Allee effect.

Effects of experimental competition and pathogen infection on plant performance

During the experiment only 1.21% of the plants died. The proportion of reproductive plants over all four treatment combinations in the greenhouse experiment was 49%, which is similar to the proportion of 56% measured in the field in 2000 and 40% in 2001 (Galeuchet 2003).

Plants of different populations of origin differed in both vegetative measures of plant size ($P < 0.001$) and in plant diameter of reproductive plants ($P < 0.001$), plant height ($P < 0.001$), and biomass ($P < 0.05$; Table 4).

In competition with the grass *Anthoxanthum odoratum* fewer plants were reproductive ($P < 0.001$; Table 4). Moreover, all size measures of vegetative and reproductive plants (plant diameter, rosette diameter, plant height, number of reproductive branches, total number of flowers, number of uninfected flowers, and biomass) were significantly reduced by competition (Table 4). The negative effect of competition on diameter of vegetative plants was largest for plants from populations with higher heterozygosity (competition-by-heterozygosity interaction, $P < 0.05$; Table 4). Moreover, when competition was applied, plant diameter of reproductive plants from isolated populations increased with increasing population size while no trend could be observed for diameters of plants from non-isolated populations ($P < 0.05$; Table 4), suggesting that such a genetic Allee effect only becomes apparent in isolated populations under competitive stress.

Forty-six percent of the reproductive plants treated with *M. violaceum* had visibly infected flowers. On average, 73% of the side branches and 75% of the flowers of an individual were affected. Plants treated with *M. violaceum* had significantly more flowers but fewer uninfected flowers, fewer reproductive branches, and less biomass ($P < 0.05$; Table 4). The interaction between population identity and pathogen infection was significant for the number of flowers ($P < 0.001$) and biomass ($P < 0.05$; Table 4). The proportion of reproductive plants was significantly lower for plants that had not been treated with the pathogen that originated from populations with a higher humidity index ($P < 0.01$). Increased observed heterozygosity of the population of origin led to a significantly higher percentage of infected plants and a higher percentage of visibly infected flowers ($P < 0.05$; Fig. 3; Table 4). Generally, the competition-by-pathogen-infection interaction was not significant, indicating that these treatments affected plant performance largely independently from one another. An exception was the number of uninfected flowers, which was more reduced by pathogens without competition than under competition, probably simply because there were more flowers without competition (Table 2). Moreover, the significant three-way interaction of the treatment combination with population size for rosette diameter of vegetative plants and plant diameter of reproductive plants ($P < 0.05$; Table 4) suggests that plants from smaller populations may be more affected by the combination of both treatments than plants from larger populations.

DISCUSSION

Genetic population differentiation in fitness measures

The significant variation between plants of different populations of origin observed for most fitness measures in the greenhouse indicates a pronounced genetic structure between populations (Table 4). Our greenhouse was lower in altitude (420 m) than the natural populations and more similar in climate to populations at lower altitudes, which may explain the observed fitness advantages for plants from lower populations, which were most prominent for germination percentage

TABLE 3. Extended.

	Reproductive plants					
df	Plant diameter	Rosette diameter	Plant height	No. reproductive branches	No. flowers	Biomass
1	0.197	13.597†	809.00*	1.9790	209.60	0.3161
1	7.965	1.904	74.54	0.0000	476.00	1.5675
1	22.577	0.893	31.44	9.7070	1086.10*	1.0070
1	0.451	3.453	266.94	2.5680	1419.50*	0.3360
1	5.683	1.444	251.24	0.1510	467.10	0.8025
1	6.275	0.984	285.37	0.0340	101.20	0.0441
20	12.899**	3.883	124.05	4.3750	218.40	0.5420
201	5.950	4.614	86.31	6.0460	213.70	0.3948

(Table 1) and the proportion of reproductive plants (Tables 3 and 4).

Among the most important results was that habitat fragmentation, mainly expressed as effects of population size, affected offspring fitness (Table 3). Reproductive fitness, measured as number of flowers of the offspring plants in the greenhouse, decreased with decreasing population size of the population of origin (Fig. 2b), indicating a genetic Allee effect. In accordance with the present greenhouse experiment we observed increased reproductive fitness measured in terms of the number of seeds per capsule with increasing population size in our field survey (Galeuchet 2003). Reduced reproduction of plants from small populations has been observed in studies on rare species (e.g., Oostermeijer et al. 1994, Fischer and Matthies 1998*a*, Menges 1999, Fischer et al. 2000*a*, Paschke et al. 2002). With more common species, different relationships between population size and reproduction were observed. Leimu and Syrjanen (2002) did not detect a decrease in reproductive traits in *Vincetoxicum hirundinaria* in naturally fragmented habitats. Hooftman et al. (2003) observed a reduction of reproductive fitness in plants from smaller isolated habitat remnants compared to larger remnants for *Carex davalliana* and the opposite for *Succisa pratensis*.

The positive correlation of observed heterozygosity per population with reproductive fitness (Tables 1 and 3, Figs. 1 and 2a) may be explained by inbreeding depression (Perret 2003). Reduced plant performance in genetically less variable smaller populations was reported for several rare species (Oostermeijer et al. 1994, Ouborg and Van Treuren 1994, 1995, Fischer and Matthies 1998*b*, Paschke et al. 2002, Fischer et al. 2003). Further evidence for inbreeding depression in *L. flos-cuculi* is given by Biere (1991) and Hauser and Loeschcke (1994, 1996). In contrast, a negative relationship of increased observed heterozygosity with reproductive fitness was observed in the field (Galeuchet 2003), suggesting different habitat quality among more or less heterozygous populations.

Effects of experimental competition and pathogen infection

Experimental competition with the naturally co-occurring grass *Anthoxanthum odoratum* reduced most measured fitness traits, indicating that competition is an important biotic factor for the performance of this species. The percentage (46%) of inoculated reproductive plants that became visibly infected as indicated by fungal spores in flowers was similar to other studies in which other closely related *Silene* species were exper-

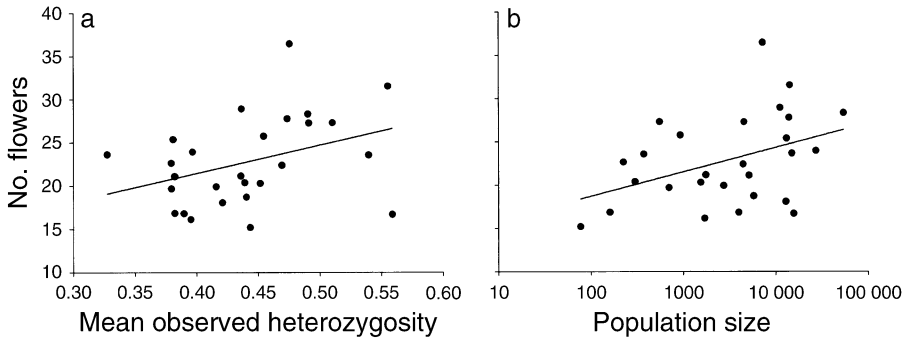

FIG. 2. Positive relationship between the number of flowers of *Lychnis flos-cuculi* offspring grown in the greenhouse and (a) mean observed microsatellite heterozygosity measured on maternal plants and (b) size of the population of origin. Both relationships were significant at the 5% level (ANOVA; Table 3).

TABLE 4. Effects of competition and pathogen-infection treatments on offspring plants from 27 populations of *Lychnis flos-cuculi* in a greenhouse.

Source of variation	All plants	Vegetative plants		Reproductive plants	
	Percentage of reproductive plants‡	Plant diameter	Rosette diameter	Plant diameter	Rosette diameter
Altitude	82.276***	10.72	84.454*	2.812	15.129
Moisture indicator value	3.498	21.96	12.616	5.203	8.221
Heterozygosity	1.969	51.50	0.019	30.145	24.63†
Population size	1.319	1.17	11.351	21.79	13.392
Isolation	0.279	37.73	4.719	50.539	11.914
Population size × Isolation	0.068	28.05	2.273	10.978	1.962
Population of origin	2.089	31.92***	11.864***	17.236***	6.588
Competition	707.025***	2180.87***	321.786***	444.895***	49.772**
× Altitude	2.706	17.31†	0.246	0.372	0.103
× Moisture	0.713	0.02	0.600	17.482†	9.431
× Heterozygosity	2.612	20.87*	6.271†	8.396	6.472
× Population size	0.006	8.19	0.195	3.921	3.262
× Isolation	0.135	1.65	0.006	0.019	6.318
× Population size × Isolation	0.862	6.13	0.183	24.248*	3.274
× Population	2.060	4.46	2.016	5.377	5.209
Pathogen	0.796	29.57†	15.269†	0.229	4.578
× Altitude	0.000	0.28	3.277	0.438	1.721
× Moisture	9.738**	2.66	2.987	1.115	4.098
× Heterozygosity	0.010	1.84	3.584	2.384	1.271
× Population size	2.565	6.66	0.086	4.405	0.047
× Isolation	0.647	1.67	0.026	0.421	0.923
× Population size × Isolation	0.019	0.37	8.156	5.254	1.715
× Population	0.906	7.27	4.025	5.068	3.913
Competition × Pathogen	0.267	3.18	0.002	10.569	0.003
× Altitude	5.556†	0.12	0.684	4.254	5.573
× Moisture	0.004	22.45	2.049	13.977	0.619
× Heterozygosity	0.011	0.32	1.541	5.979	2.891
× Population size	0.123	32.67†	17.318*	33.284*	4.985
× Isolation	2.189	10.79	3.364	17.394	4.5
× Population size × Isolation	···	0.17	0.222	2.166	1.182
× Population	1.295	8.56	3.464	6.266	5.701
Residual	1.464	8.25	4.176	6.081	4.65

Notes: Covariates are altitude, mean moisture indicator value, and mean observed heterozygosity of the population of origin. Population size and isolation represent effects of habitat fragmentation. Measured variables are percentage of reproductive plants, plant and rosette diameter for vegetative plants, plant and rosette diameter, plant height, number of reproductive branches, number of flowers, and biomass for reproductive plants. Values shown are mean squares, except for percentage of reproductive plants for which mean deviance changes are shown. All variables have df = 1 except the following: df = 20 for population of origin and its interaction with competition and pathogen. For the interaction of competition × pathogen × population of origin, df = 21 for all plants, 19 for vegetative plants, and 16 for reproductive plants. For residuals, for all plants df = 919; for vegetative plants, df = 553; for reproductive plants, df = 539 for plant and rosette diameter, 536 for plant height, 522 for plant biomass, 538 for number of reproductive branches and number of flowers, and 264 for plant infected and percentage of infected flowers.

† $P < 0.1$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

‡ See Table 3.

imentally infected (Alexander 1989, Shykoff and Kaltz 1997). The infection treatment with *M. violaceum* reduced plant fitness (Table 4), even of those reproductive plants that did not produce fungal spores (data not shown), suggesting that these plants were also infected or that they had costs of inducing successful resistance to infection. With the exception of the number of uninfected flowers, which was higher without competition and also more reduced there by pathogens than in plants with competition, the treatments acted independently of one another as observed for the treatment combination of competition and herbivory in *Hypochaeris radicata* (Weiner 1993).

In contrast to other fitness measures, the total number of flowers per plant was increased by the infection treatment (Table 4). This finding is in agreement with other studies on different species of the Caryophyllaceae (Biere and Honders 1996, Shykoff and Kaltz 1997). We assume that the infection with *M. violaceum* stimulates the production of flowers in the plant to increase the output of fungal spores and does not constitute a fitness increase for the plant because all infected flowers are sterilized by the fungus and no seeds will be produced. This is clearly supported by the significantly reduced number of uninfected flowers under pathogen infection (Table 4).

In the field, larger populations are more likely to be diseased (e.g., *Viscaria vulgaris* [Jennersten et al. 1983], *Silene alba* [Antonovics et al. 1994], and our study populations [Galeuchet 2003]). However, habitat

TABLE 4. Extended.

Reproductive plants						
Plant height	Biomass	No. reproductive branches	No. flowers	No. uninfected flowers	Plant infected	Percentage of infected flowers
1017.49†	0.0567	0.814	68.9	10.8	1.565	0.0108
185.05	0.7645	0.009	71	463.9*	0.29	0.1458
42.06	1.7051	2.886	1798.1*	138.7	7.99*	1.037*
0.02	0.0499	2.602	918.9	495.5*	1.131	0.1169
15.59	0.0692	2.269	298.7	146.2	1.168	0.0654
126.02	0.125	3.486	174.7	406.7*	3.206	0.4359
265.99***	0.5982*	4.422	330.7	64.0	1.621	0.165
5724.55***	41.7573***	325.651***	35 275.2***	16 762.6***	9.087*	0.2264
0.86	0.1568	0.032	63.3	122.4	0.5	0.0314
3.56	0.0615	2.272	3.2	61.0	1.862	0.072
2.59	0.1694	2.76	283.2	60.7	1.218	0.052
50.36	0.0488	0.622	356.1†	203.8	0.009	0.0146
7.46	0.0132	0.743	7.2	2.3	0.014	0.0527
8.82	0.0723	0.067	59.7	73.6	0.449	0.0523
62.24	0.1762	2.021	105.3	79.9	1.648	0.2747
340.91†	1.9072*	11.536*	1416*	9284.9***		
1.42	0.5315	1.381	198.9	145.4		
0.14	1.1273	0.132	531.1	130.9		
2.11	0.0507	3.558	0	526.2		
157.97	0.4508	0.225	120.7	3.1		
391.42†	1.0172	4.957	389.9	1.5		
151.37	0.075	1.269	52	262.6		
130.61†	0.5102*	3.969	522.7***	166.9		
6.29	0.1423	0.539	228.4	483.3*		
6.4	0.2067	0.084	0.1	43.7		
0.03	0.1411	0.773	7.4	2.6		
1.88	0.1751	2.659	191.3	104.3		
99.11	0.2124	3.958	214	97.6		
2.11	0.0683	2.2	4.7	211.8		
38.94	0	0.058	13.8	0.1		
56.99	0.1128	1.553	99.4	84.1		
90.33	0.2862	2.806	222	110.2	543.825	0.1995

fragmentation did not affect susceptibility to experimental infection with the anther smut in our common environment study. Thus, there is no evidence for adaptation of larger plant populations to the smut fungus nor for lower resistance of plants from small populations due to genetic drift or inbreeding. In a study of the relationship between inbreeding levels of different lines of *Silene alba* and effects of experimental infection with *Microbotryum violaceum* on these lines, Oubborg et al. (2000) concluded that inbreeding effects on the response to the anther smut were highly unpredictable.

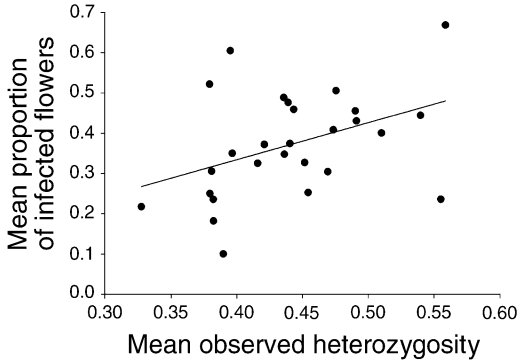

FIG. 3. Positive relationship of mean proportion of infected flowers of experimentally inoculated offspring and mean observed microsatellite heterozygosity of maternal plants ($P > 0.05$; Table 4).

Plants from more heterozygous populations produced more flowers and were also more likely to be visibly infected by *Microbotryum violaceum* (Fig. 3, Table 4), which is in line with observational and experimental evidence that plants that grew more flowers were more likely to be infected by *M. violaceum* (Alexander and Antonovics 1988, Alexander 1989). This indicates a trade-off between reproductive fitness and infection risk in *L. flos-cuculi*.

There was hardly any indication that treatment effects on plant performance depended on habitat fragmentation. Thus, the main conclusions from our comprehensive study of offspring plants from 27 populations of *L. flos-cuculi* are that genetic effects of habitat fragmentation negatively affected plant performance even of this common species and that there is very little effect of habitat fragmentation on plant response to competition and pathogens.

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APPENDIX

A table presenting populations of origin of seeds of *Lychnis flos-cuculi* used in the greenhouse experiment is available in ESA's Electronic Data Archive: *Ecological Archives* E086-054-A1.