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Kaisa Mustajärvi

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Human induced changes in the landscape and resulting habitat destruction is endangering the survival of numerous plant species. In this thesis I used *Lychnis viscaria* (Caryophyllaceae), a perennial herb, as a model plant to study some of the genetic and ecological factors related to habitat fragmentation that may influence the viability of small plant populations. *L. viscaria* is common in Southern Finland but occurs in small, endangered populations in Central Finland. Small and isolated populations had less genetic variation, measured with allozymes, but the viability of individuals in these populations (germination rate, seedling mass or seed yield) was not lower than in large populations. However, in longer time scale the loss of genetic variation in small populations may endanger their ability to adapt to changing environment. Although levels of allozyme variation do not necessarily reflect the levels of adaptively significant variation, in *L. viscaria* a relationship between levels of allozyme variation and morphological variation was found. However morphological population differentiation did not reflect the allozyme differentiation. Habitat fragmentation can also affect the mutualistic plant-pollinator interactions. The pollinator visitation rates were higher in large and, surprisingly, sparse populations. Higher visitation rates in sparser populations were probably due to their larger area and inflorescence size. Pollinator behaviour was also affected by plant density. The changes in the plant-pollinator relationship did not directly affect reproductive success of the plants, but probably lowered the quality of seeds by increasing inbreeding depression. The study on the expression of inbreeding depression revealed that inbred populations may be adapted to inbreeding, expressing less inbreeding depression at early life stages (germination), but may still express relatively high inbreeding depression at later stages. This study provides information to be applied in modern conservation biology, when designing management plans and policies for endangered plant populations.

Key words: Genetic variation; fragmentation; inbreeding depression; *Lychnis viscaria*; morphological variation; plant-pollinator interaction; population size.

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CONTENTS

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following publications, which will be referred in the text by their Roman numerals. I have performed a significant proportion of work for papers I-V. I have planned a significant portion and personally written papers II-IV. For paper V I have been the main writer.

I Lammi, A., Siikamäki, P. & Mustajärvi, K. 1999. Genetic diversity, Population size, and fitness in central and peripheral populations of a rare plant *Lychnis viscaria*. Conservation Biology 13: 1069-1078.

II Mustajärvi, K. & Siikamäki, P. Allozyme and morphological variation in relation to population size in common garden and natural populations of *Lychnis viscaria*. Manuscript submitted.

III Mustajärvi, K., Siikamäki, P. Comparing distribution of morphological and allozyme variation in locally rare plant, *Lychnis viscaria*. Manuscript.

IV Mustajärvi, K., Siikamäki, P., Rytkönen, S. & Lammi, A. 2001. Consequences of plant population size and density for plant-pollinator interactions and plant performance. Journal of Ecology (in press)

V Mustajärvi, K., Siikamäki, P. & Åkerberg, A. Expression of inbreeding depression in *Lychnis viscaria*: effects of population mating history and nutrient availability. Manuscript.

1 INTRODUCTION

Due to activities of man, an ever-increasing number of plant populations are becoming small and fragmented. In many parts of the world habitat destruction and fragmentation has been an increasingly dominant process that has shaped landscapes over the last 100-150 years, and has endangered the survival of numerous plant species. Habitat fragmentation may have dramatic impact on both ecological and evolutionary dynamics of the species (Holsinger 1993). Although most plant species are at least at some scale patchily distributed due to their sedentary habit and the spatial heterogeneity of the environment, the patchiness has been further increased through habitat destruction and fragmentation by humans (Olesen & Jain 1994, Schemske et al. 1994). Formerly connected populations are restricted to small fragments separated by unsuitable habitats. These remnants face increased risk of extinction, because reduced size and increased isolation between populations increase the effects of stochastic environmental, demographic, and genetic processes that influence population persistence and make them more vulnerable to environmental catastrophes (Shaffer 1981, 1987). Reduced population size and increased isolation may also lower population viability by affecting the ecological interactions between plants and its flower visitors (Olesen & Jain 1994), herbivores (Kareiva 1985, Bach 1988) and pathogens (Jennersten et al. 1983). In this thesis I use a locally rare perennial, animal-pollinated plant *Lychnis viscaria* as a model plant to study the genetic (reduced variation, population differentiation, inbreeding, reduced population viability) and ecological (plant-pollinator interactions) consequences of reduced population size.

1.1 Peripheral vs. central populations

Currently great deal of resources are spent protecting peripheral populations of species that are not threatened globally. These peripheral populations have several characters in common; they are small, isolated, and occur in ecologically marginal habitats. The conservation value of these populations is sometimes

questioned as, due to their characteristics, they are considered less viable and depleted of genetic variation. Lesica and Allendorf (1995) have suggested that because peripheral population occur in ecologically marginal habitats, they may posses genetic variation and adaptations not present in the main distribution area, and serve as candidate populations for local adaptation and even speciation events. Thus, these populations would be of great conservation value. If the environment changes dramatically (e.g. global warming) the survival of a species, or applicable character for an economically important species, may be depended on the adaptations that have arisen in these peripheral areas. Therefore in addition to focusing on saving the evolutionary potential of endangered species, the issue is relevant for identifying genetically divergent populations of widespread species.

1.2 Genetic variation (I, II, III)

Previously conservation strategies for endangered species were aimed primarily at preservation of the individuals and their habitats, and such aims are still the most crucial ones to secure the existence of species in the short term. Recently however, interest have arisen in the genetic effects of population reduction, such as inbreeding and genetic erosion, and their consequences for fitness, population viability and regional species persistence (Barrett & Kohn 1991, Ledig 1992, Young et al. 1996). Population genetic theory predicts that small isolated populations will loose genetic variation and become increasingly differentiated due to founder effects, increased random genetic drift, and reduced inter-population gene flow. Increased inbreeding may lower heterozygosity and cause inbreeding depression.

Millar and Libby (1991) have explained how the loss of variation can affect fitness and viability in every level of organisation of genetic variation: 1) at the level of individuals, loss of variation (increasing homozygosity) can lead to inbreeding depression that is often related to severe decline in individual viability (I and V), 2) at the population level, where variation is often related to local environments, reduction in genetic variation or other disruptions of the local gene pool may decrease the chance of adapting to new environment (II), 3) at the species level, loss of diverse populations reduces the potential of the species to respond to environmental changes at regional and global scales (III).

Studies on levels and distribution of genetic variation using neutral markers (e.g. allozymes) provide also valuable information on the history and genetic structure of populations, the mating system, and extent of reproductive isolation between populations. Thus knowledge on the distribution and levels of genetic variation within and between populations is crucial, if informed management decisions are to be made and the evolutionary potential of the species to be preserved.

1.3 Allozyme variation and fitness (I)

Numerous studies have used allozymes to study the distribution and levels of genetic variation within and between populations (reviewed in Loveless & Hamrick 1984, Govindaraju 1988, Hamrick & Godt 1990). Measurement of genetic variation with allozymes provides several advantages: 1) allozymes have discrete Mendelian inheritance and interpretation of genetic variation can be made without concern of environmental influences on the expressed genotype, 2) codominance of alleles allows direct calculation of allele and genotype frequencies, 3) the same allozyme loci can be analysed in several populations or across related species, which allows direct comparison of the levels and distribution of genetic variation (Hamrick et al. 1991). The large number of previous studies also give an opportunity to compare levels of variation among species.

In paper I we studied the levels and distribution of allozyme variation in *Lychnis viscaria* and the consequences of reduced population size and peripherality of population for genetic variation and fitness. Currently the importance of genetic diversity, population size, geographic location of populations, and the effects of these traits on fitness of individuals within a population, and thus population viability, is unclear (Schemske et al. 1994, Vrijenhoek 1994, Fischer & Matthies 1998). However, fitness components such as germination rate, seedling growth, and seed production, which are subjected to selection, are crucial for population persistence (e.g. Menges 1991).

1.4 Morphological variation (II, III)

When concerned about the evolutionary potential and the ability of a population to adapt to environmental changes, it is important to know about the levels and distribution of adaptive significant variation. It has been argued that allozyme variation may not be well correlated with ecologically and evolutionary relevant variation, such as variation in quantitative traits (Lande & Barrowclough 1987, Goodnight 1988, Podolsky & Holtsford 1995). Neutral allozyme variation within populations may vary with population size differently than adaptively significant variation such as that in genes that control for some morphological traits (II). Population differentiation measured with allozymes may give divergent results to differentiation observed for morphological traits (III). This is expected partly due to genetic basis of these traits (allozymes controlled by single loci, quantitative traits by multiple loci) and the fact that selection is expected to be the major force in shaping adaptively significant traits, while random genetic processes such as genetic drift, founder effects and bottlenecks are considered as the predominant factors in shaping neutral marker variation.

However, the measurement of morphological variation without the

confounding effects of environmental variation requires labour-intensive common garden studies, and neutral markers are used today as they would reflect the variation for adaptive traits as well. However, relatively few studies are available on the relationship between morphological and allozyme variation, especially on the level of variation preserved within populations and relationship between population size and morphological variation (II, III).

1.5 Plant-pollinator interactions (IV)

In addition to genetic effects, fragmentation can affect the ecological interactions between plants and other organisms, such as the mutualistic relationship with pollinators (McKey 1989, Ratchcke & Jules 1993, Aizen & Feinsinger 1994). In addition to reducing population size (individual numbers), habitat fragmentation often reduces population density (increase distance between individuals). Both size and density of a population may affect pollinator visitation rates, as smaller and sparser populations are expected to be less attractive to pollinators. This may result in reduced pollination success and as a consequence low seed set (Lamont *et al.*1993, Fischer & Matthies 1998).

The changes in plant-pollinator relationship may also have genetic consequences. If pollination success is reduced in self-compatible plants, selfing will increase. In sparse population pollinators are also more likely to move within an inflorescence than to switch between plants, increasing geitonogamous self-fertilisation (reviewed in Handel 1983, de Jong et al. 1993). Thus in small and sparse populations inbreeding may be increased and inbreeding depression may lower population viability.

Thus both reduced population size and density can affect both quality and quantity of seeds. Both the size and density of a population are know to affect pollination and subsequent reproductive performance (Sih & Baltus 1987, Feinsinger et al. 1991, Kunin 1993, 1997, Boch & Waser 1999), but due to strong correlations between these two factors in natural populations (Ågren 1996) experimental manipulations are needed to separate their effects. Such experimental studies are currently rare, thus in paper IV we studied these effects experimentally.

1.6 Inbreeding depression (V)

When plant population size reduces, the probability and level of inbreeding may increase. In self-compatible species, inbreeding is likely to increase due to both reduced number of possible mating partners and increased level of selfing as a result of reduced pollination success or increased geitonogamy (pollination between flowers within an individual) (Olesen & Jain 1994). The negative effects of inbreeding have been documented widely (Charlesworth &

Charlesworth 1979, Lloyd 1979, Lande and Schemske 1985 and references therein). However, predicting the magnitude of inbreeding depression in any given population remains difficult (Husband & Schemske 1996), partly due to the limited understanding of the genetic basis of inbreeding depression (Charlesworth & Charlesworth 1987, Charlesworth et al. 1990) and partly the fact that the magnitude of inbreeding depression may vary according to mating history, life span, current and past population size and the environmental conditions where it is measured (e.g. Dudash 1990, Norman et al. 1995).

Inbreeding depression may be caused by the expression of deleterious recessive alleles, overdominant loci, or both. Current evidence enforces the role of partially recessive deleterious alleles in determining the level of inbreeding depression (Charlesworth & Charlesworth 1987, Johnston & Schoen 1995, Willis 1999). Through generations of inbreeding deleterious recessive alleles are exposed to selection, and therefore may be purged (Lande and Schemske, 1985). Thus, selfing populations or populations with history of inbreeding may express lower levels of inbreeding depression after selfing than predominantly outcrossing populations (e.g. Barrett & Charlesworth 1991, Husband & Schemske 1996). However, some recent studies have also reported considerable inbreeding depression in selfing populations (Byers & Waller 1999 and references there in) and that purging may be an inconsistent force within populations (Byers & Waller 1999, Willis 1999).

Inbreeding depression in early life history traits have been suggested to be caused by a few recessive lethal alleles and that are effectively purged through inbreeding, while inbreeding depression in later life stages of life-cycle is often caused by mildly deleterious alleles that are not as effectively purged (Husband and Schemske 1996). Thus, in outbred populations inbreeding depression after selfing is expected to be expressed both late (growth and reproduction) and early (germination) in life cycle. While selfers are expected to express inbreeding depression late in life-cycle. Thus inbreeding depression should be measured over the whole life-cycle of the plant (Charlesworth & Charlesworth 1987).

The environmental conditions can also affect the levels of observable inbreeding depression. Usually stronger inbreeding depression is expressed harsher conditions (Dudash 1990, Schmitt & Ehrhardt 1990, Eckert & Barret 1994), but this in not always the case (see Hauser & Loeschcke 1996, Crnokrak & Roff 1999 and references therein). The studies on inbreeding depression should preferably be conducted in natural habitats (Schemske 1983, Eckert & Barrett 1994) or at least over different environmental conditions to estimate the effect of environment on the expression of inbreeding depression (e.g. Schemske 1983, Dudash 1990).

2 MATERIALS AND METHODS

2.1 Study species and areas

Lychnis viscaria L. (*Viscaria vulgaris* Bernh., Caryophyllaceae) is a perennial herb that was much more common in Finland a century ago, when old fashioned agriculture constantly created and maintained suitable dry meadows for the species to occupy. Now a few peripheral populations, situated on rocky outcrops, exist in Central Finland (Välivaara et al. 1991); of the 29 previously known populations known only 8 were found to still exist. In the Southern Finland *L. viscaria* is still fairly common occupying dry meadows and roadsides. It occurs in fairly distinct patches of a few to thousand individuals throughout northern and central Europe, the main distribution area extending up to the 62nd latitude. Few isolated populations are found up to the 68th latitude. The flowers of *L.viscaria* are protandrous and pollinated by insects, mainly bumblebees and butterflies (Wilson et al. 1995,Jennersten 1988), but despite protandry also self-pollination occurs (Jennersten et al. 1988, K. Mustajärvi, unpublished data). The plant produces 1-50 flowering stems each bearing about 20-25 flowers. The seeds are dispersed by gravity. *L. viscaria* over-winters as a green rosette.

The distribution area and characteristics of *L. viscaria* are typical of several plant species that occur in northern Europe and America. As a consequence it can be considered as a suitable model organism for several rare, perennial, and hermaphroditic plants that have colonised their habitats since the last glaciation. In this thesis, populations located both in the central and in the peripheral distribution area of *L. viscaria* were studied. In the surroundings of Tampere, (61° 30'N, 23° 45'E) situated at the main distribution area, three populations were sampled (Nokia, Epilä, Kalevankangas, hereafter cited as central populations) and in peripheral area, in the surroundings of Jyväskylä (62° 15'N, 25° 45'E), 150 km NE of Tampere all eight currently existing populations were studied (hereafter cited as peripheral populations).

2.2 Genetic variation

2.2.1 Electrophoretic assay (I, II, III, V)

Several seedlings per maternal plant were grown in a laboratory, and about 30 (range 21-30) seedlings per population (from 1 to 2 seedlings from different maternal plants) were randomly chosen for analysis, except for peripheral population Kotimäki, where all 7 flowering individuals where sampled. The level of genetic variation in the populations was assessed by starch gel electrophoresis as described in Wendel and Weeden (1989) and May (1992). Three gel and electrode buffer systems, as described in Siikamäki et al. (1999), were used to resolve the isozymes, and a total of 13 enzymes and 17 loci were screened. Six of the screened loci (F-EST-1, F-EST-2, GPI-2, PGM-2, SKD-1, SOD-1) were polymorphic. Genetic diversity in each population was assessed as Nei's (1978) unbiased estimate of expected heterozygosity (H_{exp}, I, II, III, V), observed heterozygosity (H_{obs}, I), percentage of polymorphic loci (P, I), and mean number of alleles per locus (A, I). The distribution of variation within and among populations, and estimation of inbreeding coefficients were studied with F-statistics according to Weir and Cockerham (1984)(I, III). To illustrate the genetic relationships between populations an UPGMA dendrogram using Nei's genetic distance was constructed (I, III). The association between (Nei's, 1972) genetic distance and the geographic distance between populations was analysed with Mantel's test.

2.2.2 Genetic variation, population size and fitness (I)

The population size was determined as the number of flowering rosettes in summer 1996. To study the relationship between fitness of individuals within populations and the amount of genetic variation, we measured the mean seed germination percentage, seedling dry biomass, and seed number per capsule of the 11 *L. viscaria* populations. In addition to populations measured for genetic variation, we measured plants in four additional central populations for the above-mentioned fitness traits to get a larger sample when studying the relationship between population size and fitness.

2.2.3 Morphological variation in common garden and in natural populations (II, III)

To study how allozyme variation within and among *Lychnis viscaria* populations is related to the amount and distribution of morphological variation, the same seed material collected to measure genetic diversity with allozyme electrophoresis was used to grow seedlings to establish the common garden in the Laukaa Research and Elite Plant Station (62° 15'N, 25° 30'E). Seedlings were grown randomly arranged in standard greenhouse conditions (21°C, 16 hours light, 8 hours darkness). In spring 1996 about 40 seedlings (1-2 per maternal plant) per population were transplanted outdoors. Because only

some of the plants flowered in 1996, the morphological characters were measured in 1997 when all the plants flowered. In 1997, 40 randomly chosen wild individuals in each population were marked with small flags. These individuals and the plants in the common garden were measured for several growth related (rosette width, leaf length etc.), flower size related (e.g. petal length, ovary length), and reproductive (flower number) characters to estimate the amount of morphological variation within population in relation to population size (II) and the distribution of morphological variation between populations (III). The measurements were conducted, both in common garden and in natural sites, at approximately the same phenological stage in all populations.

2.2.4 Genetic variation, morphological variation and population size

The level of morphological variation within the 11 studied *L. viscaria* populations for the 15 characters were measured as CV (coefficient of variation). The populations were ranked according to their CV values for each trait and the mean of rank the values was used as a measure of total morphological variation within population. The mean CV ranks in common garden and natural sites were then compared to each other, and in relation to population size and level of allozyme variation.

2.2.5 Distribution of morphological and genetic variation

The distribution of morphological variation was compared to distribution of allozyme variation by calculating Mahalanobis distances between populations (separately for common garden and natural sites) based on morphological data and comparing them with Nei's genetic distances with Mantel's test. Mantel's test was also used to test the association between geographic distances and Mahalanobis distances between populations. UPGMA dendrograms based on Mahalanobis distances of morphological traits were constructed separately for common garden and natural sites, and visually compared to a dendrogram based on genetic data. To find applicable and reliable methods for conservation purposes I focused on the relationships among population size, allozyme and morphological variation (II) and population differentiation with respect to different measures of variation (III).

2.3 Population density and population size: consequences for plant-pollinator interactions and plant fitness? (VI)

To study the effects of plant population size and density on pollinator visitation rate, pollinator behaviour, and consequent plant reproductive success, I established artificial *L. viscaria* populations in Laukaa Research and Elite Plant Station. Greenhouse grown seedlings from seeds collected from population Vaaruntie, were planted to form large (100 individuals) and small (10

individuals) populations of two densities (dense = distance between individuals 20cm, sparse = distance between individuals 80cm). Three replicates of each population type: small dense (total area 0.84m^2), small sparse (4.64 m^2), large dense (6.40 m^2), and large sparse (54.88 m$^{2)}$ were established, separated by at least 200m.

Two kinds of observations were conducted to study pollinator visitation rates and pollinator behaviour. First, the diversity and visitation rates of pollinators were examined by observing patches of ten individuals in different types of populations. Second, the behaviour of pollinators (the number of plants visited, the number of flowers probed per plant, and visitation time) was studied. After the flowering season we measured the reproductive success of the plants as the percentage of flowers that developed into capsules (fruit set), mean seed weight, number of seeds set per capsule, and seed production (no. of seeds per capsule x no. of capsules in the longest flowering stem).

2.4 Inbreeding depression

I studied the differences in the expression of inbreeding between populations Kanavuori (population size = 82, H_{exp} = 0.028), Iso-Salmijärvi (population size = 124, H_{exp} = 0.002), and Vaarunvuori (population size = 250, H_{exp} = 0.058). In summer 1996, five flowers were pollinated with self-pollen and five flowers with cross-pollen in each of 15-16 bagged maternal plants in each population. The seeds were collected when ripen, and the expression of inbreeding depression in the three populations was measured as the difference between inbred and outbred progeny in germination percentage, seedling growth rate, seedling size, seedling survival, over-wintering survival, stem number, capsule number, and seed number.

To determine germination percentage, 50 outcrossed and 50 selfed seeds per maternal plant were germinated in Petri dishes. To determine seedling growth rate, seedling size and mortality, seedlings were grown under standard greenhouse conditions in Laukaa Research and Elite Plant Station. The effect of nutrient availability on the expression of inbreeding depression was determined by fertilising half of the seedlings with liquid standard fertiliser after six weeks of growth. In May 1997, the seedlings were planted out in the field in Laukaa Research and Elite Plant Station. To prevent pollen from mixing between populations, the seedlings were planted separately in three similar locations separated by 250 m of open field. The fertiliser treatment was continued in the field by adding fertiliser sticks close to the roots of formerly fertilised seedlings three weeks after planting. Since only some of the plants flowered 1997, the reproductive success as stem, capsule, and seed number was measured in 1998, when all the plants flowered.

The data was analysed with split-plot ANOVA with population, cross and fertiliser treatment as fixed factors and maternal family as random factor nested within populations. In addition, we calculated inbreeding depression as

$$\delta = 1 - \frac{w_s}{w_o}, \text{ if } w_s \leq w_o$$

$$\delta = 1 - \frac{w_o}{w_s}, \text{ if } w_s > w_o$$

for each of the measured fitness components separately and for cumulative fitness of each population means. To study if the inbreeding coefficients deviated significantly from zero also family level inbreeding depression coefficients were calculated.

2.5 Data analysis

I used SPSS version 9.0 or 10.01 for Windows for all data analysis, except for cluster analysis and calculation of Mahalanobis distances, which I calculated with SAS. Non-parametric test were applied when the assumptions of parametric tests were not met. In the analysis of allozyme data I used BIOSYS program, except for F-statistics, which were calculated by J. Goudet with FSTAT.

3 RESULTS AND DISCUSSION

3.1 Genetic diversity, population size, and fitness (I)

The overall level of genetic variation in *Lychnis viscaria* (grand mean: H_{exp}= 0.056, central populations: H_{exp} = 0.114, peripheral populations H_{exp} = 0.034) was low compared to other species with similar life-history (listed by Hamrick & Godt 1989), but the levels varied substantially among populations (H_{exp}= 0.000 – 0.116). F-statistic values suggested low gene flow and stronger differentiation between populations than could be expected by their geographic distance (mean F_{st} = 0.430). No association was found between genetic distances and geographic distances between populations.

The low level of genetic variation in *L. viscaria* is probably due to bottlenecks in connection with local extinctions and re-colonisation events as a result of Quartenary glaciations (Haraldsen & Wesenberg 1993). Low levels of variation, strong differentiation between populations and lack of correlation between genetic and geographic distances suggests that genetic drift plays a major role in shaping the genetic variation in *L. viscaria*, and populations are differentiating independently of each other and their geographic area or distances. Differentiation was expected, however, as the distances between populations are significant; given the restricted pollen and seed dispersal mechanisms of *L. viscaria* (Wilson et al. 1995) gene flow between populations is likely to be very limited.

The population size was positively correlated with genetic diversity; the smaller populations had less genetic variation than the large ones, presumably because of genetic drift, founder effects, and bottlenecks. The increased inbreeding due to weak pollination success in smaller populations (see IV) may have in part affected the depletion of variation. Our results are in accordance with previous studies (Furnier & Adams 1986, Godt & Hamrick 1993, Kuittinen et al. 1997) and genetic models (Garcia-Ramos & Kirkpatrick 1997) that indicate lower genetic variation in peripheral populations. In addition to other aspects of peripherality, this may be in part caused by the small size of peripheral

populations. Thus the effects of size and peripherality are hard to separate. However, both of the higher isolation and size have probably affected the depletion of variation from the small and peripheral populations of *L. viscaria.* These populations have most likely been isolated for quite long time, and such strong correlation between population size and genetic variation may not be found in populations that have only recently been fragmented and connected to other with frequent gene flow.

The population size and genetic variation were not associated with the fitness traits we measured and no difference in these traits were found between the central and peripheral populations. However, in this study we did not measure longevity or other long term effects on fitness. Previous studies on these relationships between population size and fitness (e.g. Heschel & Paige 1995 vs. Van Treuren et al., Hauser & Loeschcke 1994), and that between allozyme variation and fitness (Ouborg & Van Treuren 1995 vs. Fischer & Matthies 1998) have given controversial results. Correlations between genetic variation and fitness have been stated to be only coincidentally found (Vrijehoek 1994) as the dynamics of allozymes and genes controlling fitness traits may be different (Hedrick & Savolainen 1996).

The viability of small peripheral *L.viscaria* populations is good news to conservation biologists, as also small and genetically depauperate populations may be viable (Van Treuren et al. 1993, Widen 1993, Hauser & Loeschcke 1994, Ouborg & Van Treuren 1995).

3.2 Levels of morphological variation, allozyme variation, and population size (II)

Morphological variation was correlated with the level of allozyme variation. Although the level of allozyme variation within populations was related to population size, the morphological variation was not. The lack of correlation was expected, as the quantitative traits that may be selected for are expected to react to changes in population size differently than variation in neutral single locus traits (Lande & Barrowclough 1987, Foley 1992, Lynch 1996). However, some indication of relationship between population size and morphological variation existed, as the relationship between allozyme variation and morphological variation in natural sites disappeared when population size was controlled for. Thus, the relationship between morphological variation in natural populations and allozyme variation was probably caused by the larger environmental heterogeneity in larger populations that increased phenotypic variation. In common garden genetic variation and morphological variation were correlated even when population size was controlled for, indicating that the relationship between levels of morphological variation and allozyme variation may have some genetic basis. Ouborg et al. 1991 found a similar relationship for *Salvia columbaria* and *Scabiosa pratensis* and stated that this relationship is likely to arise when stochastic effects such as genetic drift,

bottlenecks, and founder events, instead of selection, are the major factors affecting levels of variation.

The level of variation a population expressed in natural environment did not reflect the level of variation in common garden. Thus variable range of phenotypes in natural sites did not reflect high genetic variability for morphological traits, indicating that in *L. viscaria* allozyme data better predicted levels of genetic variation also for morphological traits than measuring phenotypic variation from wild individuals.

3.3 Distribution of morphological variation

The population differentiation was stronger when measured with neutral allozymes (F_{st} = 0.430) than by morphology (Q_{st} = 0.046-0.287 in natural sites, Q_{st} = 0.019-0.207) suggesting that genetic drift instead of selection plays a major role in genetic differentiation of populations in *L. viscaria*. As expected, no connection was found between the distribution morphological variation and distribution of allozyme variation among populations. The clustering of populations based on morphological data (both in common garden and in natural sites) resulted in different population groupings than cluster analysis based on allozyme data, and Mahalanobis distances were unrelated to Nei's genetic distances. The lack of association between the distribution of allozyme and morphological data suggests that relying only on allozyme data when making assumptions on the population differentiation and genetic relationships between populations is questionable. Allozyme data should therefore be used in connection with other type of data (such as life-history, geographical, environmental or morphological).

Neither geographic distance nor any environmental factor (habitat type, shading regime, herbivory) could explain the morphological differentiation patterns of the populations in common garden or in natural populations. The patterns observed in common garden were not related to patterns of morphological diversity observed in natural sites. Thus, obtaining data on differences in quantitative traits for conservation purposes requires common garden studies that are often time- and labour intensive, but according to our results, necessary.

3.4 Plant population size and density: consequences on plant-pollinator interactions and plant fitness

Plants in the large populations were visited significantly more frequently by the bumblebees than plants in the small populations. Thus, as expected but not very often experimentally recorded, the pollination success of plants in small populations may be lower than in large populations. Surprisingly, the visitation rates per plant were higher in sparser populations. Further analysis revealed

that this was mostly due to the larger inflorescence size in sparse populations, but the larger area of sparse populations also probably played an important role in attracting pollinators to these populations.

The pollinators probed significantly more flowers within inflorescence in the sparse populations, which is in accordance with optimal foraging theory (Charnov 1976). As the flight distance between individuals increases, it is more profitable for the pollinators to probe more flowers within an individual than to move between plants. However, the behaviour of pollinators was probably also influenced by the larger inflorescence size in sparse populations as pollinators tend to probe more flowers in larger inflorescences (Klinkhamer et al. 1989, Klinkhamer & de Jong 1990).

The density had effect on reproductive success: plants in sparse populations tended to produce fewer but significantly heavier seeds (per capsule), had higher fruit set, and due to their larger inflorescences, the total seed production was higher in sparse populations. But although visitation rates were higher in large populations, reproductive output of plants was not affected by population size. Thus, pollination success was probably not as important factor as resource availability in determining the reproductive success in self-compatible *L. viscaria*. In self- incompatible species the reduced visitation rates in small populations and increased geitonogamy in sparse populations, would have probably had more severe effects on reproduction.

The better resource availability in sparse populations first increased the visitation rates and influenced pollinator behaviour through larger inflorescences and after fertilisation, granted better seed and capsule production. In nature, such resource mediated density effects may be observed in a population where plants grow with few interspesific competitors (e.g. *L. viscaria* in cracks of rocky cliff, or pioneer species). However, many small fragmented plant populations grow in deteriorating habitats, where density is low due low habitat quality and individuals have small inflorescences. Applied to such occasions, our results suggest that small inflorescences (along with population size and density) are likely to be an important factor in reducing pollination success.

3.5 Expression of inbreeding depression in three *L. viscaria* populations (IV)

The level of inbreeding depression was quite high (cumulative inbreeding: 0.057 - 0.629) for a plant with a mixed mating system, where some degree of inbreeding must be common in all the studied populations. The most pronounced difference between populations in expression of inbreeding depression, reflecting purging of deleterious alleles, was observed for germination percentage. The homozygous and thus probably most inbred population Iso-Salmijärvi, expressed significantly lower inbreeding depression for germination rate than smaller but more heterozygous Kanavuori and large

Vaarunjyrkkä. Thus some purging may have taken place for germination, which was also found for experimental purging experiments by Willis (1999). Inbreeding depression for germination is likely to be to some extent determined by recessive deleterious alleles. Thus, these alleles are easier to purge than those causing inbreeding depression in later stages, which are suggested to be alleles of mildly deleterious effects (Husband and Schemske 1996). Our results on the later stages were consistent with this prediction as no clear differences between populations in expression of inbreeding was found. The population level inbreeding depression varied with the nutrient levels and the effect of fertiliser differed between populations and life stages, but no clear trend of fertilising either increasing or decreasing inbreeding depression could be found. For two of the populations the cumulative inbreeding depression decreased with fertilisation, but for the third population the inbreeding depression was non-existent for the unfertilised plants while fertilised plants expressed relatively high inbreeding depression. Thus, inbreeding depression should be measured under several environments and predictions on the magnitude of inbreeding depression in different environments should be made cautiously.

4 CONCLUSIONS

In this thesis I aimed to study the ecological and genetic consequences of small population size, low population density, and increase isolation between populations. I used *Lychnis viscaria* as a model plant for threatened species with similar life histories. Since *L. viscaria* occurs both in small, threatened peripheral populations and in large central populations, I was also able to compare ecology and genetics between populations of different sizes and location in relation to the main distribution area. Currently, throughout Europe several meadow vascular plants face similar threats as *L. viscaria* as changes in farming practises deteriorate old meadow habitats, reducing population sizes, and increasing their isolation. However, the threatened marginal populations of *L. viscaria* I have studied have been isolated probably for long time and probably always been smaller than the populations in central area. Thus, results obtained here for genetic effects may not directly apply to populations that have only recently been fragmented and are still connected with gene flow. But these results may give indication what might be expected for fragmented populations in the future if their isolation increases and continues. Thus, with these limitations the results of this thesis may be applied to modern conservation biology, when giving management recommendations or prioritising populations of threatened plants for conservation.

The genetic variation in *L. viscaria* was generally low, and correlated with population size, and levels of morphological variation. The low levels of variation were, however, mostly due to small isolated and peripheral populations, in which level of variation was closer that observed for inbreeding species. This indicates that random genetic processes, such as genetic drift, founder events, and bottlenecks leading to loss of variation have indeed stronger influence in smaller populations.

Although the fitness of individuals (seed set per capsule, seedling mass, and seed weight) in small populations was not lower along with decreased genetic variation, the capability of these populations to buffer against environmental fluctuations and to adapt to changing environmental conditions remains questionable. Integrating genetic, demographical, and ecological data with modelling or into population viability analyses may provide information

on the survival of a population in an evolutionary time scale.

The loss of variation and increased homozygosity in smaller populations may also be influenced by the increased inbreeding due to increased levels of selfing in *L. viscaria* as a result of disrupted plant-pollinator interactions. We did not measure outcrossing rates in our study populations, but the reduced pollinator visitation rates in small populations and the pollinators' habit of visiting more flowers within an individual in sparse populations are likely to promote selfing. Thus although the lowered visitation rates did not affect the reproductive success of self-compatible *L. viscaria,* the quality of seeds may be diminished if plants are forced to compensate low pollination success with selfing. More serious reduction in seed set may be expected for self-incompatible species.

Increased inbreeding may purge deleterious alleles and small populations may become more tolerant of inbreeding. However, as in *L. viscaria,* purging is likely to reduce inbreeding depression only for early traits, whereas serious inbreeding depression maybe expressed in the later life stages. As a result also small and inbreeding populations may express strong inbreeding depression endangering their survival.

In this thesis I also studied the relationship between allozyme variation and variation in morphological traits, as neutral allozymes may not reflect adaptive variation that is often primary of interest for conservation purposes. Although the neutral marker diversity and adaptive quantitative traits may react to changes in population size differently, the level of morphological variation measured in common garden correlated with allozyme diversity. This indicates that allozymes may in fact be used in estimating, or at least in predicting the levels of quantitative trait variation within populations where genetic drift has been the major factor in shaping the level of genetic variation. However, allozymes and morphological variation did not show similar distribution of variation and genetic relationships among populations. Thus, although neutral allozymes provide valuable information on genetic processes in and between populations (e.g. gene flow, mating systems, history), they may not be as useful when populations are evaluated for their suitability to particular restoration or reintroduction site, or when populations are evaluated for conservation on the basis of their genetic divergence.

As shown in this thesis, both ecological and genetic processes within populations are affected by population size. Thus, combination of these approaches is necessary when predictions on the fate of a population are cast. Data on these processes can then be applied for example in viability analyses and extinction models to provide valuable information for management planning and decision making in conservation of rare plants.

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YHTEENVETO

Ihmistoiminnasta johtuva elinympäristöjen pirstoutuminen on uhka useiden kasvilajien ja populaatioiden säilymiselle. Etenkin pienet ja eristyneet populaatiot, joissa uhanalaiset kasvit yleensä esiintyvät, ovat herkkiä erilaisille stokastisille, demografisille ja geneettisille tekijöille. Luonnonsuojelubiologian teorioiden mukaan pienistä populaatioista häviää nopeasti geneettistä muuntelua erilaisten satunnaisten prosessien (mm. geneettinen ajautuminen) seurauksena, jolloin populaation kyky sopeutua muuttuviin ympäristöolosuhteisiin heikentyy. Joidenkin tutkimusten mukaan geneettisen muuntelun häviäminen saattaa myös suoraan vaikuttaa populaatioiden yksilöiden elinkykyyn. Pienissä ja eristyneissä populaatioissa myös ekologiset vuorovaikutussuhteet kasvin ja pölyttäjien välillä voivat häiriintyä, jolloin siementuotto heikkenee.

Tutkin geneettisten ja ekologisten tekijöiden vaikutusta pienten populaatioiden elinkykyyn mäkitervakolla (*Lychnis viscaria*, Caryophyllaceae). Mäkitervakko on aikaisheteinen, mutta itsepölytykseen kykenevä monivuotinen kukkakasvi. Se on harvinaistunut viimeisen sadan vuoden aikana vanhanaikaisten viljelymenetelmien, ennen kaikkea ketojen, häviämisen myötä. Laji on suhteellisen yleinen Eteläisessä Suomessa, mutta jo Keski-Suomen korkeudella laji on luokiteltu paikallisesti harvinaistuneeksi (enää 8 populaatiota 29:stä viime vuosisadalla tunnetusta populaatiosta jäljellä). Koska mäkitervakko esiintyy suurissa ja pienissä populaatiossa, se on sopiva laji populaatiokoon vaikutusten tukimiseen.

Geneettinen vaihtelu, jota mitattiin allotsyymielektroforeesimenetelmällä, oli hyvin vähäistä tutkituissa mäkitervakkopopulaatioissa. Esiintymien välillä on luultavasti vain vähän geeninvaihtoa, sillä ne olivat hyvin erilaistuneita toisiinsa verrattuna. Kuten oletettiinkin, äärilevinneisyysalueen pienissä populaatiossa oli vähemmän muuntelua kuin keskeisen levinneisyysalueen suurissa populaatiossa. Kasvien elinkyky esiintymissä, mitattuna siementen keskimääräisenä itävyytenä, taimien kasvukykynä ja kasvien siementuottona, ei ollut kuitenkaan riippuvainen populaatiokoosta tai geneettisen vaihtelun määrästä. Pienet ääripopulaatiot olivat siis yhtä elinkykyisiä kuin keskusalueen suuret populaatiot. Pienten populaatioiden kyky sopeutua muuttuviin ympäristöolosuhteisiin saattaa kuitenkin olla heikentynyt koska geneettisen vaihtelun määrä on vähentynyt.

Allotsyymimuuntelun määrä ei kuitenkaan anna välttämättä parasta kuvaa populaation sopeutumiskykyyn vaikuttavasta geneettisestä muuntelusta, vaikka sitä käytetäänkin yleisesti kuvaamaan geneettistä vaihtelua. Koska luonnonvalinta ei yleensä vaikuta allotsyymimuunteluun, allotsyymimuuntelun suhde populaatiokokoon voi olla erilainen kuin luonnonvalinnalle alttiina olevan muuntelun, esim. kasvin morfologisissa piirteissä. Siksi mittasimme myös morfologisen muuntelun määrää ja populaatioiden morfologisia eroja samoissa populaatioissa, joissa ensimmäinen osatutkimus oli tehty. Morfologisen muuntelun määrä populaation sisällä ei korreloinut populaatiokoon kanssa, mutta yllättävää kyllä, allotsyymimuuntelun määrän kanssa. Niinpä

ainakin mäkitervakolla allotsyymimuuntelu kuvasi myös morfologisen muuntelun määrää populaation sisällä. Populaatioiden välinen erilaistuminen allotsyymeillä mitattuna ei kuitenkaan vastannut niiden morfologista erilaistumista. Siksi allotsyymitutkimuksesta saatua tietoa ei yksin tulisi käyttää tehtäessä päätelmiä populaatioiden välisistä geneettisistä eroista. Esimerkiksi jos halutaan suojella mahdollisimman paljon toisistaan eroavia populaatioita tai valita mahdollisimman paljon alkuperäistä muistuttavia populaatiota ennallistamissuunnitelmia varten.

Väitöskirjani neljännessä osatutkimuksessa käsittelin kasvipopulaation koon ja yksilötiheyden vaikutusta kasvi-pölyttäjä vuorovaikutussuhteeseen. Tätä tutkimusta varten perustimme keinotekoisia populaatioita Laukaan maataloudentutkimus- ja tervetaimiaseman pelloille. Pienissä populaatioissa vieraili merkitsevästi vähemmän pölyttäjiä kasviyksilöä kohden kuin suurissa populaatiossa. Kasvien siementuotto ei kuitenkaan eronnut pienten ja suurten populaatioiden välillä. Koska mäkitervakko kykenee tuottamaan siemeniä myös ilman pölyttäjiä, pienten populaatioiden kasvit kykenivät luultavasti itsepölytyksellä kompensoimaan huonomman pölytysmenestyksensä. Yllättävä tulos oli, että harvoissa populaatiossa vieraili enemmän pölyttäjiä kuin tiheissä kasvipopulaatioissa. Pölyttäjien oli ilmeisesti helpompi löytää suuremmalle alalle levittäytyvät harvat populaatiot. Koska harvoissa populaatiossa yksilöiden välinen kilpailu oli vähäisempää kuin tiheissä populaatioissa, kasvoivat kukinnot suuremmiksi, mikä myös houkutteli pölyttäjiä harvoihin populaatioihin. Populaation tiheys vaikutti myös pölyttäjien käyttäytymiseen. Harvoissa populaatiossa pölyttäjät vierailivat useammassa kukassa per kasviyksilö kuin tiheissä populaatiossa, sillä pölyttäjien on kannattavampaa vierailla useammassa kukassa kukinnon sisällä kuin lentää yksilöiden välillä, kun kasviyksilöiden välinen etäisyys on suuri. Myös kukintojen suuri koko harvoissa populaatiossa vaikutti tähän pölyttäjien käytökseen. Vaikka pölyttäjien käytös pienissä ja harvoissa populaatiossa ei suoranaisesti heikentänyt kasvien lisääntymismenestystä, pölyttäjien käytös saattoi kuitenkin lisätä autogamista ja geitonogamista itsepölytystä ja huonontaa siementen laatua.

Pienissä populaatioissa sukusiitos voi yleistyä, lisääntyneen geitonogamisen ja autogamisen itsepölytyksen lisäksi, myös siksi että mahdollisia lisääntymiskumppaneita on vähän ja sukulaiset lisääntyvät lopulta välttämättä keskenään. Neljännessä osatutkimuksessa käsittelin sukusiitosheikkouden ilmenemistä kolmessa mäkitervakkopopulaatiossa. Vaikka sukusiitoksesta seuraava sukusiitosheikkous onkin hyvin tutkittu ja yleiseksi havaittu ilmiö, on sukusiitosheikkouden voimakkuuden ennustaminen vaikeaa. Sukusiitosheikkouden voimakkuuteen ja ilmenemisen ajankohtaan vaikuttaa mm. populaation lisääntymishistoria ja ympäristö, sekä sukusiitosheikkouden geneettinen tausta. Tutkimuksessa todettiin että populaatiossa, jossa sukusiitos on ollut yleisintä itsesiitoksesta seuraava sukusiitosheikkous oli vähäisempää elinkierron alkuvaiheessa (siementen itävyys) kuin muissa populaatiossa. Tämä johtuu luultavasti siitä että populaatiot voivat "sopeutua" sisäsiitokseen siten että elinkierron alkuvaiheessa sukusiitosheikkoutta aiheuttavat hyvin haitalliset tai jopa letaalit resessiiviset alleelit ovat luonnon valinnan kautta hävinneet sukupolvia jatkuvan itsepölytyksen myötä. Elinkierron myöhäisemmässä

vaiheessa sukusiitosheikkous johtuu sen sijaan yleisemmin lievästi haitallisista alleelleista, jotka eivät "puhdistu" sukusiitoksen myötä yhtä helposti kuin hyvin haitalliset mutaatiot. Myös mäkitervakolla havaittiin, että myöhemmissä elinkierron vaiheissa (siementuotto) ei populaatioiden välillä ollut eroja sukusiitosheikkouden ilmenemisessä, niiden erilaisesta lisääntymishistoriasta huolimatta. Niinpä pienissä populaatioissa lisääntyneestä sukusiitoksesta johtuva sukusiitosheikkous saattaa heikentää yksilöiden elinkykyä ja populaation riski hävitä kasvaa, vaikka osa sukusiitosheikkoutta aiheuttavista alleeleista karsiutuukin populaatiosta.

Pienestä populaatiokoosta seuraavat geneettiset ja ekologiset tekijät siis vuorovaikuttavat toistensa kanssa ja saattavat heikentää populaation elinkykyä. Tässä tutkimuksessa saatuja tuloksia voidaan hyödyntää luonnonsuojelubiologiassa mm. suunniteltaessa strategioita pienten kasvipopulaatioiden suojelemiseksi.

REFERENCES

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

Ågren, J. & Shemske, D. 1993. Outcrossing rate and inbreeding depression in two annual monoecious herbs, *Begonia hirsuta* and *B. semiovata.* Evolution 47:125-135.

Aizen, M.A. & Feinsinger, P. 1994. Forest fragmentation, pollination, and plant reproduction in a Chaco dry forest. Ecology 75: 330-351.

Bach, C.E. 1988. Effects of host plant patch on herbivore density: patterns. Ecology 69: 1090-1102.

Barrett, S.C.H. & Charlesworth, D. 1991. Effects of a change in the level of inbreeding on the genetic load. Nature 352: 22-524.

Barrett S.C.H. & Kohn, J.R. 1991. Genetic and evolutionary consequences of small population size in plants: implications for conservation. In: Falk, D.A. & Holsinger, K.E. (eds), Genetics and conservation of rare plants: 3-30. Oxford University Press, New York.

Byers, D. L. & Waller,D. M. 1999. Do plant populations purge their genetic load? Effects of populaton size and mating history on inbreeding depression. Annual Review in Ecology and Systematics. 30: 479-513.

Bosch, M & Waser N.M. 1999. Effects of local density on pollination and reproduction in Delphinium nuttalianum and Aconitum columbianum (Ranunculaceae). American Journal of Botany 86: 871-879.

Charlesworth, D. & Charlesworth, B. 1979. The evolutionary genetics of sexual systems in flowering plants. Proceedings of the Royal Society, Biological Sciences London B 205: 513-530.

Charlesworth, D. & Charlesworth, B. 1987. Inbreeding depression and its evolutionary consequences. Annual Review of Ecology and Systematics18: 237-268.

Charlesworth, D., Morgan M.T. & Charlesworth, B. 1990. Inbreeding depression genetic load, and the evolution of outcrossing rates in a multilocus system with no linkage. Evolution 44: 1469-1489.

Charnov, E. 1976. Optimal foraging: the marginal value theorem. Theoretical Population Biology, 9: 129-136.

Crnokrak P. & Roff D.(1999) A. Inbreeding depression in the wild. Heredity 83: 260-270.

de Jong, T.J., Waser, N.M. & Klinkhamer, P.G.L. (1993) Geitonogamy: the neglected side of selfing. Trends in Ecology and Evolution 8: 321-325.

Dudash M. (1990) Relative fitness of selfed and outcrossed progeny in a self-compatible, protandrous species, Sabatia angularis L. (Gentianaceae): a comparison in three environments. Evolution 44, 1129-1139.

Eckert, C. G., & Barrett S.C.H. 1994. Inbreeding depression in partially self-fertilising Decodon verticillatus (Lythraceae): Population-genetic and experimental analyses. Evolution 48: 952-964.

Feinsinger, P., Tiebout III, H.M. & Young, B.E. 1991. Do tropical bird-pollinated plants exhibit density-dependent interactions? Field experiments. Ecology

72: 1953-1963.
Fenster, C.B. & Dudash, M.R. 1994. Genetic cosiderations for plant population restoration and conservation. in Bowles, M.L. & Whelan, C. (eds), Recovery and restoration of endangered species: 34-61. Cambridge University Press, Oxford, England.
Fischer, M. & Matthies, D. 1998. RAPD variation in relation to population size and plant fitness in the rare *Gentianella germanica* (Gentianaceae). American Journal of Botany 85: 811-819.
Foley, P. 1992. Small population genetic variability at loci under stabilising selection. Evolution 46: 412-429.
Furnier, A., & Adams, W.T. 1986. Geographic patterns of allozyme variation in Jeffrey pine. American Journal of Botany 73:1009-1015.
Garcia-Ramos, G. & Kirkpatrick, M. 1997. genetic models of adaptation and gene flow in peripheral populations. Evolution 51: 21-28.
Godt, M.J. & Hamrick 1993. Genetic diversity and population structure in Tradescantia hirsuticaullis (Commenaliceae). American Journal of Botany 80: 959-966.
Goodnight, C.J. 1988. Epistasis and the effect of founder events on the additive genetic variance. Evolution 42: 441-545.
Govindaraju, D.R. 1988. Relationship between dispersal ability and levels of gene flow in plants. Oikos 52: 31-35.
Hamrick, J.L. & Godt, J.W. 1990. Allozyme diversity in plant species. In: Brown, A.H.D., Clegg, M.T. Kahler, A.L. & Weir, B.S. (eds) Plant population genetics, breeding and genetic resources: 43-63. Sinauer Associates, Sunderland, Massachusetts.
Hamrick, K.E., Godt, M.J., Murawski, D.A. & Loveless, M.D. 1991. Correlations between species traits and allozyme diversity: implications for conservation biology. In: Falk, D.A. & Holsinger K.E. (eds). Genetics and conservation of rare plants: 76-86. Oxford University Press, Oxford, UK.
Handel, S.N. 1983. Pollination ecology, plant population structure, and gene flow. In: Real, L. (ed), Pollination biology: 163-211. Academic Press.
Haraldsen, K.B. & Wesenberg, J. 1993. Population genetic analyses of an amphi-Atlantic species: Lychnis alpina (Caryophyllaceae). Nordic Journal of Botany 13:377-387.
Hauser, T. P. & Loeschcke, V. 1994. Inbreeding depression and mating distance dependent offspring fitness in large and small populations of Lychnis flos-cuculi (Caryophyllaceae). Journal of Evolutionary Biology 7: 609-622.
Hauser, T. P. & Loeschcke, V. 1996. Drought stress and inbreeding depression in Lychnis flos-cuculi (Caryophyllaceae). Evolution 50: 1119-1126.
Hedrick, P.W. & Savolainen, O. 1996. Molecular and adaptive variation a: perspective for endangered plants. In: J. Maschinski, Hammond, H.D., Holter, L. (eds) Southwestern rare and endangered plants: proceedings of the second conference 1995: 92-102. U.S. Forest Service, Rocky Mountain Forest and Range Experiment Station, Fort Collins, Arizona.
Heschel, S.M., & Paige, K.N.1995. Inbreeding depression, environmental stress, and population size variation in scarlet gilia (Ipomopsis aggregata). Conservation Biology 9:126-133.

Holsinger, K.E. 1993. The evolutionary dynamics of fragmented plant populations. In: Kareiva, P. & Kingsolver, J. (eds), Biotic interaction and global change: 198-216. Sinauer Associates, Sunderland, Massachusetts.

Husband , B.C. & Schemske D.W. 1996. Evolution of magnitude and timing of inbreeding depression in plants. Evolution 50: 54-70.

Jennersten, O. 1988. Pollination of Viscaria vulgaris (Caryophyllaceae): the contributions of diurnal and nocturnal insects to seed set and seed predation. Oikos 52:319-327.

Jennersten, O., Nilsson, S.G. & Wästljung. 1983. Local populations as ecological islands: the infection of Viscaria vulgaris by the fungus Ustilago violacea. Oikos 41: 391-395.

Jennersten, O. Berg, L. & Lehman, C. 1988. Phenological differences in pollinator visitation , pollen deposition and seed set in the sticky catchfly, Viscaria vulgaris. Journal of Ecology 76:1111-1132.

Johnston, M.O. & Schoen, D.J. 1995. Mutation rates and dominance levels of genes affecting total fitness in two angiosperm species. Science 267:226-228.

Kareiva, P. 1985. Finding and losing host plants by Phyllotreta: patch size and surrounding habitat. Ecology 66: 1809-1816.

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 Bruyn, G. (1989) Plant size and pollinator visitation in cynoglossum officinale. Oikos 54: 201-204.

Kuittinen, H., Mattila, A. & Savolainen, O. 1997. Genetic variation at marker loci and in quantitative traits in natural populations of arabidopsis thaliana. Heredity 79: 144-152.

Kunin, W.E. 1993. Sex and single mustard: population density and pollinator foraging and plant bahavior effects on seed-set. Ecology 74: 2145-2160.

Kunin, W.E. 1997. Population size and density effects in pollination: pollinator foraging and plant reproductive success in experimental arrays of Brassica kaber. Journal of Ecology 85: 225-234.

Lamont, B.B., Klinkhamer, P.G.L. & Witkowski, E.T.F. 1993. Population fragmentation may reduce fertility to zero in Banksia goodii - a demonstration of the Allee effect. Oecologia 94: 446-450.

Lande, R. & Barrowclough, G.F. 1987. Effective population size, genetic variation. and their use in population management. In: Soule, M.E. (ed) Viable populations for conservation: 69-86. Cambridge University Press, Cambridge.

Lande, R. & Schemske D.W. 1985. The evolution of self fertilisation and inbreeding depression in plants. I. Genetic models. Evolution 48: 965-978.

Ledig, F.T. 1992. Human impacts on genetic diversity in forest ecosystems. Oikos 63: 87-108.

Lesica, P. &Allendorf F.W. 1995. When are peripheral populations worth conserving? Conservation Biology 5: 158-164.

Loveless, M.D. & Hamrick J.L. 1984. Ecologica determiants of genetic structure in plant populations. Annual Review in Ecology and Systematics 15: 65-95.

Lynch, M. 1996. A quantitative-genetic perspective on conservation issues. - In: Avise, J. C. and Hamrick, J.L. (eds), Conservation Genetics: Case Histories from Nature. Chapman and Hall, New York, pp. 471-501.

May, B.1992. Starch gel electrophoresis of allozymes. In: Hoelzel, A.R. (ed.) Molecular genetic analysis of populations: 1-27. Oxford University Press, Oxford, England.

McKey, D. 1989. Population biology of figs: applications for conservation. Experientia 45: 661-673.

Menges, E. 1991. Seed germination percentage increases with population size in a fragmented prairie species. Conservation Biology 5: 158-164.

Millar, R.H., & Libby, W.J. 1991. Strategies for conserving clinal, ecotypic, and disjunct population diversity in widespread species. Pages 149-170 in D. A. Falk and K. E. Holsinger

Nei, M. 1972. Genetic distance between populations. American Naturalist 106:283-292.

Nei, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89: 583-590.

Norman J. N., Sakai A. K, Weller S.G. & Dawson T. E. (1995) Inbreeding depression in morphological and physiological traits of *Schiedea lydgatei*. Evolution 49: 297-306.

Olesen, J.M. & Jain S.K. 1994. Fragmented plant populations and their lost interactions. In: Loeschcke, V. & Jain , S.K. (eds) Conservation genetics: 417-426. Birkhäuser, Basel, Switzerland.

Ouborg, N.J. & Van Treuren, R. 1995. Variation in fitness-related characters among small and large populations of Salvia pratensis. Journal of Ecology 83:369-380.

Ouborg, N.J., van Treuren, R. & van Damme, J.M.M. 1991. The significance of genetic erosion in the process of extinction II. Morphological variation and fitness components in populations of varying size of Salvia pratensis L. and Scabiosa columbaria L. Oecologia 86: 359-367.

Podolsky, R.H. & Holtsford, T.P. 1995. Population structure of morphological traits in Clarkia dudleyana I. comparison of F_{st} between allozymes and morphological traits. Genetics 140: 733-744.

Ratchcke, B.J. & Julcs, E.S. 1993. Habitat fragmentation and plant-pollinator interactions. Current Science 65: 273-277.

Schemske, D.W. 1983. Breeding systems and habitat effects on fitness components in three neotropical Costus (Zingiberaceae). Evolution 37: 523-539.

Schemske, D.W. 1984. Population structure and local selection local selection in Impatiens pallida (Balsaminaceae) a selfing annual. Evolution 38: 817-832.

Schemske, D.W., Husband, B.C., Ruckelhaus, M.H. Goodwillie, C. Parker, I.M. & Bishop, J.G. 1994. Evaluating approaches to conservation of rare and endangered plants. Ecology 75: 584-606.

Shaffer, M.L. 1981. Minimum population sizes for species conservation. Bioscience 31: 131-134.

Shaffer, M.L. 1987. Minimum viable populations: coping with uncertainty. In: Soulé, M.E. (ed), Viable population for conservation: 69-86. Cambridge

University Press, Cambridge.
Sih, A. &Baltus, M.-S. 1987. Patch size, pollinator behavior, and pollination limitation in catnip. Ecology 68:1679-1690.
Schmitt, J. & Ehrhardt, D. W. (1990) Enhancement of inbreeding depression by dominance and suppression in *Impatiens capensis*. Evolution 44: 269-278.
Välivaara, R., Raatikainen, T., Saari, V., Halinen, P., Salminen, M. & Raatikainen, M. 1991. The threatened vascular plants in Central Finland. Publications A2. Keski-Suomen Liitto, Jyväskylä.
Van Treuren, R., Biljsma, R.,Ouborg, N.J. & Van Delden, W. 1993. The significance of genetic erosion in the process of extinction III. Inbreeding depression and heterosis effects due to selfing ans outcrossing Scabiosa columbaria. Evolution 47: 1669-1680.
Vrijenhoek, R.C. 1994. Genetic diversity and fitness in small populations. In: Loeschcke, V., Tomiuk, J. & Jain, S.K. (eds), Conservation genetics: 37-53. Birkhäuser Verlag, Berlin.
Weir, B.S., & Cockerham, C.C. 1984. Estimating F-statistics for the analysis of population structure. Evolution 38: 1358-1370.
Wendel, J.F., & Weeden, N.F. 1989. Visualization and interpretation of plant isozymes. In: Soltis, D.E. & Soltis, P.S. (eds), Isozymes in plant biology: 5-45. Dioscorides Press, Portland, Oregon.
Widen, B. 1993. Demographic and genetic effects on reproduction as related to population size in a rare, perennial herb, Senecio integrifolius (Asteraceae). Biological Journal of the Linnean Society 50: 179-195.
Willis J. H. 1999. The role of genes of large effect on inbreeding depression in Mimulus Guttatus. Evolution 53: 1678-1692.
Wilson, G.B., Wright, J., Lusby, P., Whittington, W.J. & Humphries, R.N. 1995. Biological flora of the British Isles, No. 188. Lychnis viscaria L. (Viscaria vulgaris Bernh.). Journal of Ecology 83: 1039-1051
Young , A., Boyle, T. & Brown, T. 1996. The population genetic consequences of habitat fragmentation for plants. Trends in Ecology and Evolution 11: 413-418.

I

Genetic diversity, population size and fitness in central and peripheral populations of a rare plant *Lychnis viscaria*

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II

Allozyme and morphological variation in relation to population size in common garden and natural populations of *Lychnis viscaria*

by

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Manuscript (submitted)

Allozyme and morphological variation in relation to population size in common garden and natural populations of *Lychnis viscaria*

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Abstract

Genetic variation, the evolutionary potential for the future, is considered especially important for management purposes of rare species that usually have small and isolated populations. Commonly, the genetic variation is assessed with neutral markers, whose use in management purposes is currently debated, because they may respond differently to decreases in population size than adaptively significant variation, such as variation in morphological traits. We studied the relationships between allozyme variation, morphological variation, and population size in 11 populations of *Lychnis viscaria*, a locally rare perennial herb. Morphological variation was measured from natural populations and also from common garden to estimate how well variation in natural sites predicts variation observed in common garden. In common garden, where the level of morphological variation more reliably reflects its genetic basis than in natural sites, the level of morphological variation was correlated with the level of allozyme variation, even after exclusion of the effect of population size. This suggests genetic drift to be a predominant factor in determining both allozyme and morphological variation in small populations of *L. viscaria*. However, in natural populations the correlation between morphological and allozyme variation disappeared when the effect of population size was excluded, suggesting that the correlation was probably caused by larger microhabitat variability in larger populations. There was no relationship between levels of variation between common garden and naturally grown populations. As expected, different factors determine the levels of phenotypic variation expressed in common garden and natural environments, emphasizing the use of common garden when determining levels of morphological variation within plant populations. Moreover, our results indicate that frequently used cost-efficient allozymes seem to be useful tools for estimating, or at least making predictions about, the levels of ecologically relevant variation.

Introduction

Population extinction is a process strongly influenced by stochastic demographic, environmental and genetic factors (e.g. Schaffer 1981, 1987). Small populations tend to be a subject to an increased probability of stochastic extinction (e.g. Soulé 1987). Environmental stochastity combined with demographic factors is generally accepted to be the most important component (Lande and Barrowclough 1987, Lande 1988, Menges 1991), however the role of genetic factors in the population extinction process has been the subject of recent studies (Barrett and Kohn 1991, Ouborg et al. 1991, Young et al. 1996, Buza et al. 2000, Schmidt & Jensen 2000). Small populations are expected to suffer from high levels of genetic drift and inbreeding, leading to decreased genetic variation due to increased homozygosity and random loss of alleles (Barrett and Kohn 1991, Ellstrand and Elam 1993).

Loss of genetic variation may endanger the long-term survival of populations, as their ability to adapt to changing environments is directly related to the level of genetic variation within population (Ellstrand and Elam 1993, Lammi et al. 1999). On shorter time scales increased inbreeding depression (Jiménez et al. 1994, Keller et al. 1994), and loss of heterozygote vigour (Barrett and Kohn 1991) may decrease the viability of a population. This process, known as "genetic erosion", may increase the population extinction risk (Frankham 1995, Saccheri et al. 1998). Thus, the study of existing levels of genetic diversity and the maintenance of these levels are debatable issues in conservation biology (Schaal et al. 1991). Data on genetic diversity of populations are also valuable when setting conservation priorities, and thus help to maximise the benefits of limited conservation effort and resources when aiming for the preservation of evolutionary potential of a species.

Traditionally genetic variation within plant populations has been assayed by allozyme electrophoresis (e.g. Schwaegerle and Schaal 1979, Hamrick 1989, Hamrick and Godt 1990). This technique is used because it is relatively inexpensive, and the results are easy to compare across populations and easy to interpret without concern for environmental effects in gene expression (Hamrick 1989). The genetic variation assayed by allozymes has often been reported to decrease with decreasing population size (e.g. Holsinger 1993, Frankham 1996, Weidema et al. 1996, Lammi et al. 1999), although this is not always the case (see Ellstrand and Elam 1993). However, it has been argued that allozyme variation may not correlate well with variation in ecologically relevant traits, such as quantitative traits (Lande and Barrowclough 1987, Goodnight 1988, Podolsky and Holtsford 1995, Hedrick and Savolainen 1996, Lynch 1996). As the allozyme variation is usually considered selectively neutral, it may react to changes in population size differently than adaptively significant genetic variation. Variation in morphological characters is more likely to be adaptive and thus prone to selection and thus, contribute to the evolutionary potential of a population.

In natural populations, the phenotypic variation in the morphology of individuals is a result of both genetic variation and environmental

heterogeneity. The genetic component of variation in morphological traits can be measured at the phenotypic level using a common garden approach. As the effect of environmental differences on phenotypic expression has been minimised, the remaining variation between individuals should be genetically based (Bradshaw 1984). However, the possibility of maternal effects must be taken into account. Compared to electrophoresis, measuring morphological variation in common garden is, unfortunately, time consuming and labour intensive. Because of uncertainty of the usefulness of allozymes in measuring ecologically relevant morphological variation, conservation biologists may be tempted to measure morphological variation in natural populations, when time and resources are too limited to establish a common garden.

The lack of evidence on correlation between allozyme variation and adaptive variation poses a problem in using allozymes as in a conservation context (Milligan 1994 et al., Lynch 1996). Relatively few studies concerning the relationship between morphological and allozyme variation have been conducted and most of them have concentrated on the distribution of variation (Podolsky and Holtsford 1995, Black-Samuellsson et al. 1997, Knapp and Rice 1998 and references therein, Waldman and Andersson 1998) and not on the levels of genetic variation compared to population characteristics (but see Ouborg et al. 1991). Both population divergence and level of genetic diversity within populations contribute to total diversity within species, so studies on comparing levels of variation are needed. Knowledge of genetic variation within individual populations can be considered especially important when designing population level management of small isolated populations of endangered plants.

Study on allozyme variation in 3 central and 8 peripheral *Lychnis viscaria* populations showed that the level of genetic variation was correlated with population size, and large central populations had significantly more variation than the small peripheral populations (Lammi et al. 1999). However, the fitness of individuals within the populations was not correlated with level of allozyme variation within populations.

In this study, we estimated the level of morphological variation within these same populations both in their natural environment and in the common garden. As relatively few earlier studies concentrated on levels of variation, instead of distribution of variation between populations, we will focus on levels of morphological variation within populations. We examine the relationships between variation in morphological traits, allozyme variation, and population size. We compare the usefulness of allozyme variation and morphological variation measured from natural populations in predicting the morphological variation in common garden. We will also discuss the importance of sources of phenotypic variation in these populations.

Materials and methods

Study species

Lychnis viscaria L. (*Viscaria vulgaris* Bernh., Caryophyllaceae) is a perennial herb that occurs in open sunny habitats such as dry meadows and south sloping rocky outcrops. The long-lived rosette produces several flowering stalks, each bearing 20-25 purple, protandrous flowers. The flowers are pollinated by insects, mainly bumblebees and butterflies (Wilson et al. 1995, Jennersten 1988). Seeds are dispersed by gravity. The species occurs in fairly distinct patches of a few to thousands of individuals throughout northern and central Europe, the main range extending to 62nd latitude, but few isolated populations are found up to 68th latitude (Hultén 1971, Wilson et al. 1995).

In this study, morphological variation was measured in populations located both in the central and in the peripheral distribution area of *L. viscaria* in Finland. We sampled three populations (Nokia, Epilä, Kalevankangas) from surroundings of Tampere (61° 30'N, 23° 45'E) within the central range of the species (Hultén 1971). In this area the species occurs in larger populations mainly on roadsides and dry meadows. In the area around Jyväskylä (62° 15'N, 25° 45'E), 150 km NE of Tampere, *L. viscaria* is at its northern range and occurs in rather small and isolated patches on rocky cliffs. In this marginal area, morphological variation was measured in eight populations (for more information on the population characteristics, see Siikamäki and Lammi 1998, Lammi et al. 1999). Population sizes were determined as the number of flowering individuals during the peak flowering period.

Common garden

Seed material for the common garden was acquired by collecting five random, mature but unopened capsules of 30 randomly chosen plants in each population in summer 1995, except for Kotimäki, in which seeds were collected from all 7 flowering individuals. After six months storage at 5°C, four seeds per pot / 1-3 pots per maternal plant were sown in plastic pots containing mixture of vermiculite and peat moss. In population Kotimäki 4 pots per maternal plant was sown. If several seeds per pot germinated, we removed all but one randomly selected seedling per pot. The seedlings were randomly arranged in a greenhouse and grown in standard greenhouse conditions (21°C, 16 hours light 8 hours darkness) until the beginning of June 1996, when about 40 seedlings per population were transplanted out to establish a common garden. Because only some of the plants flowered in 1996, morphological characters were measured in 1997 when all the plants flowered. The greenhouse and the common garden were situated in central Finland, in the Laukaa Research and Elite Plant Station.

Measurements in the field and common garden

In the beginning of June 1997, 40 randomly chosen wild individuals were marked with small flags in natural sites of each study population. These individuals and the common garden plants were measured for several morphological characters during the growing season in summer 1997. Measurements were conducted, both in common garden and in natural sites, at approximately the same phenological stage in all populations.

Length of the longest leaf (LL) from base to apex and maximum width of that leaf (LW), together with the rosette diameter (RD) from the base of the lowest rosette leaves were measured before flowering. During flowering, the following characters were measured from the longest flowering stem: length of the flowering stem (FSL) from stem base to the base of the top flower, number of flowers (FNO), number of leaf nodes (NNO), number of flowering branches (BNO), the distance between the two lowest branches in an inflorescence (BDI), and length of the tar strip on the stem (TAR). The number of flowering stems (FSNO) was also calculated from each individual. One randomly chosen flower from each plant was taken into the lab to accurately measure the following floral traits: sepal length (SL), maximum length (PL) and width (PW) of the longest petal, petal lobule length (PLL), and ovary length (OL). All the length and width characters were measured using digital callipers rules, except the length of the flowering stem, which was measured with a ruler.

The coefficient of variation: $CV = \frac{s}{x} * 100$,

where s is the standard deviation and $\bar{x}$ the population mean, was calculated for each measured character in each population, for natural site and common garden separately and used as a measure of morphological variation. CV values of populations were ranked for each character and the mean of these rank values indicated the relative measure of morphological variation within a population.

Allozyme variation

The same seed material collected to establish the common garden was used to grow seedlings for electrophoresis. Several seedlings per maternal plant were grown in the laboratory, and approximately 30 (range 21-30) seedlings per population (all from different maternal plants) were randomly chosen for analysis. The level of genetic variation in the populations was determined by starch gel electrophoresis as described in Wendel and Weeden (1989) and May (1992). Three gel and electrode buffer systems, as described in Siikamäki & Lammi (1998), were used to resolve the isozymes, and a total of 13 enzymes and 17 loci were screened. Six loci (F-EST-1, F-EST-2, GPI-2, PGM-2, SKD-1, SOD-1) were polymorphic. Genetic diversity in each population was assessed as Nei's (1978) unbiased estimate of expected heterozygosity (H_{exp}). The data set used is the same as in Siikamäki & Lammi 1998 and Lammi et al. 1999.

In our previous study on genetic variation we found that the allozyme variation is correlated with population size in these populations of *L. viscaria*,

(Lammi et al. 1999). Morphological variation is also likely to be correlated with population size, especially in natural populations, if large population extend over wider range of microhabitats. Thus the correlation between morphological and allozyme variation could arise only through a connection between present population size rather than common history or genetic relationship between the two froms of variation. Therefore population size had to be controlled for when studying the relationship between allozyme and morphological variation. In addition to Spearmans' correlation between the mean of CVs' for each character in natural sites and common garden, Kendall's correlation coefficient T was used to study the relationships among mean rank of CVs' and allozyme variation, because it allows the calculation of a partial correlation for non-parametric data (Siegel and Castellan 1988) and thus, exclusion of the population size effects.

Results

Plants in common garden were larger than plants in natural populations (paired t-test for population means for number of flowering spikes: df = 10, t = -10.92, P<0.001; for the height of longest flowering spike df =10, t = -7.24, P<0.001). CVs differed considerably among populations, both in common garden and in natural sites (Appendix 1, 2). Morphological variation was significantly higher in natural sites for only 7 of 15 characters (Table 1), but the overall trend, tested with combined probabilities (Fisher's χ^2 method), indicates significantly higher overall morphological variation in natural populations (df = 30, χ^2 = 110.60, P<0.001).

Heterozygosity was correlated with morphological variation both in common garden (Fig.1a) and natural sites (Fig. 1b), but population size was not (Fig. 2a and b). When population size was controlled with Kendall's partial correlation, the relationship between heterozygosity and morphological variation was no longer significant in natural populations (N = 11, T = 0.408, NS), but remained significant in the common garden (N = 11, T = 0.627, P < 0.05)

No clear relationship between the mean rank of CV in the common garden and in natural environment was found (Fig. 3). Only CVs of leaf length were correlated between the two environments (Table 1). This indicates that the amount of phenotypic variation observed in populations in their natural environment is not related to the actual genetic variation for morphological traits measured in common garden.

In the natural populations, one (leaf length) out of 15 characters tended to be more variable in the central populations than in the peripheral populations. In common garden, only one (node number) out of 15 characters was significantly more variable in central populations (Table 2).

Discussion

We found a significant correlation between allozyme variation and morphological variation in common garden, but none in natural sites, when the effect of population size was excluded. This difference, as well as the lack of correlation between CV-values measured in common garden and in natural sites, suggests that different factors are controlling the expression of phenotypic variation in common garden compared to natural sites. In the natural sites, the correlation between the morphological and allozyme variation was probably partly the effect of present day population size, which is related to wider microhabitat variation. In the common garden, instead, the effect of environment is much diminished, and as a result it gives more reliable measure on the actual genetic basis of the morphological variation. Therefore the observed correlation between allozyme and morphological variation, in particularly when the effect of population size is controlled for, should have at genetic basis. This relationship between marker diversity and morphological variation is expected to arise only when stochastic effects such as genetic drift, bottlenecks and founder events, instead of selection, are the major factors affecting the levels of genetic variation. This is because variation at allozyme loci has been argued to be largely selectively neutral and thus highly susceptible to genetic drift, while morphological characters, especially reproductive characters are considered to be influenced by natural selection. Thus genetic drift, instead of selection, may be the predominant factor in determining the levels morphological variation in small *L. viscaria* populations. This explanation was also suggested for *Scabiosa columbaria* and *Salvia pratenis*, where Ouborg et al. (1991) found a concordance between the two measures of variation. This correlation is expected to be found only for predominantly selfing species, because of the stronger persistence of linkage disequilibrium (Price at al. 1984).

Although allozyme variation in these populations was related to population size (see also Lammi et al. 1999), morphological variation was not. Lack of correlation may be partly due to small sample size, but there are several theoretical considerations why the morphological variation may react to changes in population size differently than allozyme variation (Lande and Barrowclough 1987, for review see Lynch 1996). If the environment is either spatially variable or variable in time, the shifting selection pressures favour different genotypes and maintain variation within population (Ennos 1984). Quantitative morphological characters under polygenic control, are expected to react to changes in population size more slowly than single-locus characters, such as allozymes, as the population size necessary to maintain variation following a bottleneck is much smaller for quantitative traits (Lande and Barrowclough 1987). The faster mutation rate of quantitative characters results in a more rapid recovery of genetic variation after bottleneck (Lynch 1988). As selection also acts differently on monogenic traits, like allozymes, compared to polygenic traits, effects of reduced population size on these two types of traits may differ (Foley 1992). However, only few earlier experimental studies exist

on the relationship between level of morphological variation and population size. Ouborg et al. (1991) found a significant correlation between morphological variation and population size in *S. pratensis* and *S. columbaria*. In *Scabiosa,* which experiences more inbreeding than *Salvia*, the relationship disappeared when only reproductive characters were included in the analysis. According to allozyme data (Lammi et al. 1999) the level of inbreeding is probably quite high in *Lychnis viscaria* populations, which makes it more comparable to *Scabiosa.* A small population of *Senecio integrifolius* contained actually more adaptive quantitative genetic variation in morphological traits than a large population (Widen and Andersson 1993). The authors suggested that sub-structuring of the small population into isolated patches might have influenced the retention of heritable variation in this population. *L. viscaria* also occurs in very patchy environments, especially in the marginal area where the small populations are found. Thus, the high population subdivision may also have preserved variation in these populations.

Only a few of the studied characters were significantly more variable in the central populations, thus central location within the distribution area seemed to have no significant effect on the level morphological variation. However, number of populations sampled in the central area is very small, which affects the power of the statistical tests and prevents us from making reliable conclusions about the differences between central and marginal populations.

In *L. viscaria* populations, the morphological variation was higher in natural sites. Although, with the current data we cannot precisely separate and identify the factors responsible for this observation, the data suggest that in the natural populations the environmental heterogeneity resulted in increase of phenotypic variation. In natural populations the observed phenotypic variation is a result of environmental heterogeneity, genotypic variation, and genotype-environment interactions. Therefore in common garden, where environmental heterogeneity is minimized, phenotypic variation in morphological traits should is typically lower than that in natural populations. Phenotypic variation may also decrease in common garden if the genes underlying the traits are expressed only in harsh conditions. On the other hand, if environmental heterogeneity is of minor relevance, the variation might actually be lower in natural sites compared to common garden, for example if in harsh natural conditions only certain genotypes are able to establish themselves. This could be true especially in peripheral populations that are expected to occur in less favourable habitats. If strong phenotypic selection also suppresses expression of variation in some characters, the observed phenotypic variation in natural conditions could be less than the genotypic potential within th population might allow (Gebhardt-Henrich and Van Noordvijk 1991, see also Hoffman and Merilä 1999). In the latter cases, the phenotypic variation would be higher in common garden than in natural sites, since no selection for the seedling establishment occurs as the environment is favourable. In common garden the resources are practically unlimited, and the expression of traits to their fullest extent should not be limited by environmental factors.

The higher variability in natural populations may also be partly due to the

fact that in common garden all individuals were of same age, while in natural populations we could not control for the variability caused by age differences between plants. The lack of family data to get information on variance of traits within family and thus lack of knowledge about the adaptive nature of the measured traits, also restrains us from making definite conclusions about the sources of variation in natural and common garden.

It must be kept in mind that the interpretation of results of this kind of studies is to some extent complicated by maternal effects (Roach and Wulff 1987). We cannot rule out the possibility of maternal effects on the morphological variation, but we expect them to be of minor relevance, since the plants were grown in common garden for two growing seasons before the measurements. The strongest maternal effects are usually observed at the earlier stages of the development (Roach and Wulff 1987, Ouborg et. al 1991), while their relevance decreases in the later life stages and the offspring's own genotype begins to contribute significantly to the variation (Roach and Wulff 1987). So, we suggest that the variation present in our common garden would be mostly attributed to genetic background, which is further supported by the fact that the phenotypic variability in natural sites was not related to variability of population in common garden.

Some recent theoretical studies indicate that the use of neutral markers may also be valuable for sampling selected genes for ex situ conservation or giving priority to populations for conservation purposes (Bataillon 1996 et al., Petit et al. 1997). The results presented here indicate that allozymes may in fact be useful tools in conservation genetics for detecting variable populations for conservation purposes, because there was a correlation between variation in allozymes and morphological traits in common garden. This is good news for conservation biologists, since allozyme analysis requires only a fraction of the time and effort compared to laborious common garden assays. The results of this study suggest that when the time and resources are limited, the use of allozymes in assaying levels of genetic variation also give in fact more reliable results on the levels of genetically based morphological variation than measuring morphological variation of wild individuals, because phenotypic variation in natural sites gave no indication of real levels of genetic variation for morphological traits.

However, it must be noted that the lack of allozyme variation does not necessary indicate loss of fitness. Our earlier study indicates that the individuals in small *L. viscaria* populations are equally fit as in the large populations although they posses much less allozyme variation than large populations (Lammi et al. 1999). Recent studies comparing allozyme and quantitative trait data studying the distribution of genetic variation and genetic differentiation between populations (see Knapp and Rice 1997 and references therein) also indicate that the connection between morphological and genetic variation may only be found in some cases, where special conditions are met. Experimental evidence on the relationship between neutral markers and adaptive genetic variation are still scarce, but in the case of *L. viscaria* the levels of allozyme variation reflect also the variation in morphological traits.

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References

Barrett, S. C. H. and Kohn, J. R., 1991. Genetic and evolutionary consequences of small population size in plants: Implications for conservation. - In: Falk, D.E., Holsinger, K.E. (eds.), Genetics and Conservation of Rare Plants. Oxford University Press, New York, NY, pp. 45-61.

Bataillion, T. M., David, J. L. and Schoen D. J. 1996. Neutral genetic markers and conservation genetics: simulated germplasm collections. - Genetics 144: 409-417.

Black-Samuelsson, S., Eriksson, G., Gustafsson, L. and Gustafsson, P. 1997. RAPD and morphological analysis of the rare plant species *Vicia pisiformis* (Fabaceae). – Biol. J. Linn. Soc. 61: 325-343.

Bradshaw, A. D. 1984. Ecological significance of genetic variation between populations. - In: Dirzo, R .and Sarukhan, J. (eds.) Perspectives on plant population. Sinauer, Massachsetts, pp. 213-228.

Buza, L., Young, A. and Thrall, P. 2000. Genetic erosion, inbreeding and reduced fitness in fragmented populations of the endangered tetraploid pea *Swainsonia recta*. - Biological Conservation 93: 177-186.

Ellstrand, N. C. and Elam, D. R. 1993. Population genetic consequences of small population size: implications for plant conservation. – Annu. Rev. Ecol. Syst. 24: 217-243.

Ennos, R. A. 1984. Maintenance of polymorphism in plant populations. – Evol. Biol. 16: 129-156.

Foley, P. 1992. Small population genetic variability at loci under stabilising selection. - Evolution 46: 412-429.

Frankham, R. 1995. Inbreeding and extinction: a threshold effect. – Conserv. Biol. 9: 792-799.

Frankham, R. 1996. Relationship of genetic variation to population size in wildlife. – Conserv. Biol. 10:1500-1508.

Geberhard-Henrich, S. G., Van Noordwijk, A. J. 1991. Nestling growth in the Great Tit. I. Heritability estimates under different environmental conditions. – J. Evol. Biol. 3: 341-362.

Gilpin, M. E. and Soule, M. E. 1986. Minimum viable populations: processes of species extinction. - In: Soule, M. E. (ed.) Conservation biology: the science of scarcity and diversity. Sinauer, Sunderland, Massachusetts, pp. 19-34.

Goodnight, C. J. 1988. Epistasis and the effect of founder events on the additive genetic variance. - Evolution 42: 441-545.

Hamrick, J. L. 1989. Isozymes and the analysis of genetic structure in plant populations. - In: Soltis, D. E. and Soltis, P. S. (eds.) Isozymes in Plant Biology. Dioscorides Press, Oregon, pp. 87-105.

Hamrick, J. L. and Godt, M. J. W. 1990. Allozyme diversity in plant species. - In: Brown, H. D., Clegg, M. T., Kahler, A. L. and Weir, B. S. (eds.), Plant population genetics, breeding, and genetic resources. Sinauer, Sunderland, Massachusetts, pp. 43-63.

Hedrick, P. W. and Savolainen, O. 1996. Molecular and adaptive variation: a perspective for endangered plants.- In: Maschinski, J., Hammond, D. H. and Holter, L. (eds.) Southwestern rare and endangered plants: Proceedings of Second conference; 1995 September 11-14; Flagstaff, AZ. Gen. Tech. Rep. RM-GTR-283 Forth Collins, Co:U.S. Department of Agriculture, Forest service, Rocky Mountain Forest and Range Experiment Station, pp. 92-102.

Hoffman, A. A. and Merilä, J. 1999. Heritable variation and evolution under favourable and unfavourable conditions. - Trends Ecol. Evol. 14: 96-101.

Holsinger, K. E. 1993. The evolutionary dynamics of fragmented plant populations. - In: Kareiva, P. and Kingsolver, J. (ed.) Biotic interaction and global change. Sinauer Associates, Sunderland, Massachusetts, pp.198-216.

Hultén, E. 1971. Atlas of the distribution of vascular plants in northwestern Europe. -Generalstabens litografiska anstalts förlag, Stockholm.

Jennersten, O. 1988. Pollination of *Viscaria vulgaris* (Caryophyllaceae): the contributions of diurnal and nocturnal insects to seed set and seed predation. - Oikos 52: 319-327.

Jiménez, J. A., Hughes, K. A., Alaks, G., Graham, L. and Lacy, R. C. 1994. An experimental study of inbreeding depression in natural habitat. - Science 266: 271-273.

Keller, L. F., Arcese, P., Smith, J. N. M., Hochachka, W. M. and Stearns, S. C. 1994 Selection against inbred songsparrows during a natural population bottleneck. - Nature 372: 356-357.

Knapp, E. E. and Rice, K.J. 1997. Comparison of isozymes and quantitative traits for evaluating patterns of genetic variation in purple needlegrass *(Nassella pulchra)*. – Conserv. Biol. 12: 1031-1041.

Lammi, A., Siikamäki, P. and Mustajärvi, K. 1999. Genetic diversity, population size and fitness in central and peripheral populations of a rare plant *Lychnis viscaria* (Caryophyllaceae). – Conserv. Biol. 13: 1069-1078.

Lande, R. 1977. On comparing coefficients of variation. – Syst. Zool. 26: 214-217.

Lande, R. and Barrowclough, G. F. 1987. Effective population size, genetic variation, and their use in population management. - In: Soulé, M. E. (ed.) Viable populations for conservation. Cambridge University Press, Cambridge, pp. 69-86.

Lande, R. 1988. Genetics and demography in biological conservation. - Science

241: 1455-1460.
Lynch, M. 1996. A quantitative-genetic perspective on conservation issues. - In: Avise, J. C. and Hamrick, J. L. (eds.) Conservation Genetics: Case Histories from Nature. Chapman and Hall, New York, pp. 471-501.
Lynch, M. 1988. The rate of polygenic mutation. – Gen. Res. 51: 137-148.
May, B. 1992. Starch gel electrophoresis of allozymes. - In: Hoelzel (ed) Molecular genetic analysis of populations. Oxford University Press, Oxford, United Kindom, pp. 1-27.
Menges, E. S. 1991. The application of minimum viable population theory to plants. - In: Falk, D. E., Holsinger, K. E. (eds.) Genetics and conservation of rare plants. Oxford University Press, New York, NY, pp. 45-61.
Milligan, B. G., Leebens-Mack, J. and Strand, A. E. 1994. Conservation genetics: beyond the maintenance of marker diversity. – Mol. Ecol. 3: 423-435.
Nei, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. - Genetics 89: 583-590.
Ouborg, N. J., van Treuren, R. and van Damme J. M. M. 1991. The significance of genetic erosion in the process of extinction II. Morphological variation and fitness components in populations of varying size of *Salvia pratensis* L. and *Scabiosa columbaria* L. - Oecologia 86: 359-367.
Petit, R. J., El Mousadik, A. and Pons, O. 1997. Identifying populations for conservation on the basis of genetic markers. – Conserv. Biol. 12: 844-855.
Podolsky, R. H. and Holtsford, T. P. 1995. Population structure of morphological traits in *Clarkia dudleyana* I. comparison of F_{st} between allozymes an morphological traits. - Genetics 140: 733-744.
Price, S. C. K. M., Shumaker, A. L., Kahler, R. W., Allard, R.W. and Hill, J.E. 1984. Estimates of population differentiation obtained from enzyme polymorphisms and quantitative characters. – J. Hered. 75: 141-142.
Roach, D. A. and Wulff, R. D. 1987. Maternal effects in plants. – Annu. Rev. Ecol. Syst. 18: 209-235.
Saccheri, I., Kuussaari, M., Kankare, M., Vikman, P., Fortelius, W. and Hanski, I. 1998. Inbreeding and extinction in a butterfly metapopulation. - Nature 392: 491-494.
Schaal, B. A., O'Kane, S. L. L. and Rogstad, S. H. 1991. DNA variation in plant populations. - Trends Ecol. Evol. 6: 329-333.
Schwaegerle, K. E. and Schaal, B. A. 1979. Genetic variability and founder effect in the pitcher plant *Sarracenia purpurea* L. - Evolution 33: 1210-1218.
Schmidt, K. and Jensen, K. 2000. Genetic structure and AFLP variation of remnant populations in the rare plant *Pedicularis palustris* and its relation to population size and reproductive components. - Am. J. Bot. 87: 678-689.
Shaffer, M. 1981. Minimum population size for species conservation. - BioScience 31: 131-134.
Shaffer, M. 1987. Minimum viable populations: coping with uncertainty. - In: Soulé, M. E. (ed.) Viable populations for conservation. Cambridge University Press, Cambridge, pp. 69-86.
Siegel, S. and Castellan, N. J. Jr. 1988. Nonparametric statistics for behavioral sciences. - Mc-Graw-Hill International Editions, Statistics Series, Singapore, Second edition.

Siikamäki, P. and Lammi, A. 1998. Fluctuating asymmetry in central and marginal populations of *Lychnis viscaria* in relation to genetic and environmental factors. - Evolution 52: 1285-1292.

Soulé, M. E. 1987. Where do we go from here? - In: Soulé, M. E. (ed.) Viable populations for conservation. Cambridge University Press, Cambridge, pp. 69-86.

Waldman, P. and Andersson, A. 1998. Comparison of quantitative genetic variation and allozyme diversity within and between populations of *Scabiosa canescens* and *S. columbaria.* - Heredity 81: 79-86.

Weidema, I. R., Siegismund, H. R. and Philipp, M.1996. Distribution of genetic variation within and among Danish populations of *Armeria maritima*, with special reference to the effects of population size. - Hereditas 124: 121-129.

Wendel, J. F., Weeden, N. F. 1989. Visualization and interpretation of plant isozymes. - In: Brown, H. D., Clegg, M. T., Kahler, A. L. and Weir, B. S. (eds.) Plant population genetics, breeding, and genetic resources. Sinauer, Sunderland, Massachusetts, pp. 43-63.

Widen, B. and Andersson, S. 1993. Quantitative genetics of life-history and morphology in a rare plant, *Senecio integrifolius.* - Heredity 70: 503-514.

Wilson, G. B., Wright, J., Lusby P., Whittington W. J. and Humphries R. N. 1995. *Lychnis viscaria* L. (*Viscaria vulgaris* Bernh.) (Biological flora of the british isles No. 188). – J. Ecol. 85:1039-1051.

Young, A. Boyle, T. and Brown, T. 1996. The population genetic consequences of habitat fragmentation for plants. – Trends Ecol. Evol. 11: 413-418.

TABLE 1 Comparison of populations' CV-values of in natural environment compared to corresponding CV-values in common garden (paired samples t-test, positive t-value indicates larger CV-value in natural populations), and correlation between CV- values in the two environments.

	t	r
Leaf length	1.18	0.655*
Leaf width	0.54	0.473
Rosette width	12.29***	-0.318
Flower stem length	3.97***	-0.009
Flower number	5.66***	0.409
Node number	2.49*	0.664*
Branch number	4.20**	-0.018
Branch distance	5.90***	-0.264
Tar length	0.38	-0.009
Flower stem number	5.03***	0.200
Sepal length	1.58	0.273
Petal length	2.05	-0.73
Petal width	0.79	0.500
Petal lobule length	0.21	-0.255
Ovary length	2.20	0.173

*P<0.05, **P<0.01, ***P<0.001

TABLE 2 The t-values for comparisons of CV-values between central and marginal populations both in natural populations and in common garden (negative value indicates larger CV value in marginal populations)

	Natural	Common
Leaf length	1.92	0.30
Leaf width	-0.43	0.18
Rosette diameter	-0.46	2.07!
Flower stem length	0.76	0.90
Flower number	1.42	0.98
Node number	1.55	3.31**
Branch distance	0.43	0.29
Branch distance	0.21	-0.81
Tar	0.81	-0.61
Flower stem number	1.45	0.55
Sepal length	0.86	0.09
Petal length	1.03	-0.93
Petal width	0.39	0.82
Petal lobule length	-0.29	-0.12
Ovary length	1.67	0.15

*P<0.05, ** P<0.01, ***P<0.001

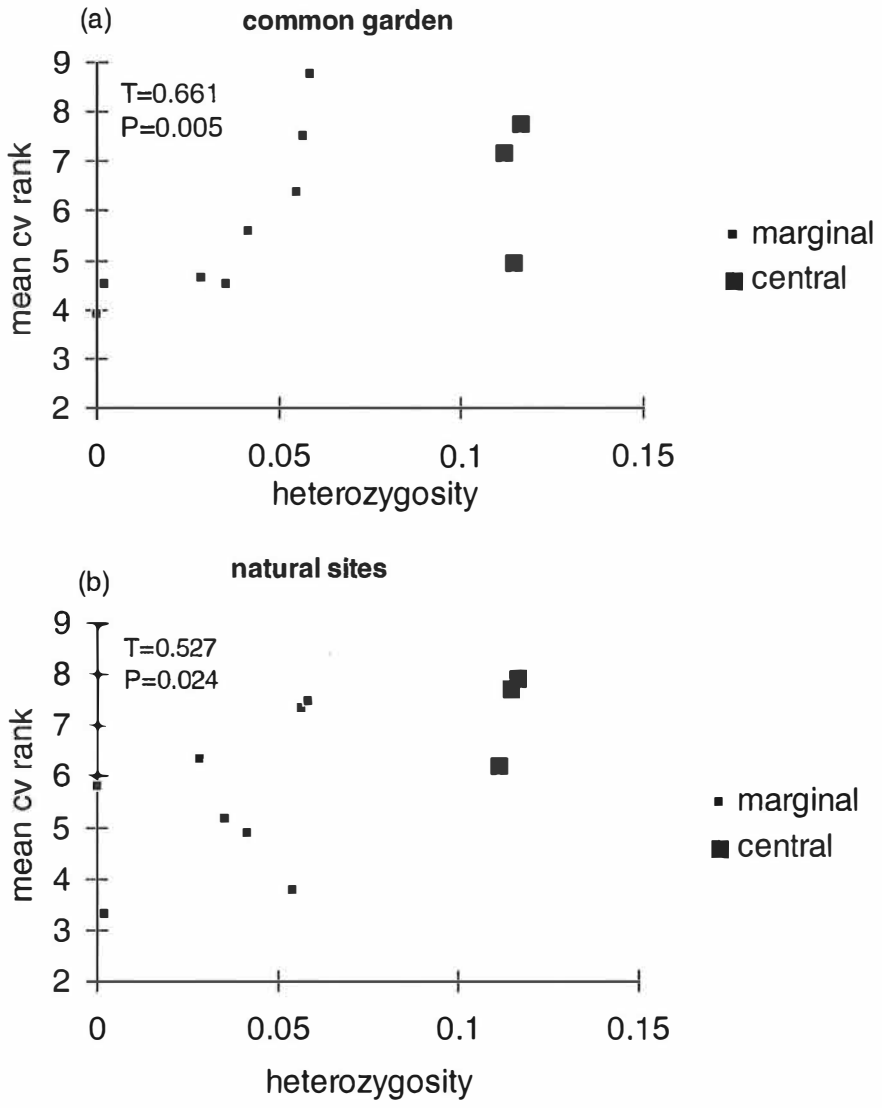

FIGURE 1 Correlation between the amount of allozyme variation and morphological variation in common garden (a) and in natural populations (b).

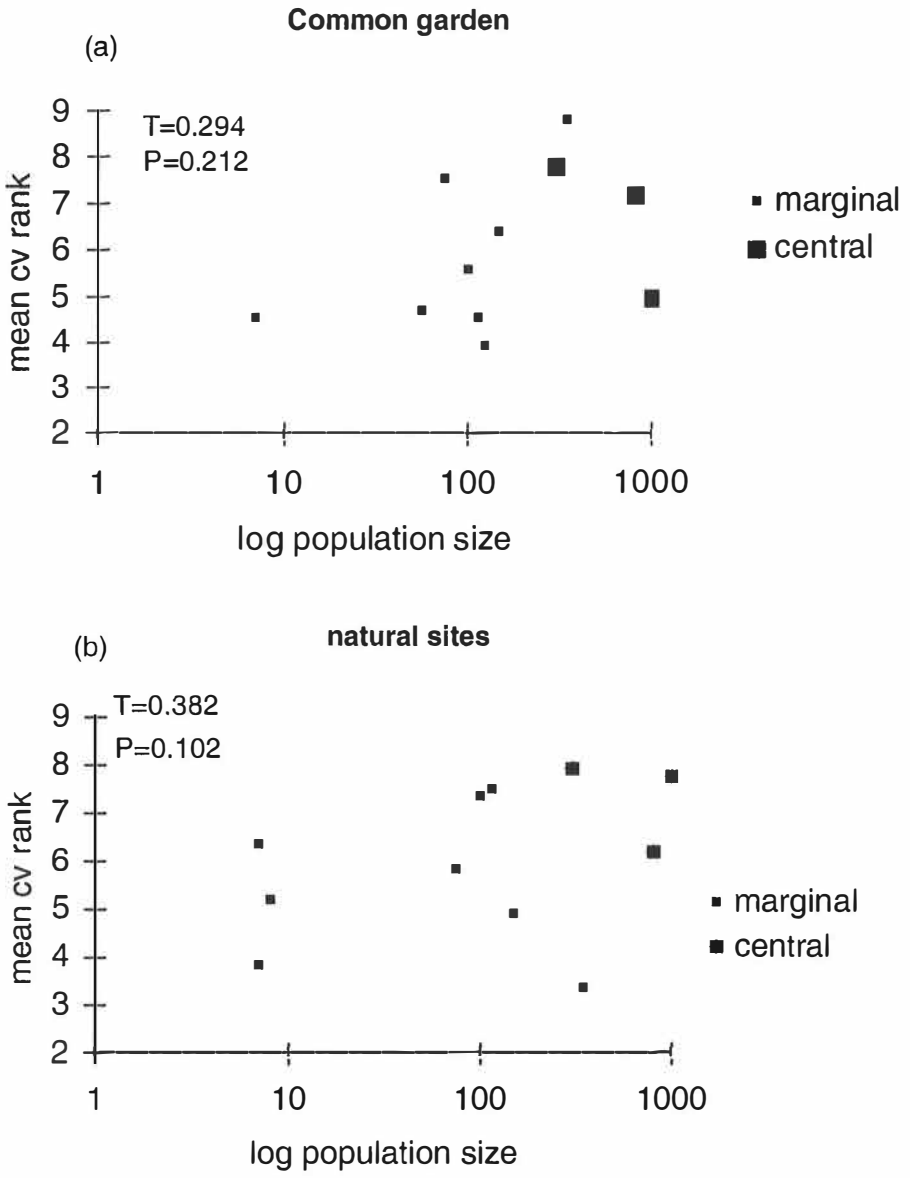

FIGURE 2 Mean rank of morphological variation measured in common garden (a) and in natural populations (b) plotted against population size (logarithmic scale).

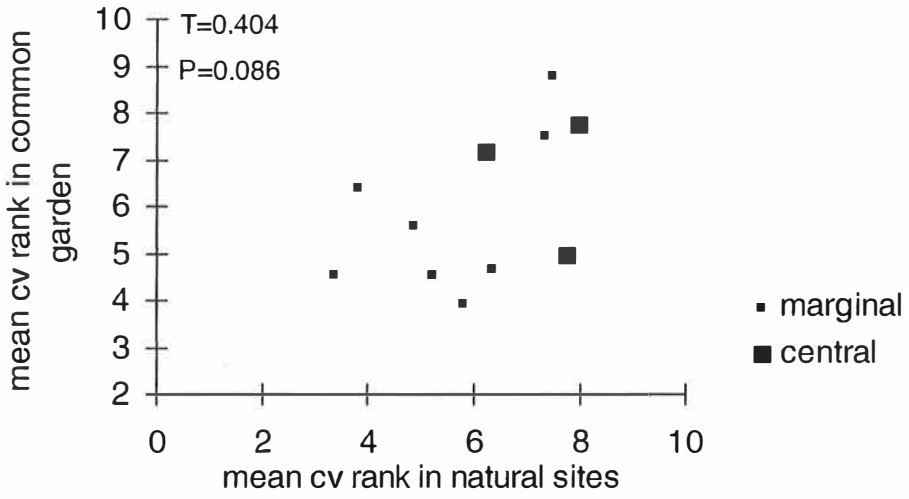

FIGURE 3 Relationship between mean of CV-rank values of morphological variation in common garden and in natural sites.

APPENDIX 1 The coefficients of variation and mean (in brackets) in natural sites for each trait in each of the studied populations.

	Marginal populations											
	Iso-salmijärvi		Kanavuori		Kettuvuori		Kotimäki		Lullinvuori		Vaarunjyrkkä	
LL	15.7	(80.8)	23.1	(78.3)	24.2	(84.9)	21.4	(101.0)	18.4	(108.2)	24.1	(97.1)
LW	17.7	(5.9)	32.5	(5.5)	28.1	(6.0)	18.6	(9.2)	23.7	(7.5)	24.3	(5.6)
RW	66.2	(21.5)	66.9	(56.4)	63.3	(40.3)	62.4	(31.4)	73.8	(40.1)	64.5	(43.1)
FSL	39.3	(202.2)	43.1	(191.1)	39.4	(292.4)	31.9	(346.7)	29.1	(281.5)	35.2	(275.1)
FNO	47.7	(12.2)	54.1	(7.8)	55.8	(19.2)	23.7	(11.0)	41.4	(13.9)	57.1	(18.4)
NNO	20.2	(3.2)	26.2	(2.6)	27.4	(3.1)	15.6	(3.5)	21.3	(3.2)	28.9	(2.6)
BNO	33.1	(2.6)	38.7	(2.2)	26.8	(2.8)	19.4	(2.7)	21.6	(3.2)	23.3	(3.2)
BDI	57.3	(18.5)	41	(35.6)	74.7	(30.9)	63.1	(45.3)	67	(39.0)	66.8	(30.8)
TAR	45.5	(15.7)	77.6	(11.2)	43.1	(16.8)	25.3	(28.7)	40.2	(24.6)	64.2	(16.2)
FSNO	!1.8	(1.8)	94.3	(3.4)	94.4	(3.3)	55.8	(1.5)	90.2	(3.7)	66.5	(1.9)
SL	10.8	(11.8)	6.3	(10.3)	11.1	(11.7)	14	(11.3)	8.9	(11.2)	9.4	(9.5)
PL	18.5	(6.2)	7.6	(4.9)	17.7	(6.3)	9.3	(6.2)	15.1	(6.5)	22.2	(5.4)
PW	19.7	(4.1)	8.7	(4.6)	14.4	(4.3)	9.6	(5.0)	12.7	(4.1)	26.9	(3.5)
PLL	21.3	(2.5)	27.3	(2.2)	17.8	(2.8)	6.3	(2.8)	19.8	(3.1)	22.5	(2.6)
OL	18.	(4.1)	10.1	(3.9)	17.4	(3.7)	4.9	(4.6)	13.9	(3.6)	16.0	(3.0)
size		124		57		75		7		100		300

	Marginal populations				Central populations					
	Vaaruntie		Vällynhoilo		Nokia		Kalevankangas		Epilä	
LL	22.8	(99.2)	21.7	(83.3)	23.3	(90.9)	27.9	(91.6)	24.2	(96.7)
LW	24.9	(5.9)	23.0	(6.0)	25.1	(5.7)	21.6	(4.7)	21.8	(4.9)
RW	52.9	(41.6)	76.9	(26.5)	71.6	(57.5)	56.2	(45.9)	68.3	(75.3)
FSL	18.2	(383.3)	17.8	(282.3)	41.4	(0.8)	44.1	(0.8)	24.8	(0.7)
FNO	59.9	(24.4)	46.3	(11.6)	63.0	(11.7)	67.1	(19.8)	47.6	(19.9)
NNO	21.0	(3.1)	26.0	(2.9)	31.5	(2.0)	30	(2.2)	22.7	(2.4)
BNO	23.9	(3.2)	26.8	(2.5)	28.3	(2.9)	27.2	(2.8)	19.2	(3.2)
BDI	46.5	(39.9)	63.3	(22.7)	54.5	(39.9)	58.9	(41.9)	70.9	(32.9)
TAR	32.9	(23.6)	34.6	(15.0)	52.4	(15.7)	49	(19.7)	60.3	(17.4)
FSNO	52.4	(2.3)	61.2	(1.5)	80.3	(3.9)	77.5	(3.1)	111.7	(6.9)
SL	4.5	(12.5)	12.8	(11.3)	8.9	(12.3)	14.1	(11.4)	11.5	(11.5)
PL	11.5	(8.5)	15.9	(6.0)	21.3	(7.8)	21.2	(5.7)	12.1	(6.3)
PW	19.1	(5.2)	17.2	(4.2)	16.2	(5.2)	23.3	(4.6)	13.3	(4.4)
PLL	16.8	(3.3)	13	(3.3)	18.8	(2.9)	21.2	(2.9)	10.8	(3.2)
OL	13.2	(3.9)	16	(3.9)	17.6	(4.6)	16.6	(4.0)	21.4	(4.7)
size		150		115		1000		300		800

APPENDIX 2 The coefficients of variance and means (in brackets) of "common garden" populations for each measured character.

	Marginal populations											
	Iso-Salmijärvi		Kanavuori		Kettuvuori		Kotimäki		Lullinvuori		Vaarunjyrkkä	
LL	12.9	(123.9)	18.1	(109.7)	27.4	(84.6)	21.9	(89.4)	22.4	(91.1)	24.9	(109.2)
LW	22.9	(6.0)	25.4	(5.7)	30.1	(5.1)	19.8	(5.3)	24.4	(5.7)	26.9	(5.0)
RW	11.2	(59.8)	27.6	(48.0)	22.1	(47.6)	18.8	(48.1)	22.2	(54.2)	26.8	(50.7)
FSL	18.4	(428.0)	16.8	(492.3)	24.6	(390.4)	10.8	(511.6)	19.5	(493.4)	20.1	(455.4)
FNO	30.4	(37.8)	35.2	(33.5)	31.7	(36.0)	29.1	(33.9)	24.9	(31.6)	36.4	(36.4)
NNO	14.6	(3.5)	18.9	(3.6)	27.2	(2.9)	16.6	(3.5)	16.8	(3.6)	20.4	(3.3)
BNO	18.5	(3.8)	14.8	(3.6)	18.6	(3.9)	15.6	(3.6)	20.1	(3.7)	19.3	(4.3)
BDI	38.3	(37.2)	34.5	(55.9)	31.1	(45.2)	37.7	(40.0)	36.3	(48.1)	40.9	(47.3)
TAR	40.9	(31.4)	67.6	(21.0)	158.5	(8.2)	49.9	(23.2)	30.4	(35.1)	32.8	(21.7)
FSNO	49.5	(31.6)	51	(28.3)	48	(17.2)	40.6	(17.8)	51.2	(25.5)	52.1	(30.8)
SL	5.3	(14.0)	6.6	(12.0)	10	(12.2)	8.3	(11.9)	9.3	(11.9)	13.4	(11.0)
PL	10.7	(7.0)	11.5	(6.4)	14.5	(6.2)	13	(7.2)	11.3	(6.9)	15.6	(6.6)
PW	16	(4.9)	9	(5.2)	12.8	(4.0)	15.6	(4.9)	15	(4.6)	18.6	(4.2)
PLL	12.3	(2.9)	15.0	(3.0)	19.4	(2.8)	19.3	(2.8)	18.8	(3.8)	24.2	(3.6)
OL	11	(4.7)	10.5	(4.1)	14.8	(3.8)	8.7	(4.6)	11.4	(3.8)	15.5	(3.9)
size	124		57		75		7		100		350	

	Marginal populations				Central populations					
Population	Vaaruntie		Vällynhoilo		Nokia		Kalevankangas		Epilä	
LL	21	(114.9)	20.6	(111.2)	18.5	(94.9)	25	(105.3)	22.5	(120.5)
LW	21.6	(6.0)	23.3	(5.5)	22.5	(5.7)	22.3	(5.6)	29.3	(6.0)
RW	20	(54.9)	17.7	(54.5)	24.8	(62.0)	35.2	(34.4)	25.2	(40.6)
FSL	22.6	(432.4)	24.1	(529.7)	26.3	(482.6)	21.5	(413.9)	18.9	(469)
FNO	39.8	(35.9)	25.4	(45.7)	27.6	(39.8)	37.3	(43.0)	41.3	(51.6)
NNO	18.9	(3.0)	16.1	(3.4)	23.5	(3.4)	29	(2.2)	28.8	(2.2)
BNO	18.4	(4.2)	18.9	(4.0)	22.5	(3.6)	17.4	(3.9)	15.6	(4.8)
BDI	50.3	(48.1)	33	(64.5)	34.4	(50.2)	35.3	(77.0)	35.1	(86.3)
TAR	35.1	(27.8)	37.9	(26.3)	40.8	(30.9)	49.6	(25.3)	32.5	(27.1)
FSNO	47.1	(27.7)	52.5	(25.7)	45.3	(21.4)	54.9	(16.9)	51.5	(18.9)
SL	8.2	(11.8)	6.7	(12.6)	7.1	(12.6)	10.1	(12.4)	8.7	(12.8)
PL	14.6	(7.5)	10.5	(7.1)	9.9	(7.3)	12.5	(6.8)	12.2	(7.7)
PW	20.2	(4.5)	11.7	(5.3)	13.4	(5.4)	18.1	(4.7)	18.8	(4.6)
PLL	14.3	(3.4)	15.9	(3.6)	16.8	(2.9)	14.8	(3.1)	19.7	(3.1)
OL	14.8	(4.0)	12.3	(4.1)	9.6	(4.1)	14.8	(4.4)	13.5	(4.3)
size	150		115		1000		300		800	

III

Comparing distribution of morphological and allozyme variation in a locally rare plant, *Lychnis viscaria*

by

Kaisa Mustajärvi and Pirkko Siikamäki

Manuscript

Comparing distribution of morphological and allozyme variation in locally rare plant, *Lychnis viscaria*

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Abstract

Knowledge of the levels and distribution of genetic variation is considered important for management purposes of rare species. The use neutral markers for the analysis of the levels and distribution of genetic variation is currently debated, because the divergence of neutral markers may be different from adaptively significant variation, such as variation in morphological traits. We compared the distribution of variation, level of isolation, and genetic differentiation between populations of *Lychnis viscaria*, a locally rare plant. Variation was measured, a) with allozymes, b) as morphological variation in natural sites, c) as morphological variation in common garden. Populations were differentiated for both isozyme and morphological traits but, surprisingly, more differentiated by neutral isozymes than by morphological variation. This suggests that genetic drift is the major factor affecting population differentiation. Cluster analysis based on morphological and allozyme data did not result in the same population groupings, which was further emphasised by the lack of correspondence of genetic distances and Mahalanobis distances (Mantels' test). None of the distance measures correlated with geographic distance of the populations and no evident environmental factor could explain the groupings of the populations. This suggests that genetic drift is probably the major factor affecting population differentiation, and morphological traits and allozymes are differentiating independently of each other. The lack of correlation between allozyme and morphological differentiation suggests that obtaining data on patterns of quantitative trait variation requires common garden studies that are often time- and labour intensive, but according to these results, necessary.

Introduction

Human induced changes in the landscape during this century are an ever-increasing threat to many plant species. Especially small isolated populations face an increased risk of extinction as a result of environmental, demographic and genetic stochastity. Although it is generally accepted that environmental stochastity is the major threat, the relative importance of random genetic processes for demography and extinctions of rare plants is still under debate. The future evolutionary adaptations depend on the existence of genetic variation. Thus, genetic variation is the prerequisite for long term survival of a species, and therefore obtaining information about the overall level of genetic diversity and its distribution within and among populations is a crucial issue in conservation biology. One of the goals of conservation genetics is to use this information to implement conservation policies for a given species, and in particular to identify areas for on-site conservation (Millar & Libby 1991) to preserve variation over species range.

Currently great deal of conservation resources are spent protecting small and threatened peripheral populations of species that are not endangered globally. In addition to focusing on saving the evolutionary potential of endangered species, the issue is relevant for identifying genetically divergent populations of widespread species. Lesica and Allendorf (1995) have suggested that as the peripheral populations of widespread species are situated in ecologically marginal habitats, they may posses alleles and adaptations not present in the main distribution area and serve as candidate populations for local adaptations and speciation events. Therefore they may be of great conservation value. If the environment changes dramatically (e.g. due to global warming) the survival of a species or new trait for an economically important species may depend on the adaptations that have arisen in these peripheral areas.

Traditionally, the distribution of genetic variation has been assessed with allozyme electrophoresis (see e.g. Schwaegerle and Schaal 1979; Hamrick & Godt 1990) and recently with other genetically based neutral markers such as RAPD (Wolff & Peters-van Rijn 1993, Black-Samuelsson et al. 1997) and microsatellite techniques. The underlying assumption is that the distribution of genetic variation at marker loci directly reflects the distribution of variation that influences adaptation and individual fitness.

However, since allozymes are considered to be selectively neutral, it is still unclear whether a marker-based approach to genetic conservation also leads to gains in the capture of adaptive genetic variation (Millar & Libby 1991, Milligan et al.1994). The adaptive variation is more likely to be predominantly shaped by selection pressures, while neutral marker diversity is influenced by stochastic processes such as founder effects and genetic drift. Moreover, variation in single loci marker genes are likely to respond to changes in population size differently than adaptive variation in quantitative traits that often are controlled by multiple loci (Lande & Barrowclough 1987, Foley 1992). Although some earlier studies have found a connection between marker diversity and

quantitative trait variation (reviewed in Hamrick 1989), many experimental (Podolsky & Holtsford 1995, Karhu et al. 1996, Knapp & Rice 1998, Black-Samuelsson et al. 1997) and theoretical studies (see review in Lynch 1996) have arisen to doubt this connection. Studies on the distribution of morphological divergence and quantitative genetic studies have been suggested instead, or in connection with, allozyme assays when screening for diverse populations for conservation purposes (Lynch 1996, Lande & Barrowclough 1987). At the same time, however, allozymes are used to develop sampling and management plans (Ceska et al. 1997, Petit et al. 1997, Chamberlain 1998)

But why the use of the molecular markers is so wide spread if their relationship to adaptively relevant variation is still under debate and other measures are considered more relevant for conservation purposes? The advance of neutral markers over quantitative trait variation is the possibility of directly assessing genetic variation to the allele level without the confounding influence of environmental factors, and the relative ease and non-disruptive nature of obtaining data. The assays of morphological variation do not only require "common garden" experiments, but also a series of controlled crossings which make them very labour intensive. Therefore, when time and resources are limited and the usefulness of molecular markers is questioned, conservation biologists may be tempted to measure population differentiation in quantitative traits from wild individuals in their natural habitats.

We examined the relationship between distribution of morphological variation, both in common garden and in natural sites, and allozyme variation in central and marginal populations of *Lychnis viscaria*. This species was much more common a century ago, when old fashioned agriculture constantly created and maintained suitable habitats for the species to occupy. Nowadays few peripheral populations, situated on rocky cliffs, exist in Central Finland (Välivaara et al. 1991) and in the province of central Finland the species is listed regionally endangered. The distribution area and characteristics of *L. viscaria* are typical for several plant species in northern Europe and America. As a consequence it may serve as a suitable model organism for several rare, perennial, and hermaphroditic plants that have colonised their habitats since the last glaciation. We studied morphological variation patterns among 11 populations (8 peripheral, 3 from central distribution area) of *L. viscaria* in 1) common garden and 2) in wild individuals in their natural habitats and compared these patterns of morphological variation to patterns of 3) allozyme variation in these populations. We also examined if geographical distance or environmental factors could explain the population differentiation. We aimed to examine if either variation in natural sites or allozyme variation could predict the variation observed in common garden. If genetic drift (random fluctuations of gene frequencies and loss of alleles, due to a fact that genes passed to next offspring are not perfectly representative sample of the genes in parent generation) was the major factor affecting population differentiation, and if there was a connection between neutral allozymes and morphological variation, the morphological variation in common garden and allozyme variation would be connected. On the other hand if selection would have strong effect, it might also be reflected in the morphology of wild individuals, and thus common

garden and natural site variation might be correlated. If no correlations were found, it suggests that genetic drift may be a predominat factor in shaping population differentiation and it is affecting allozyme and morphological variation differently.

Materials and methods

Study species

Lychnis viscaria L. (Caryophyllaceae) is a perennial herb, occurring in open sunny habitats like dry meadows and south sloping rocky outcrops. The flowers are protandrous and pollinated by insects, mainly bumblebees and butterflies (Jennersten 1988, Wilson et al. 1995). The seeds are dispersed by gravity. *L. viscaria* occurs in fairly distinct patches of a few to thousand individuals throughout northern and central Europe, the main distribution area extending up to the 62nd latitude. Few isolated populations are found up to the 68th latitude. In this study, morphological variation was measured in populations located both in the central and in the peripheral distribution area of *L. viscaria*, both in common garden and in natural populations. In the surroundings of Tampere, (61° 30'N, 23° 45'E) situated at the main distribution area, where the species occurs in larger populations mainly on roadsides and dry meadows (Hultén 1971), three populations were sampled (Nokia, Epilä, Kalevankangas). In the surroundings of Jyväskylä (62° 15'N, 25° 45'E), 150 km NE of Tampere, *L. viscaria* occurs in its northern range in rather small and isolated patches on rocky outcrops. In the Central Finland, there has been 29 know populations during last century (Välivaara et al.1991), but now only 8 populations are known. All these populations were included in our study (for more information on population characters see Siikamäki & Lammi 1998, Lammi et. al 1999). In this north-south direction there is a clinal change in climate and e.g. a difference of one week in the length of growing season. The genetic variation in these populations was examined earlier by allozyme electrophoresis (Lammi et al. 1999).

Common garden

The seed material for common garden was collected from 30 randomly chosen plants in each of the populations in summer 1995, except for population Kotimäki, where the seeds were collected from all the of the 7 flowering individuals. After six months storage in cold at 5°C, the seeds were sown in plastic pots containing mixture of vermiculite and peat moss in March 1996. If more than one seed germinated, extra seedlings were thinned and one randomly chosen seedling was left in the pot. The seedlings were randomly arranged in a greenhouse in the Laukaa agriculture and elite plant station, situated in the central Finland. The seedlings were kept in standard greenhouse

conditions (21°C, 12 hours light 12 hours darkness) and they received equal amounts of water and nutrients. In the beginning of June 1996, the seedlings were transplanted out into a garden bench in the research station. Since only a small fraction of the plants flowered in 1996, the morphological characters were measured in 1997 when all the plants flowered.

Measurements in the field and in the common garden

In summer 1997, 40 random individuals were marked with small flags in the natural sites of each of the study populations, except for Kotimäki where only 13 rosettes were found and all the 6 flowering individuals were measured. These individuals and the plants grown in the common garden were measured for several morphological characters during the growing season. The plants were measured at the same phenological state in all populations both in common garden and in natural sites.

The length of the longest leaf and its maximum width together with the rosette diameter were measured before flowering. The following characters were measured from the longest flowering stem during flowering: length of the flowering stem, number of flowers, number of leaf nodes, number of branches and the distance between the two lowest branches in an inflorescence. The number of flowering stems was also calculated from each individual. One randomly chosen flower from each plant was taken into a lab to measure the length of the sepal, the maximum length and width of the longest petal, and the length of the ovary.

A variance component analysis with population as a random factor was performed for each character using SPSS 7.5 and relative maximum likelihood (REML) directive. The within -population component (V_G) and between population component (V_{pop}) were used to quantify the level of population differentiation (Q_{st}) for each of the characters (Wright 1951; see also Spitze 1993; Podolsky & Holtsford 1995; Waldman & Andersson 1998). Assuming that populations are in Hardy-Weinberg equilibrium and the characters have a strong additive component the

$$Q_{st} = \frac{2V_G + V_{pop}}{V_{pop}}$$

. As this parameter is analogous to F_{st}, it allows us to compare the population differentiation in allozyme locus allele frequencies with differentiation in morphological traits. However, this measure is only a rough estimate of population differentiation, because as family data is not included, it cannot exclude the sample variation.

In natural sites the effect of environment is fully present and thus the difference between populations reflect also difference between environments and it would be expected that differentiation in natural sites is stronger than in common garden. Variation in common garden gives more reliable estimate on the genetic basis of morphological variation than variation in natural populations.

Genetic variation

The allozyme data used in this study is the same as reported in Lammi et al 1999. In late July 1995 naturally pollinated seeds were collected from 30 randomly chosen maternal plants in each population, except for Kotimäki where all the flowering individuals (7 maternal plants in 1995) were sampled. Several seedlings per maternal plant were grown in a laboratory, and usually about 30 seedlings per population (all from different maternal plants) were randomly chosen for analysis. The amount of genetic variation in the populations was determined by means of starch gel electrophoresis as described in Wendel & Weeden (1989) and May (1992) (for more detail, see Siikamäki & Lammi 1998). Totally 13 enzymes and 17 loci were screened. Six loci (F-EST-1, F-EST-2, GPI-2, PGM-2, SKD-1, SOD-1) were polymorphic.

Population structure and inbreeding coefficients were calculated by F-statistics (Wright 1969) according to the protocol of Weir & Cockerham (1984) using FSTAT (Goudet 1995). To examine genetic similarity among populations genetic identity and distance measures were also calculated for each pair of populations (Nei 1972).

Associations between genetic distances, Mahalanobis distances, and linear geographic distance were estimated using Mantel's test. To illustrate the genetic and morphological relationships among populations, UPGMA phenograms were constructed using Mahalanobis distances (with SAS) for morphological data and Nei's genetic distances (with BIOSYS) for allozyme data.

Results

Morphological variation in common garden and in natural populations

There were significant differences in the morphological traits between the populations both in the common garden and in the natural populations. The relatively high Q_{st} values, although lower than mean F_{st} value for allozyme data, for all the characters in common garden and natural populations also indicate that population are morphologically differentiated (Table 1). As expected, however, the morphological features of populations were different in natural sites and common garden. When character means of populations measured from natural sites were compared with measures from common garden, only ovary length in natural site correlated with ovary length in common garden (Table 2).

To find out which characters best discriminate between populations a discriminant analysis using all the measured characters was performed separately for data sets from common garden and from natural populations. The first two functions with eigenvalues over one were analysed further and plotted against each other to illustrate the morphological similarities between populations (Figure 1a, b). There were differences between common garden and natural populations in the loadings of different characters to the discriminant

functions. In the natural populations, the first discriminant function described 33.6% of the total variance between populations and was mostly correlated with petal length, petal form, sepal length and petal width. So this function could be best described as flower morphology. The second function explained additional 26.1% (together 60.3%) of the variance and correlated negatively with leaf width and positively with ovary length and petal width. In the common garden, the first discriminant function was mostly correlated with rosette width and the distance between the two lowest branches within an inflorescence. This function explained 30.3% of the variance between populations. The second function explained additional 22.0% (two functions together 52.3%) and was best correlated with sepal length, ovary length and petal lobule length and was therefore named flower morphology.

The common garden and natural sites data was combined and a discriminant analysis was performed for the whole data set (Figure 2). The first discriminant function clearly separated the original and common garden populations. It explained 31.6% of the total variance between populations and was positively correlated with rosette width, stem length, and flower number. Thus this variable describes plant size that was significantly larger for common garden individuals due to better resource availability in common garden than in natural habitats. The large size of individuals in natural habitats did not lead to large size in common garden, as the size order of populations changed dramatically when plants were grown in common garden (Figure 3). Second discriminant explained additional 14.4 % of variance (total: 46%) and was significantly positively correlated with branch distance (Figure 2).

The Mahalanobis distances between populations were higher in natural sites than in common garden (Figure 4a,b). Based on visual estimation, the structure of dendrogram based on Mahalanobis distances measured from natural populations data (Figure 4a) was very different from dendrogram based on the data from the common garden (Figure 4b). This was further supported by Mantel's test, that did not reveal any significant correlation between the Mahalanobis distances measured in common garden and natural populations ($z = 0.541$, $P>0.05$, no. of comparisons 56).

The morphological Mahalanobis distances between populations were independent of their geographical distance both in natural sites (Mantel's test $z = 1.618$, $P=0.284$) and in common garden (Mantel's test $z = 1.509$, $P=0.131$). Only in the dendrogram based on the common garden data a few clusters can be explained by their close geographical approximity: Vaaruntie and Vaarunjyrkkä (distance <1km), Epilä and Kalevankangas (distance 5km). These are also the geographically closest pairs in the whole data set. To see if some environmental factors that differed between the populations could explain the population groupings, we classified the populations according to habitat type (M = Meadow, RS = roadside, R = rocky) light regime (L = very exposed, M = sunny, S = shaded), and herbivory (intensive herbivory, >25% of marked flower stems cut by herbivores, most probably voles, in 1997) (Figure 4). None of the factors explained the groupings of populations either in common garden or in natural sites.

Morphological variation vs. genetic variation

The level of genetic variation was quite low in the assayed populations (mean H_{exp} = 0.056). The detailed analysis on the allozyme variation is described in Lammi et al. (1999). The high mean F_{st} (Table 1, Table 2 in Lammi et al. 1999) indicated that populations were even more differentiated according to neutral isozyme markers than with their morphological characters. The genetic distance between populations did not depend on their geographic distance (Lammi et al. 1999).

The structuring of dendrogram constructed with the Nei´s genetic distances (Figure 2 in Lammi et al. 1999) did not resemble either of the dendrograms based on morphological data (Figure 4). Mantels test also indicated that the genetic distance between populations were not related to their Mahalanobis distances either in natural sites (z = -0.300, P = 0.764) or in the common garden (z = -0.535, P = 0.596).

Discussion

Although the populations seemed to be differentiated by both allozyme variation and morphology, the patterns of allozyme variation were quite dissimilar to patterns of morphological variation both in common garden and in natural sites. Neither morphological nor allozymic patterns of genetic variation were related to geographic distance between populations and none of the environmental factors could explain the groupings of the populations by morphological traits. The allozymes showed stronger divergence between populations than any of the morphological traits suggesting that genetic drift, instead of selection, is the predominant factor in shaping the differentiation and genetic variation between populations.

In earlier studies comparing morphology and molecular markers both agreements (reviewed Hamrick 1989) and disagreements (Hamrick 1989, Podoslky & Holtsford 1995, Black-Samuelsson et al. 1997, Knapp & Rice 1998) between the two measures have been reported. The difference has been explained often by the different selective nature of molecular and morphological traits, because it is relevant to assume that both the direction and magnitude of selective forces acting on the majority of allozyme variation differ from those acting on several quantitative traits (Spitze 1993, Podolsky & Holtsford 1995). Knapp and Rice (1998) found no relationship between morphological variation and allozyme variation in *Nassella pulchra*, but on the other hand, allozyme variation was significantly correlated with geographic distance and on the other, morphological variation was correlated with climatic distance between populations. Knapp & Rice (1998) concluded that selection caused by climatic differences was probably the major factor leading population differentiation of morphological traits, while genetic drift was the major factor that shapes the allozyme variation.

In the case of *L. viscaria* the lack of correlation between allozyme variation,

geographic distance, and morphological variation suggest that the populations are highly isolated. Regardless of their relatively close proximity to each other genetic drift is acting on the differentiation independently of their geographic distance. This was expected, however, as gene dispersal in *L.viscaria* is probably very rare (Lammi et al. 1999) due to the limited pollen- (Kwak et al. 1991) and seed dispersal mechanisms (Wilson et al.1995).

One selective force acting in natural populations of *L. viscaria* could be competition affecting seedling establishment. The seedling mortality is very high at early stages (reviewed in Wilson et al. 1995). In the forested sites, this is probably due to low light regimes, as *L. viscaria* seedlings are not shade tolerant. On the other hand, seedlings in bare rocky cliffs have to adapt to low water availability and heat. The intensity of herbivory and seed predation by moths may also act as selective forces, and vary greatly among our study populations (K. Mustajärvi personal observations). These factors may vary in small local scales and are in no way related to the geographical distance between populations. As gene flow is very restricted populations may differentiate both in morphology and neutral markers independently of their geographic distance. None of the factors identified by us, however, could explain the population groupings. Thus the selective forces on morphological variation are probably not very strong.

This was further supported by the fact that population differentiation was much stronger for neutral allozymes than for morphological traits, suggesting that genetic drift is causes genetic differentiation at a faster rate than selection causes morphological differentiation. In most of the earlier studies, population differentiation has been stronger for quantitative traits than for allozymes: Podolsky & Holtsford (1995) found that several morphological traits in *Clarkia dudleyana* were much more diverged than allozymes and claimed this to be a result of selection favouring different trait optima in different areas. Lynch et al (1999) reported for *Daphnia pulex* that although average level of genetic subdivision was the same for quantitative traits as that for nuclear marker, some traits (body size) were strongly driven by local selection. Hedrick and Savolainen (1996) reported high adaptive trait variation, but very low molecular marker differentiation in populations of *Picea abies.* They stated that strong selective forces cause the adaptive trait differentiation in spite of extensive gene flow, but due to gene flow the effects of drift are non-existent and no molecular differences can be found. Higher F_{st} values than Q_{st} values in *L. viscaria* may indicate that populations are under stabilising selection. Thus, in *L. viscaria* there were large among-population differences at the molecular level, because of genetic drift, but as the populations are in relatively similar environments there is less significant morphological differences.

As reviewed by Knapp and Rice (1998), the relationship between patterns of allozyme and quantitative trait data may depend on several factors 1) the scale of population sampling over the species range (Beer 1993 et al.), 2) the evolutionary history of populations, 3) the mating system of the species (Price et al. 1984), 4) choice of the evaluated traits (Beer 1993 et al., Prout and Barker 1993, Spitze 1993, Podolsky and Holtsford 1995), and 5) the number of sampled populations. Association between quantitative trait variation and populations

should is more likely if populations are sampled over small scale over species range, instead of over whole species range (Beer 1993 et al.), and stochastic factors like bottlenecks, founder events and genetic drift, instead of selection, have been the major factors in shaping the population divergence (Bryant 1984, Knapp and Rice 1998). The relation ship is also found more often for selfing species (Price et al. 1984). Despite *L. viscaria* were sampled over small range, genetic drift is strongly affecting the populations (Lammi et al. 1999), and populations are likely to be relatively regularly selfing (Lammi at al. 1999, K. Mustajärvi personal observations), no connection between the quantitative trait variation and allozymes was found. Clearly the connection is not self evident, even for a species for which the preconditions seem likely to be fulfilled.

A number of studies has reported morphological variation between marginal and central populations (see review in Lesica & Allendorf 1995). Due to different, usually harsher, environmental conditions in marginal areas, compared to those in the central distribution areas, the marginal populations may differ from populations in central distribution area. In this study there was considerable differences among populations, but the central and marginal populations did not group into two distinctive groups. However, some marginal populations can be highly morphologically differentiated and posses local adaptations. For example population Iso-Salmijärvi was highly differentiated from others according to the common garden data. Closer examination of the data showed that individuals in this population were very vigorous (numerous long flowering stems with many flowers), but the life span of the individuals was shortest of all populations (78% died within 3 years, population mean ranges from 11% to 78%, K. Mustajärvi, unpublished data). This indicates allocation to vigorous growth and reproduction instead of longevity. With allozyme data this population showed no special divergence from other populations, and thus would not been identified as specialised.

There was no connection between morphological relationship among populations when measured in common garden and morphological relationships measured in natural sites. This suggests that sampling of wild individuals in natural populations to identify morphologically divergent populations for conservation purposes, eg. ex-situ sampling, in-situ conservation, restoration, or re-introduction is questionable. Phenotypic differentiation in natural sites may be crucially affected by environmental factors and observed divergence between populations may not reflect the genetic differences between populations. According to our results, it seems doubtful to rely solely on allozyme data when evaluating populations for conservation purposes, even when genetic drift seems to be the major factor influencing population differentiation. Like other authors (Hedrick & Savolainen 1996, Knapp & Rice 1998), we suggest that allozyme data, as well as other molecular marker data, is used in conjunction with other information including geographical, ecological, life history, historical, and morphological data from common garden experiments or quantitative genetic data. However, allozymes provide valuable information on the history and structure of a population, the mating or reproductive system of an organism or the extent of reproductive isolation between populations (Milligan et al. 1994, Hedrick &

Savolainen 1996). Therefore the combination of molecular marker data and quantitative trait data would be ideal for conservation genetic purposes.

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References

Beer, S. C., Goffreda, J., Phillips, T. D., Murphy, J. P. & Sorrells, M. E. (1993) Assesment of genetic variation in *Avena sterilis* using morphological traits, isozymes, and RFLP's. Crop Science 33: 1386-1393.

Black-Samuelsson, S., Eriksson, G., Gustafsson, L., Gustafsson, P. (1997) RAPD and morphological analysis of the rare plant species *Vicia pisiformis* (Fabaceae) Biological Journal of the Linnean Society 61: 325-343.

Bryant, E. H. (1984) A comparison of electrophoretic and morphometric variability in the face fly, *Musca autumnalis.* Evolution 38: 455-458.

Ceska, J. F., Affolter, J. M. & Hamrick, J. L. (1997) Developing sampling strategy for *Baptisia arachnifera* Based on allozyme diversity. Conservation Biology 11: 1133-1139.

Chamberlain, J.R. (1998) Isozyme variation in *Calliandra calothyrsus* (Leguminosae): Its implications for species delimitation and conservation. American journal of Botany 85: 37-47.

Foley, P. (1992) Small population genetic variability at loci under stabilising selection. Evolution 46: 412-429.

Goudet, J. (1995) FSTAT. Version 1.2. A computer program to calculate F-statistics. The Journal of Heredity 86: 485-486

Hamrick, J. L. (1989) Isozymes and the analysis of genetic structure in plant populations. In: *Isozymes in Plant Biology* (ed. Soltis DE, Soltis PS) pp 87-105. Dioscorides Press, Oregon.

Hamrick, J.L. & Godt, J.W. 1990. Allozyme diversity in plant species. In: *Plant population genetics, breeding and genetic resources* Brown, A.H.D. (ed. Clegg, M.T. Kahler, A.L. & Weir, B.S.) pp 43-63. Sinauer Associates, Sunderland, Massachusetts.

Hedrick, P.W. & Savolainen, O. (1996) Molecular and adaptive variation: a perspective for endangered plants. In: *Southwestern rare and endangered plants: Proceedings of Second conference; 1995 September 11-14; Flagstaff, AZ.*

Gen. Tech. Rep. RM-GTR-283. (ed. Maschinski J, Hammond DH, Holter L) pp.92-102. Forth Collins, Co: U.S. Department of Agriculture, Forest service, Rocky Mountain Forest and Range Experiment Station.

Hultén, E. (1971) *Atlas of the distribution of vascular plants in northwestern Europe* Generalstabens litografiska anstalts förlag, Stockholm.

Jennersten, O. (1988) Pollination of *Viscaria vulgaris* (Caryophyllaceae): the contributions of diurnal and nocturnal insects to seed set and seed predation. Oikos 52: 319-327.

Karhu, A., Hurme, P., Karjalainen, M., Karvonen, P., Kärkkäinen, K., Neale, D. & Savolainen, O. (1996) Do molecular markers reflect patterns of differentiation in adaptive traits in conifers. Theoretical and Applied Genetics 93: 215-221.

Knapp, E.E. & Rice, K.J. (1998) Comparison of isozymes and quantitative traits for evaluating patterns of genetic variation in purple needlegrass *(Nassella pulchra)*. Conservation Biology 12: 1031-1041.

Kwak, M., Kremer, P., Boerricter, E. & Van der Brand, C. (1991) Pollination of the rare species *Phyteuma nigrum*: flight distances of bumble bees. Proceedings Experimental and Applied Entomology 2:131-136.

Lammi A., Siikamäki P., Mustajärvi K. (1999) Genetic diversity, population size and fitness in central and peripheral populations of a rare plant *Lychnis viscaria* (Caryophyllaceae). Conservation Biology 13: 1069-1078.

Lande, R. & Barrowclough, G. F. (1987). Effective population size, genetic variation, and their use in population management. In: *Viable populations for Conservation*. (ed. Soulé M. E.) pp 87-123. Cambridge University Press, Cambridge.

Lesica, P. & Allendorf, F. W. (1995) When are peripheral populations valuable for conservation? Conservation Biology 9: 753-760.

Lynch, M. (1996) A quantitative-genetic perspective on conservation issues. In: *Conservation Genetics: Case Histories from Nature* (ed. Avise J.C., Hamrick J.L.). pp 471-501. Chapman and Hall, New York.

Lynch, M., Pfrender, M., Spitze, K., Lehman, N., Hicks, J., Allen, D., Latta, L., Ottene, M., Bogue, F. & Colbourne, J. (1999) The quantitative and molecular genetic architecture of a subdivided species. Evolution 53: 100-110.

May, B. (1992) Starch gel electrophoresis of allozymes. *Molecular genetic analysis of populations* (ed. Hoelzel) pp 1-27. Oxford University Press, Oxford, United Kindom.

Millar, R. H. & Libby, W. J. (1991). Strategies for conserving clinal, ecotypic, and disjunct population diversity in widespread species In: *Genetics and conservation of rare plants* (ed. D. A. Falk and K. E. Holsinger) pp 149-170. Oxford University Press, New York.

Milligan, B.G., Leebens-Mack, J. & Strand, A.E. (1994) Conservation genetics: beyond the maintenance of marker diversity. Molecular Ecology 3: 423-435.

Nei, M. (1972) Genetic distance between populations. American Naturalist 106: 283-292.

Petit, R.J., El Mousadik, A. & Pons, O. (1997) Identifying populations for

conservation on the basis of genetic markers. Conservation Biology 12: 844-855.

Podolsky, R. H. & Holtsford, T. P. (1995) Population structure of morphological traits in *Clarkia dudleyana* I. Comparison of F_{st} between allozymes and morphological traits. Genetics 140: 733-744.

Price, S. C., Shumaker, K. M., Kahler, A. L., Allard, R. W. & Hill, J. E. (1984) Estimates of population differentation obtained from enzyme polymorphisms and quantitative characters. Journal of Heredity 75: 141-142.

Prout, T. & Barker, J. S. F. (1993) F-statistics in *Drosophila buzzattii:* selection, population size, and inbreeding. Genetics 134: 369-375.

Siikamäki, P. & Lammi, A. (1998) Fluctuating asymmetry in central and marginal populations of *Lychnis viscaria* in relation to genetic and environmental factors. Evolution 52: 1285-1292.

Spitze, K. (1993) Population structure in *Daphnia obtusa*: quantitative genetic and allozyme variation. Genetics 135: 367-374.

Schwaegerle, K.E. & Schaal, B.A. (1979) Genetic variability and founder effect in the pitcher plant *Sarracenia purpurea* L. Evolution 33: 1210-1218

Välivaara, R., Raatikainen, T., Saari, V., Halinen, P., Salminen, M. & Raatikainen, M. (1991) The threatened vascular plants in Central Finland. Publications A2. Keski-Suomen Liitto, Jyväskylä.

Waldman, P. & Andersson, A. (1998) Comparison of quantitative genetic variation and allozyme diversity within and between populations of *Scabiosa canescens* and *S. columbaria.* Heredity 81: 79-86.

Weir, B. & Cockerham, C. C. (1984) Estimating F-statistics for the analysis of population structure. Evolution 38: 1358-1370.

Wendel, J.F. & Weeden, N.F. (1989) Visualization and interpretation of plant isozymes. In: *Plant population genetics, breeding, and genetic resources* (ed. Brown HD, Clegg MT, Kahler AL et al.), pp 43-63 Sinauer, Sunderland, Massachusetts.

Wilson, G. B., Wright, J., Lusby, P., Whittington, W. J. & Humphries, R.N. (1995) *Lychnis viscaria* L. (*Viscaria vulgaris* Bernh.) (Biological flora of the british isles No. 188). Journal of Ecology 85: 1039-1051.

Wolff, K. & Peters-Van Rijn, J. (1993) Rapid detection of genetic variability in chrysanthemum (*Dendranthena grandiflora* Tzvelev) using random primers. Heredity 71: 335-341.

Wright , S. (1951) the genetic structure of populations. Annual Eugenics 15: 323-354.

Wright , S. (1969) *Evolution and genetics of populations. Volume 2. The theory of gene frequencies.* University of Chicago Press, Chicago.

TABLE 1 The F_{st} value from allozyme analysis and the Q_{st} values for all the measured morphological characters in natural populations (n) and common garden (c). Both F_{st} and Q_{st} indicate population differentiation.

	Q_{ST} (n)	Q_{ST} (c)
leaf length	0.080	0.135
leaf width	0.256	0.019
leaf form (ll/lw)	0.136	0.069
rosette diameter	0.096	0.186
stem length	0.155	0.079
No. of flowers	0.105	0.082
distance between branches	0.046	0.207
tar length	0.093	0.151
No. of flower stems	0.089	0.081
sepal length	0.168	0.195
petal length	0.287	0.111
petal width	0.141	0.133
petal form (pl/pw)	0.185	0.161
length of petal lobule	0.120	0.153
ovary length	0.188	0.140
F_{st}	0.430	

TABLE 2 The correlations of population means between measures from common garden and in natural sites (n = 11).

	r_s	P
leaf length	-0.336	0.312
leaf width	-0.318	0.340
leaf form (ll/lw)	0.136	0.069
rosette diameter	-0.364	0.272
stem length	-0.082	0.811
no. of flowers	0.291	0.385
distance between branches	0.075	0.827
tar length	0.182	0.593
no. of flower stems	-0.218	0.519
sepal length	0.409	0.212
petal length	0.545	0.083
petal width	0.427	0.190
petal form (pl/pw)	0.185	0.161
length of petal lobule	0.427	0.190
ovary length	0.811	0.002

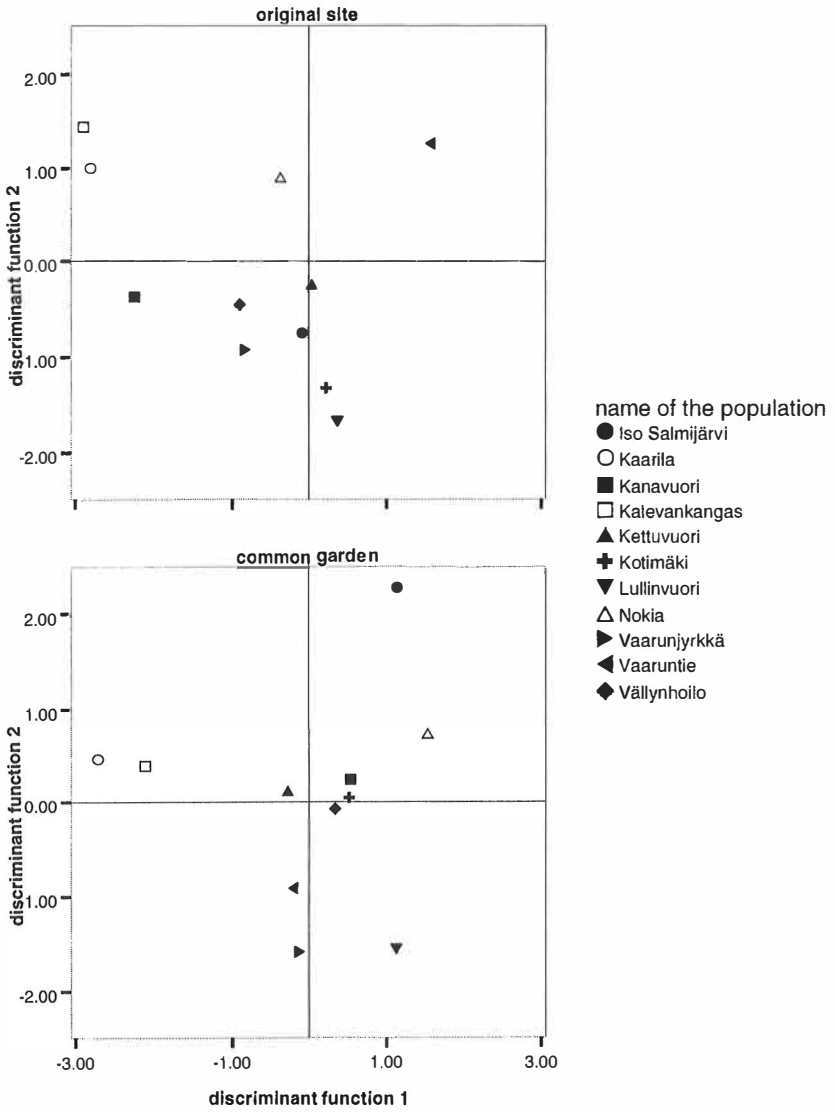

FIGURE 1 A scatterplot illustrating the morphological relationships between populations by means of discriminant functions in a) original sites b) common garden.

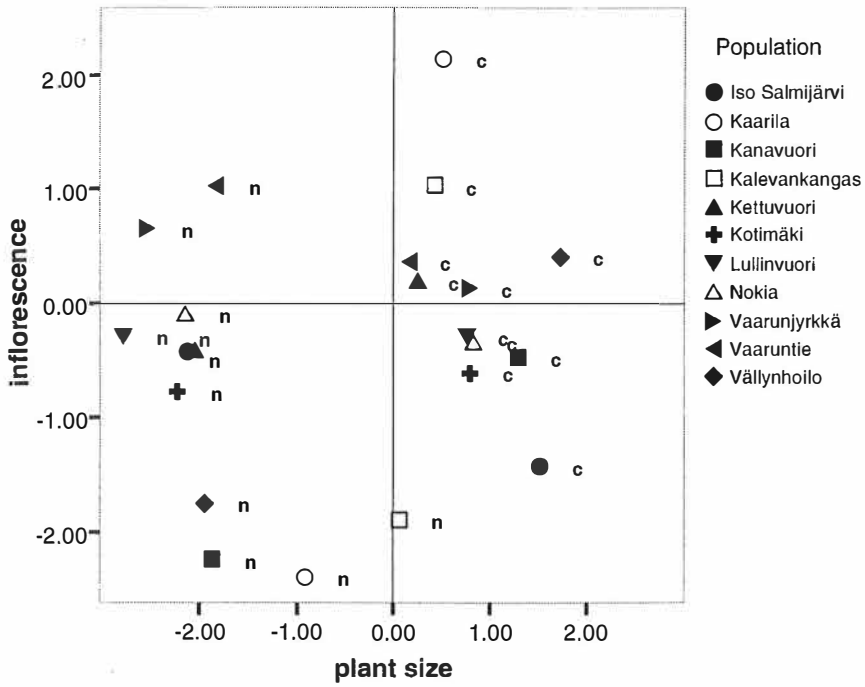

FIGURE 2 Mean discriminant scores of populations for the discriminant analysis done for combined common garden and natural site data (n = natural site, c = common garden).

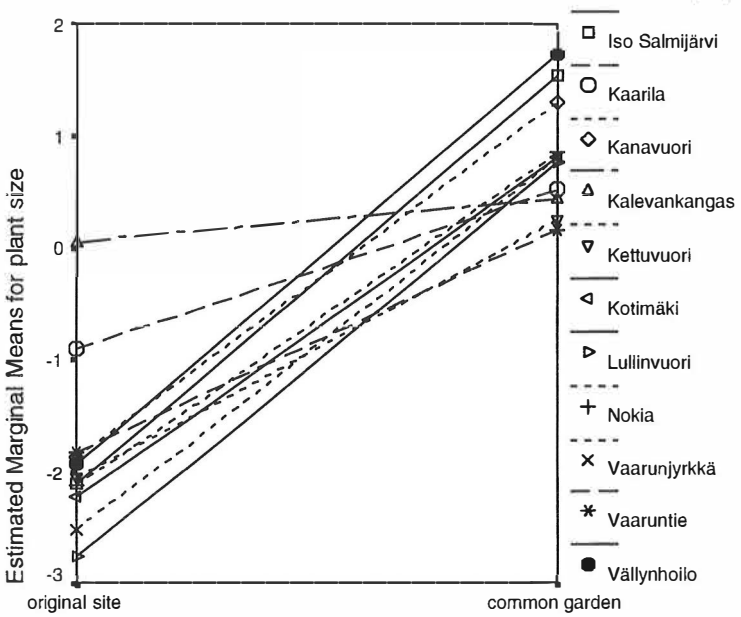

FIGURE 3 Mean flower stem number in 11 *L. viscaria* populations measured in common garden and natural sites, indicating that the order of populations change when measured in common garden compared to natural sites.

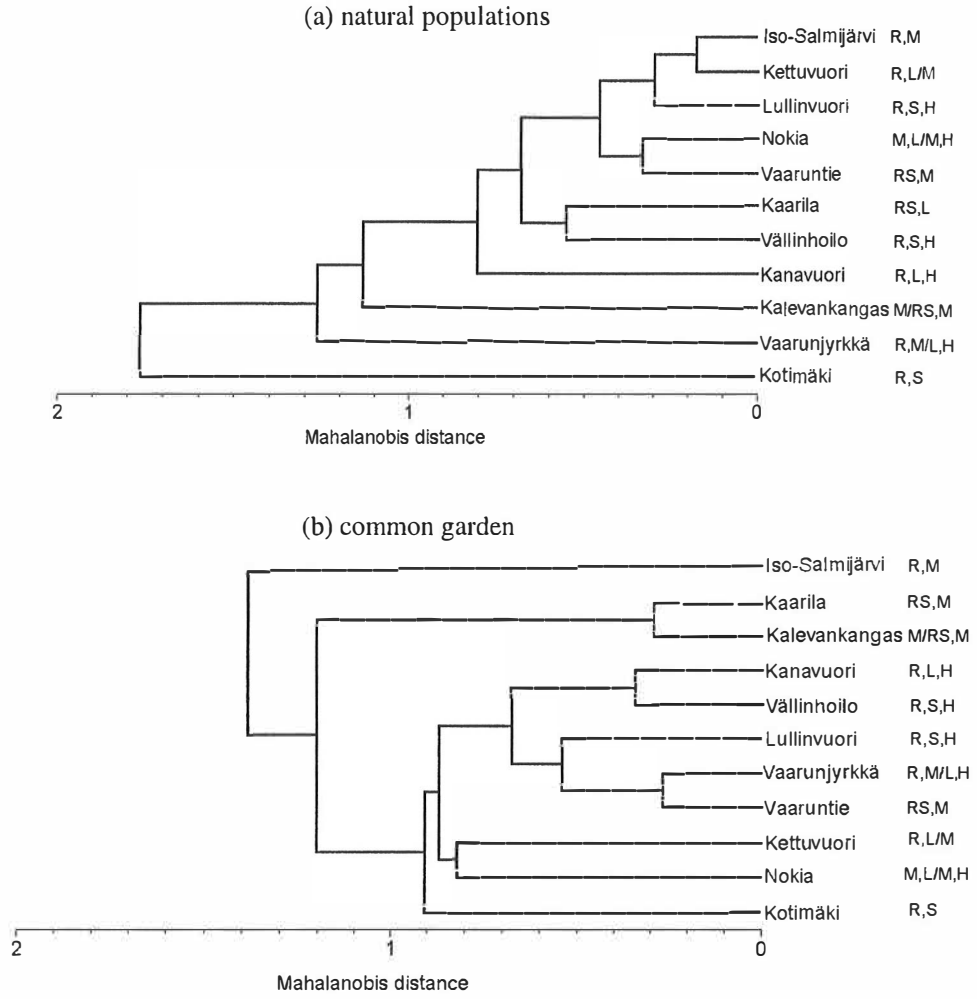

FIGURE 4 Dendrograms based on morphological characters in natural sites (a) and in common garden (b). The letters state for habitat type (M = meadow, RS = roadside, R = rocky), light regime (L = very exposed, M = sunny, S = shaded), and intensive herbivory (H).

IV

Consequences of plant population size and density for plant-pollinator interactions and plant performance

by

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V

Expression of inbreeding depression in *Lychnis viscaria*: effects of population mating history and nutrient availability

by

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