

Effects of population size on genetic diversity, fitness and pollinator community composition in fragmented populations of *Anthericum liliago* L.

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Abstract The genetic and fitness consequences of habitat fragmentation on the dry grassland species *Anthericum liliago* L. (Anthericaceae) were examined. We used random amplified polymorphic DNA (RAPD) markers to determine the distribution of genetic diversity within and among 10 German *A. liliago* populations, ranging in size from 116 to over 2 million ramets. The genetic diversity of an *A. liliago* population was highly positively correlated with its population size. The overall differentiation among populations (Φ -ST = 0.41, $P < 0.0001$, AMOVA) was considerably higher than expected for a species with a mixed breeding system. No strong correlation ($P < 0.01$) was detected between fitness parameters and population size and genetic diversity. The reproductive output (seeds per ramet) was only

highly correlated ($P < 0.001$) with the proportion of flowering ramets in a population which could be caused by a more effective pollination in large populations which are more attractive to specialized pollinators. The specialized *A. liliago* pollinator *Merodon rufus* (Syrphidae) and high abundances of solitary bees could only be found in *A. liliago* populations with more than 10,000 individuals. Genetic differentiation among the investigated *A. liliago* populations may have been caused by limited seed and pollen dispersal and a mixed mating system permitting a high selfing rate. The differentiation among the small and isolated populations lacking main pollinators seems to be caused by genetic drift.

Keywords Habitat fragmentation · *Merodon rufus* · Pollinator · Population genetic structure

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Introduction

Habitat fragmentation, the reduction of a continuous habitat into several smaller spatially isolated remnants, reduces the size and increases the spatial isolation of plant populations (Young et al. 1996). Small population size and isolation may have a number of negative effects on populations (e.g. Kery et al. 2000; Brys et al. 2004; Leimu et al. 2006). It is generally assumed that genetic diversity is reduced in small populations (e.g. Frankham 1996). Small and

isolated populations may be subjected to genetic erosion and increasing genetic divergence among populations (Culley and Grubb 2003) through random genetic drift, increased levels of inbreeding and reduced gene flow (Llorens et al. 2004). Habitat fragmentation and isolation lead to changes in the quantity and quality of pollination (Kwak et al. 1998; Steffan-Dewenter and Tschardt 1999). The modification of plant–pollinator interactions may have consequences for pollen limitation, reducing the number of insect visits, the size of pollen loads or pollen quality (Campbell and Husband 2007). In some cases, the loss of specialized pollinators will strongly select for self-pollination and reduced genetic variability in plants, resulting in a possible reduction in their evolutionary adaptability to environmental change (Allen-Wardell et al. 1998) and in their reproductive output, which is connected with an increasing extinction risk (Matthies et al. 2004).

In the agricultural landscapes of Europe, the ongoing process of habitat destruction and degradation due to the intensification and modernization of agriculture have reduced the distribution of many plant species (Jacquemyn et al. 2003) and increased the distances between the remaining populations. This is also the case for the plant species *Anthericum liliago* L. (Jackel 1999; Rosquist and Prentice 2002). In a former study of a small and isolated natural population of *A. liliago* (Halle, Kröllwitz), a low level of genetic polymorphism and a positive correlation between genetic and geographic distances in an area of a few m² were found (Peterson et al. 2002). These findings were interpreted as the result of restricted pollen and seed dispersal, and of an unusual ant pollination system connected with a high selfing rate.

In this study, we investigated 10 *A. liliago* populations (Fig. 1; study area c. 9,000 km²) in Saxony-Anhalt, Germany, on the border of the densely populated *A. liliago* southern range and the sparsely populated northern range, including some of the smallest and the largest known populations within this region. Firstly, we studied the genetic variation within and among these populations. Secondly, we looked for a possible relationship between genetic variability and population sizes. Thirdly, we investigated the influence of population size, pollinator community composition and genetic diversity on fitness parameters.

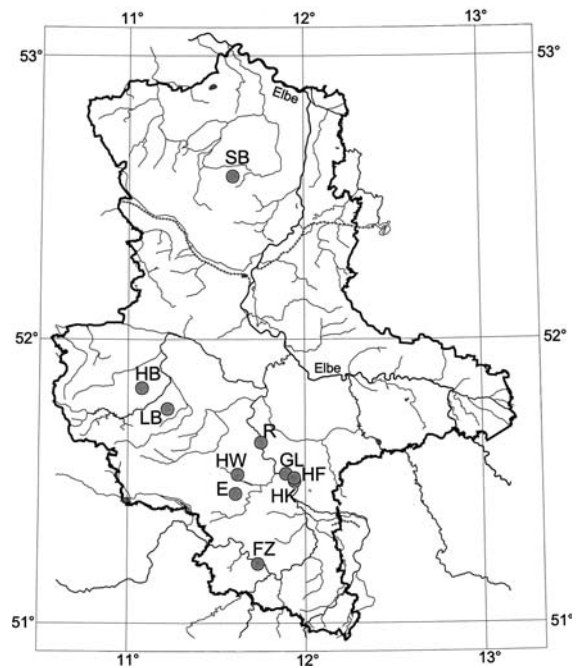


Fig. 1 Geographic map of sampling localities of *A. liliago* populations (codes in Table 1) in Saxony-Anhalt, Germany

Material and methods

Species description

The plant *A. liliago* L. (Anthericaceae) is a self-compatible predominantly outcrossing (Rosquist 2001) herbaceous perennial found in dry grassland and dry shrubs, and rarely also in open oak or pine forests. The species has one or more leaf rosettes with one inflorescence each, ranging in height from 30 to 60 cm, sprouting from a short rhizome. The species has large flowers (35–40 mm in diameter) in a racemose inflorescence. Easily accessible floral nectaries are present. The plants have oviform seed capsules with relatively large orbicular seeds. The stem of *A. liliago* is sturdy and remains stiff and erect until the seeds are mature. At this time, the upper end of the erect seed capsules burst open and the seeds are released. The seeds are gravity-dispersed, and they do not have any adaptation for dispersal with animals or wind. Solitary bees (Apoidea) and syrphid flies (Syrphidae) have been described as the main pollinators (Rosquist 2001) although field studies have shown the presence of additional unspecialized flower visitors, e.g. ants (Formicidae) (Peterson et al. 2002).

Anthericum liliago has a wide European distribution. Germany forms the northern limit of the continuous distribution range, the Danish and Swedish populations are isolated outposts.

Field study

Field studies were undertaken between 2002 and 2006. The number of *A. liliago* ramets for each large population (>500 ramets) was determined in 12 areas of 4 m² and extrapolated according to the populated area. Fitness parameters were determined from randomly selected plants in 2003 and 2005: flowering ramets (200 per population or all in HK and LB), number of flowers and seed set (100 randomly selected flowering ramets per population with the exception of the seed set in LB, SB and HF due to a loss of the fruits through herbivory; all flowering ramets in HK) and germination (all populations, with 139–1,708 seeds collected per population). Because it is difficult to break seed dormancy, the % of seed germination could only be successfully investigated with the seeds collected in 2003. According to former experiments under different storage and germination conditions, seeds were stored for 2 years at room temperature to overcome seed dormancy and then placed for germination at 4°C on moistened sheets of paper in the dark for about 2 months. The seed weight of the seeds of 2005 was determined after drying at room temperature over a period of 6 weeks. Observations of pollinators were done in 2003 and 2005, each during the main flowering period between May the 15th and June the 10th, at two sunny days for 4 h (between 11 a.m. and 3 p.m.). We measured the relative abundance and species composition of flower visiting insects. No difference in the pollinator abundance and community composition was found between both years.

RAPD analysis

Random amplified polymorphic DNA (RAPD) analysis (Williams et al. 1990) was conducted on 10 individuals from each of the 10 populations using 12 decamer oligonucleotide primers (primer sequences are listed in Peterson et al. 2002) using Ready-To-Go PCR-beads (Amersham Biosciences) in 20 µl

reactions with 10 ng DNA as described in Peterson et al. (2002). The 12 primers gave consistently reproducible bands (total sample of 238 RAPD markers) from 180 to 2,000 bp. The reproducibility of the RAPD results was checked with 14 randomly chosen individuals for eight RAPD primers; 1,754 out of 1,771 fragments were reproducible (99.04%).

Statistical analysis

A data matrix was created from the photographs of the gels by assigning 1 to present bands and 0 to absent bands. We calculated genetic diversity within populations as Shannon's indices of diversity to avoid uncertainty, associated with expected heterozygosity estimates, based on dominant marker systems (Lynch and Milligan 1994). Shannon's index was calculated for each RAPD marker *i*, separately for each population, as in Bussel (1999). The average Shannon's index $H' pop$ for a population of *A. liliago* was then calculated by averaging $H' pop (i)$ over all markers (Monaghan and Halloran 1996). Percentages of polymorphic loci were also calculated. Phi-ST coefficients of differentiation among populations were computed in ARLEQUIN ver. 2.000 (Schneider et al. 2000). This measurement of differentiation is based on squared Euclidean coefficients of variation between groups of haplotypes (Excoffier et al. 1992). We used individual patterns of presence/absence of RAPD markers as input data, following Huff et al. (1993) and estimated the partitioning of molecular variance within and among populations by AMOVA, as implemented in ARLEQUIN.

A matrix of geographic distances between populations was created using formulas given by Kirvan (1997) for converting coordinates in degrees of longitude/latitude to metric distances. The extent and significance of associations between matrices of the geographical distances and the Phi-ST coefficients was evaluated by Mantel tests (MXCOMP in NTSYS). We used Pearson correlation coefficients to check for associations between quantitative descriptors for population size, fitness parameters and genetic diversity (Shannon's index), and Kendall's tau test (SPSS rel. 14.00, Chicago: SPSS Inc.) for a three-level ordinal-scaled descriptor of flower visitation in relation to population size.

Results

Habitats, fragmentation and population sizes

Anthericum liliago inhabits different types of dry grassland vegetation in the investigated region: from species-poor atlantic *Corynephorus* grasslands on oligotrophic, highly siliceous soils, to sub-continental steppe grassland on relatively nutrient rich loess, or to xeric calcareous grassland dominated by saxicolous plant species originating from the Alps and the Mediterranean region (Table 1). Large differences between populations regarding the census number of ramets per population (from about 100 to over 2 million), the population areas (17.5 to more than 30,000 m²) or the density of plants within a population (0.17–83.5 ramets per m²; Table 1) were noticed. Generally, the population size depended on the size of the available habitat (Table 1). Habitats with relatively nutrient rich loess soils (E and HB) tend to have a higher population density (ramets per m²). The high population density in HW with nutrient poor sandy siliceous soils is probably caused by the extreme dry conditions which inhibit most of the natural plant competitors. All small populations (HK, HF, SB, and LB) were found to be isolated (Table 1), i.e. no other *A. liliago* population is known in a radius of 1 km.

Pollinator community composition

The main pollinators (Table 1) of *A. liliago* were the syrphid *Merodon rufus* Meigen as well as solitary bees of the genera *Andrena*, *Halictus*, *Lasioglossum* and *Osmia*. The highly specialized *A. liliago* pollinator *Merodon rufus* was an abundant visitor of inflorescences of the studied plant in all very large populations (FZ, HB, R, and HW). It was rare (GL) or absent (E) in middle-sized populations, and was always absent in the small populations (HK, HF, LB, and SB). This syrphid species could only be detected on flowers of *A. liliago* and was never seen visiting flowers of other plants (several individuals per population were tracked). A high pollinator abundance, especially of solitary bees, was restricted to middle and large populations (>10,000 *A. liliago* ramets). Ants visited flowers in nearly all investigated populations, but they were abundant only in the two

small populations HK and HF (Table 1). Low densities of other pollinators (Bombiliidae, Coleoptera, Diptera, Hymenoptera, and Lepidoptera) were found in all populations (data not shown).

Genetic variation and differentiation among populations

The values of Shannon's index (*I*) of within-population diversity varied from 0.098 in population HK to 0.317 in population R (Table 1). Approximately the same pattern was found in the percentage of polymorphic RAPD loci within populations (Table 1). All Phi-ST values of differentiation among populations were significant ($P < 0.05$, detailed results not shown). A considerable part of total molecular variance could be attributed to the among-population component (41.04%, $P < 0.0001$, Table 2). We found a moderate correlation between genetic (pairwise Phi-ST values) and geographic distances between the populations ($r = 0.36$, $P < 0.01$ for all 10 populations; and a relatively strong correlation ($r = 0.78$, $P < 0.001$) for the seven largest populations; Fig. 2).

Correlation analyses of genetic diversity, population size, fitness parameters and pollinators

We found a very strong and significant correlation between the logarithm of census population size and Shannon's index of gene diversity within populations ($r = 0.912$, $P < 0.001$; Fig. 3). The correlation is even greater within the range of population sizes between 10³ and 10⁶ ramets.

The fitness parameters (Table 3) were often inter-correlated (Table 4). We found a correlation between the mean number of seeds per seed capsule and the proportion of flowering ramets ($r = 0.834$, $P < 0.01$), the mean number of flowers per inflorescence ($r = 0.867$, $P < 0.001$), the germination rate ($r = 0.871$, $P < 0.001$), the seed weight ($r = 0.672$, $P < 0.05$), the mean number of seeds per ramet ($r = 0.64$, $P < 0.05$) and the population size ($r = 0.614$, $P < 0.05$). The population size was also significantly correlated with the mean seed weight ($r = 0.708$, $P < 0.05$). The mean number of seeds per ramet was strongly correlated with the proportion

Table 1 Geographic and demographic characteristics, genetic diversity and abundance of flower visiting pollinators of *A. liliago* populations

Site	Code ^a	La(N)/ Lo(E)	Ramets/ population	Area (m ²)	Ramets/ m ²	Habitat description, soil, vegetation ^b	<i>I</i>	<i>P%</i>	<i>M. rufus</i> /bees ants ^c
Langer Berg	LB*	51°45'29'' 11°13'20''	116	686	0.17	Cretaceous sandstone slope, oligotrophic siliceous soil, Spergulo-Corynephorum	0.149	24.0	0/1 1
Halle Kröllwitz	HK*	51°30'10'' 11°56'21''	127	45	2.8	Porphyry hill, oligotrophic siliceous soil, <i>Filipendula</i> <i>vulgaris</i> - <i>Helictotrichon pratense</i> association	0.098	17.3	0/0 3
Halle Fuchsberg	HF*	51°30'48'' 11°56'13''	343	17.5	19.6	Porphyry hill, oligotrophic siliceous soil, <i>Cirsio pannonici</i> - <i>Brachypodium pinnati</i>	0.110	19.2	0/1 3
Spitzer Berg	SB*	52°34'35'' 11°35'33''	3,034	474	6.4	Morainic hill, base-rich, clay- sandy soil, oligotrophic sandy soil, <i>Potentillo arenariae</i> - <i>Stipetum capillatae</i>	0.154	25.0	0/0 0
Großer Lunzberg	GL	51°31'41'' 11°53'24''	10,365	595	17.4	Porphyry hill, oligotrophic siliceous soil, <i>Thymus</i> <i>serpyllum</i> - <i>Festuca cinerea</i> association	0.174	29.8	1/3 1
Erdeborn	E*	51°27'29'' 11°36'22''	17,920	262	68.4	Sandstone slope, covered by loess soil, <i>Cirsio pannonici</i> - <i>Brachypodium pinnati</i>	0.232	39.4	0/1 1
Zscheiplitz	FZ	51°12'38'' 11°43'52''	67,200	11,200	6.0	Calcareous slope (shell lime), calcareous soil on rocky substrate, <i>Teucrio-Seslerietum</i>	0.307	54.8	3/2 1
Harslebener Berg	HB	51°49'54'' 11°4'40''	71,682	858	83.5	Cretaceous sandstone slope, covered by loess soil, <i>Cirsio</i> <i>pannonici</i> - <i>Brachypodium pinnati</i>	0.259	48.1	3/3 1
Rothenburg	R	51°38'23'' 11°45'0''	259,200	27,000	9.6	Carboniferous sandstone slope, clay-sandy siliceous soil, <i>Alysso</i> - <i>Sedion</i> : <i>Melica ciliata</i> - <i>Stipa capillata</i> grassland	0.317	59.6	3/2 1
Hasenwinkel	HW	51°31'33'' 11°37'6''	2,102,880	31,200	67.4	Bunter slope, sandy siliceous soil, <i>Xerobromion</i> : <i>Anthericum</i> <i>liliago</i> - <i>Teucrium montanum</i> association	0.291	52.9	3/2 1

Notes: *I* = Shannon's index of diversity; *P%* = percentage of polymorphic loci

^a Isolated populations (no other *A. liliago* population occur in a diameter of 1 km around) are indicated by an asterisk

^b Nomenclature according Rennwald (2000)

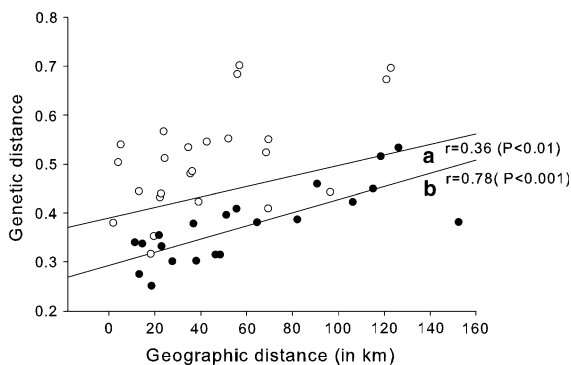
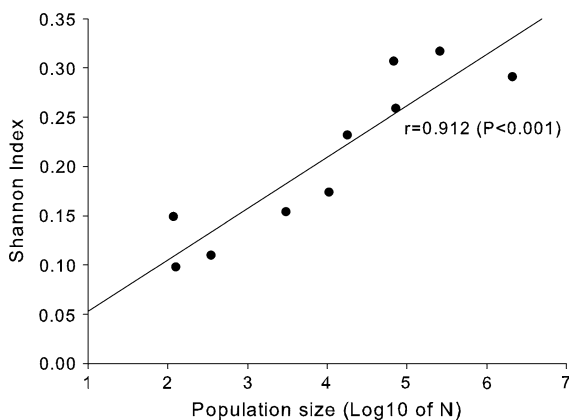
^c Abundant (3), regular (2), rare (1), absent (0)

of flowering ramets in a population ($r = 0.853$, $P < 0.001$). The mean number of flowers per inflorescence was correlated with the percentage of germinated seeds (0.769 , $P < 0.01$). Within-population Shannon's diversity index was also correlated (Table 4) with the mean seed weight ($r = 0.627$, $P < 0.05$) and marginally associated with the percentage of flowering ramets ($r = 0.508$, $P < 0.1$).

There were significantly positive correlations between population size and flower visitation by *Merodon rufus* ($\tau = 0.747$, $P < 0.01$), and solitary bees (Apoidea) ($\tau = 0.465$, $P < 0.05$). Flower visitation of no other insect group (Bombiliidae, Coleoptera, Diptera, Hymenoptera, Lepidoptera and Formicidae) was significantly associated with population size (data not shown).

Table 2 Results of AMOVA for samples of 10 populations

Source of variation	d.f.	Sum of squares	Variance component	Percentage of variation	Significance
Among populations	9	889.4	6.852	41.04	$P < 0.0001$
Within populations	134	1319.3	9.845	58.96	$P < 0.0001$
Total	143	2208.7	16.697		

**Fig. 2** Relationship between pairwise geographic and pairwise genetic distances (pairwise Phi-ST-values) of all 10 *A. liliago* populations (a) and of the seven largest populations (b), indicated by black circles**Fig. 3** Relationship between genetic diversity (Shannon's Index) and size of 10 *A. liliago* populations

Discussion

Genetic differentiation among *A. liliago* populations

Theoretically, reduced population sizes and increased spatial isolation of populations can lead to erosion of

genetic variation and increased inter-population genetic divergence (Young et al. 1996). These predicted effects were fully confirmed in our study of *A. liliago*. The pronounced genetic differentiation among the populations of *A. liliago* corresponds well with the strong isolation of their habitats in the intensively managed agricultural landscape of Middle Europe. The genetic differentiation we found among populations is correlated with the geographical distance separating them. This spatial pattern could be caused by fragmentation of a formerly larger population followed by genetic drift within the remaining relic of populations. It may also indicate that the current gene flow connects populations closer to each other more efficiently, possibly via gene flow among intermediated non-investigated populations, than populations divided by greater distances (see also Hensen et al. 2005). A strong population structure and significant relationship between genetic and geographic distances among populations was also found for *Grevillea caleyi* (Llorens et al. 2004) and *Euphrasia stricta* (Kolseth et al. 2005).

Differentiation among the highly isolated populations may be best explained by genetic drift. The area between the populations is likely to be unfavourable for extensive pollinator movement. The most agile pollinators of *A. liliago*, solitary bees, have foraging distances less than 1 km (Gathmann and Tscharnke 2002). In addition to the restricted pollinator movement, a lack of seed dispersal may have contributed to the high degree of differentiation found in *A. liliago* populations (see also Peterson et al. 2002). Although we did not investigate the possible effects of selection for specific loci or traits in our sample, these effects are unlikely to be restricted only to very small populations and to contribute considerably to their differentiation. Similar results on genetic differentiation among populations have been reported for a number of rare plant species and attributed to the absence of interpopulation gene flow, e.g. for populations of

Table 3 Fitness parameters of the analysed populations of *A. liliago*

Code	Rate of flowering ramets (in %)		Flowers per inflorescence		Rate of flowers producing seed capsules (in %)		Seeds per seed capsule		Seeds per ramet		Germination rate (in %)	Seed weight (mg/seed)
	2003	2005	2003	2005	2003	2005	2003	2005	2003	2005	2003	2005
LB	6.04	41.48	14.90	9.63	11.59	13.08	7.32	6.71	0.76	3.49	5.13	2.903
HK	0	55.80	0	12.81	0	10.41	0	8.14	0	6.07	0	3.488
HF	41.50	79.50	20.09	14.46	7.92	1.87	11.69	10.85	7.70	2.34	11.37	4.290
SB	10.50	45.50	9.00	10.48	4.76	9.92	7.33	n.d.	0.33	0	n.d.	n.d.
GL	53.00	79.00	17.98	9.94	16.57	9.96	11.46	10.10	18.09	7.90	10.68	3.721
E	73.50	73.00	13.20	13.75	16.06	3.35	10.75	12.74	16.75	4.28	14.69	4.457
FZ	66.00	40.00	13.36	9.26	9.88	9.71	10.41	9.56	9.07	3.44	6.11	4.838
HB	57.50	54.00	10.40	9.66	9.81	10.46	8.57	8.62	5.03	4.70	3.55	3.317
R	64.00	62.50	16.30	14.61	5.95	7.32	11.39	11.29	7.05	7.55	13.49	5.422
HW	34.50	52.50	17.32	12.57	7.97	4.38	8.88	13.20	4.23	3.82	13.37	5.124

Table 4 Summary of analyses on correlations between fitness and genetic estimates for the sample of 10 populations of *A. liliago*

Population viability measure	Pop size	Flowering ramets	Flowers/inflorescence	Seeded flowers	Seeds/seed capsule	Germinated seeds	Seed weight	Seeds per ramet
Flowering ramets	0.461	–	–	–	–	–	–	–
Flowers/inflorescence	0.324	0.592*	–	–	–	–	–	–
Seeded flowers	–0.013	0.094	–0.041	–	–	–	–	–
Seeds/seed capsule	0.614*	0.834**	0.867***	0.087	–	–	–	–
Germinated seeds	0.308	0.532	0.769**	–0.490	0.871***	–	–	–
Seed weight	0.708*	0.479	0.531	–0.465	0.672*	0.679*	–	–
Seeds/ramet	0.305	0.853***	0.417	0.441	0.640*	0.456	0.237	–
<i>Population diversity measure</i>								
Shannon's index	0.912***	0.508	0.185	0.231	0.512	–0.012	0.627*	0.331

Levels of significance of correlations: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

Gentianella germanica (Fischer and Matthies 1998), *Arnica montana* (Luijten et al. 2000) and *Viola pubescens* (Culley and Grubb 2003).

The size range and the degree of isolation of populations in our study are representative for the entire distributional range of *A. liliago* in Saxony-Anhalt, Germany. In Scandinavia, *A. liliago* is restricted to dry open grasslands, a type of habitat that has declined dramatically during the last 150 years (Rosquist and Prentice 2002). Similar to the situation in Germany, the Scandinavian populations are fragmented and highly disjunctive (Rosquist and Prentice 2002). Results of an allozyme study of

tetraploid *A. liliago* populations by Rosquist and Prentice (2002), where the majority of the total diversity was explained by the within-population component (89%), are in contrast with our RAPD analysis where a considerable proportion of total diversity was attributed to the among-population component. This could be caused by a stronger degree of fragmentation of the German range of this species due to a higher human population density in Central Europe in the 20th century. Another explanation could be a higher availability of open vegetation in Scandinavia. Differences could also be explained by a difference in ploidy levels. It is possible that polyploidy

buffers the effects of genetic drift, so that it takes much more time for polyploids to develop a strong differentiation between populations, than for diploids. In Germany, both tetraploids and diploids are reported (see Rosquist and Prentice 2002), but no information regarding the ploidy levels of the investigated *A. liliago* populations was available.

The overall differentiation among populations ($\Phi\text{-ST} = 0.41$) is considerably higher than the corresponding mean estimate for species with mixed breeding system subjected to RAPD analyses ($\Phi\text{-ST} = 0.27$; Nybom and Bartish 2000). This suggests a high rate of selfing. The value from our study, however, is in good congruence with the corresponding mean estimates of population differentiation for species with gravity-dispersed seeds ($\Phi\text{-ST} = 0.44$), species with regional geographic range ($\Phi\text{-ST} = 0.43$) and short-lived perennial species ($\Phi\text{-ST} = 0.39$) as summarized by Nybom and Bartish (2000).

Correlation of genetic variability and population sizes

We established a very strong and significantly positive correlation between population size and gene diversity ($r = 0.91$). Therefore, it can be assumed that, apart from population size, the genetic diversity in our sample was only weakly influenced by a number of important demographic and genetic factors. Although our results should be considered as preliminary, and more data is necessary to clarify the details of genetic structure for populations of *A. liliago* in the area of our study, we can nevertheless assume a certain degree of demographic stability and genetic equilibrium for populations from our sample, at least for those with census sizes of more than 10^3 ramets. A positive correlation between genetic variation and population size, using RAPD, was also demonstrated for *Gentianella germanica* (Fischer and Matthies 1998), *Senecio vulgaris* (Müller-Schärer and Fischer 2001), *Sesleria albicans* (Reisch et al. 2003) and *Dictamnus albus* (Hensen and Oberprieler 2005). In a meta-analysis of the relationships between plant population size, genetic variation and fitness reported in studies published between 1987 and 2005, the mean correlation between population size and genetic variation was found to be significantly positive (Leimu et al. 2006).

Plant fitness and pollinator community composition

We found no strong correlation between reproductive output, measured by the mean number of seeds per ramet, and genetic variation and population size, respectively. In addition, Jackel (1999) also found no positive correlation between the reproductive output (fruit and seed set) and population size of 5 *A. liliago* populations ranging in size from 35 to 5,000 flowering ramets. In contrast to our results, a positive correlation of genetic variation and fruit and seed set was found in *Gentianella germanica* (Fischer and Matthies 1998) and *Cochlearia bavarica* (Paschke et al. 2002). Population size had a significantly positive effect on reproductive success in *Primula vulgaris* (Brys et al. 2004), *Primula veris* and *Gentiana lutea* (Kery et al. 2000), *Arnica montana* (Luijten et al. 2000) and *Aquilegia canadensis* (Mavraganis and Eckert 2001). Leimu et al. (2006) found that the mean correlation between plant population size and fitness was generally significantly positive in self-incompatible plant species, but not significant in self-compatible plant species.

Our detailed analysis of fitness parameters in the sampled populations of *A. liliago* revealed numerous other interrelated associations. Some of these could be a result of greater pollination success in larger populations. Although no strong correlations were found between the reproductive output and population size or genetic diversity, the number of flowering individuals and number of flowers per inflorescence seem to influence the relationships between population size and seed set success. This may suggest disrupted pollination when the abundance of flowering individuals and flowers is reduced. Noteworthy, flower visits by the specialized pollinator species *Merodon rufus* (Jentzsch and Köberlein 2000) and by solitary bees were relatively frequent and significantly associated with population size. Following the trends of increased isolation and decreased numbers of ramets in populations of *A. liliago*, both abundance and species richness of flower visiting solitary bees decreased and *Merodon rufus* is completely absent in small populations. In small populations mainly unspecialized pollinators like ants can be found (see also Peterson et al. 2002). Our results therefore strongly indicate that the pollinator community composition, which depends on the population size

of *A. liliago* affects the pollination success and is associated with the reproductive output.

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