

Genetic structure and gene flow in an endangered perennial grass, *Arctophila fulva* var. *pendulina*

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Abstract

Arctophila fulva var. *pendulina* is a rare endemic perennial grass confined to seashore and riverbank meadows around the Bothnian Bay, the northernmost part of the Baltic Sea. The number of *A. fulva* populations has decreased during the last few decades in Finland and Sweden, and nowadays there are only eight populations left in the drainage area of the Bothnian Bay. We investigated the distribution of genetic variation within and between six subpopulations in the largest remaining population at Liminka Bay, Finland, using amplified fragment length polymorphism (AFLP) markers. Relatively high amounts of variation were found in the subpopulations, the mean Nei's expected heterozygosity being typical (0.267) for an outcrossing species. Despite the fact that no seedlings or viable seeds of *A. fulva* have been found in the previous field studies, the observed high genotypic diversity suggested that sexual reproduction has played an important role at some time during the history of the studied *A. fulva* population. Analysis of population structure revealed a low level of genotypic differentiation ($\Phi_{ST} = 0.046$) between subpopulations, and also significant sub-structuring within subpopulations. Isolation-by-distance between subpopulations was present on scales larger than 1 km. The overall pattern of genetic variation within and between subpopulations suggest that the population has characters of both stepping-stone and metapopulation models. Because our results suggested that subpopulations are more or less ephemeral, the conservation and management effort in this species should be targeted to conservation of the required habitat of the species instead of extant subpopulations.

Introduction

Arctophila fulva var. *pendulina* is a rare endemic perennial grass confined to seashore and riverbank meadows around the Bothnian Bay, the northernmost part of the Baltic Sea. In the Red Book of Finland *A. fulva* var. *pendulina* is regarded as critically endangered (Rassi et al. 2001), and it is also listed in European Union's Habitat Directive as a species that requires a designation of special areas of conservation. The number of populations

has declined during the last few decades, and at present there are only eight populations left in Sweden and Finland (Ericson and Wallentinus 1979; Siira 1994). There are also reports on marked reduction of the area of *A. fulva* populations (Ericson and Wallentinus 1979). The largest population of *A. fulva* var. *pendulina* at the Liminka Bay in Finland covers an area of approximately 3000 m², whereas the other populations cover from less than one square metre to some dozens of square metres. Decline in the

number and size of populations may be a consequence of a change in agricultural practices putting an end to the traditional cattle grazing and hay making which has kept the meadows open. Moreover, long-term data on the water quality of the Liminka Bay has indicated pronounced eutrophication (Siira 1994). These factors may have led to the competitive exclusion of some species, such as *A. fulva* (e.g. Markkola 1993).

A. fulva spreads clonally from perennial underground rhizomes as a dense front of roots, rhizomes and aboveground tillers, and hence usually forms almost pure monocultural patches. Although *A. fulva* flowers regularly, its sexual reproduction seems to be unsuccessful in the Liminka Bay population. No viable seeds have been found in seed germination and seed bank studies (Rautiainen et al. 2004), and no seedlings have been observed during the 20-years of field work (J. Siira, pers. comm.). These observations, together with reports of nonviable seed sets despite regular inflorescence formation in North Alaska (Dobson 1989), suggest that the main means for dispersal of *A. fulva* is by floating fragments of rhizomes, moved by water currents and waves, establishing at suitable habitats along the shoreline (Rautiainen et al. 2004). However, the above observations do not rule out successful occasional present or past sexual reproduction. Clonal reproduction may have several population genetic consequences both within and between population level (e.g. Balloux et al. 2003; Bengtsson 2003). High rates of clonal reproduction will positively affect allelic diversity, whereas genotypic diversity decreases with increasing rates of clonal reproduction. In other words, asexual populations are expected to maintain higher genetic diversity at each single locus but a lower number of different genotypes (Balloux et al. 2003). Maintenance of genetic diversity in asexually reproducing species requires that the structure of such populations should be recognized for *in-situ* conservation, in re-establishing extinct populations, and in sampling for *ex-situ* conservation (Frankham et al. 2002).

The decline in number and size of populations in *A. fulva* may have led to genetically small and isolated populations with lowered genotypic diversity, a phenomenon potentially enhanced through the evidently uncommon sexual reproduction. Small isolated populations are subject to

genetic drift, which will affect their evolutionary potential and erode genotypic diversity among clones (Young et al. 1996; Dudash and Fenster 2000). Knowledge of the amount of genetic diversity in the populations of endangered species is crucial for their management. However, at this moment we do not have any information on the amount of genetic variation in any of the endangered populations of *A. fulva*. Knowledge of population structuring in this apparently clonal species would also provide valuable guidelines for conservation strategies and management. Reliable estimates of population differentiation are crucial in conservation purposes because it is necessary to understand whether the populations or subpopulations are genetically isolated from each other, and if so, to what extent. The exchange of genes between populations homogenizes allele frequencies between populations and determines the relative effects of selection and genetic/clonal drift. On the other hand, gene flow introduces new polymorphisms into the populations, and increases local effective population size (the ability to resist random changes in allele frequencies), thereby opposing random genetic drift, generating new gene combinations on which selection can potentially act (e.g. Balloux and Lugon-Moulin 2002).

Estimation of the spatial dynamics of propagule movement with respect to extant landscape features may aid conservation biologists in predicting the demographic and genetic responses of a species to population subdivision (Sork et al. 1999). *A. fulva* grows usually in discrete monoculture patches and is restricted to a specific coastal habitat. Several basic hypotheses of migration patterns with contrasting predictions can be derived for population structure among such linearly arranged plant subpopulations (see e.g. Tero et al. 2003). First, despite the apparent spatial subpopulation structure the patches may form a single panmictic unit with free gene flow throughout the genetically uniform population. This kind of panmictic population structure (model 1) would increase local effective population size thus lowering the effects of genetic drift and possibility of "genetic erosion". Second, the patches may represent only fragments of formerly continuous population without any present-day migration between the subpopulations. In this kind of fragmented population (model 2) loss of gene flow would expose the subpopulations to

severe genetic drift and accumulation of deleterious mutations. Third, there may be migration only between the adjacent subpopulations i.e. the migration pattern may follow the classical stepping-stone model (model 3). This kind of migration pattern may differentiate the most distant subpopulations, which should be taken into account when planning translocations or reintroductions. Fourth, the patches of *A. fulva* may represent a source-sink metapopulation (model 4) in which more permanent, high quality source subpopulations are the net exporters and ephemeral low quality subpopulations the net importers of the migrants. In this case recognizing and maintaining the source populations would be critical for long time survival of the species. Fifth possible population structure model for *A. fulva* is the “classical” metapopulation model (model 5) with ephemeral subpopulations and diverse migration between them. If the subpopulations appear to be ephemeral, the conservation efforts of this species may be targeted to conservation of the required habitat instead of conserving existing subpopulations.

These population structure models provide different testable hypotheses concerning the distribution of genetic variation within and between subpopulations. The first three ones (models 1–3) are equilibrium models predicting that heterozygosity in subpopulations is inversely related to effective subpopulation size because of the balance between genetic drift and migration (e.g. Nei 1987). The panmixia model (model 1) predicts that populations will exhibit genetic uniformity over all spatial scales, while the fragmentation model (model 2) suggests that all subpopulations should show significant differentiation because of a lack of concurrent gene flow. In the stepping-stone model (model 3) genetic distance between subpopulations should increase monotonically with increasing geographical distance (Kimura and Weiss 1964; Hutchinson and Templeton 1999), and genetic diversity is expected to be higher near the centre of the habitat than near the ends (Wilkins and Wakeley 2002). Both metapopulation models (models 4 and 5) predict that recent population bottlenecks and extinction-replacement dynamics can considerably increase linkage disequilibrium between loci within subpopulations (Ohta 1982; McVean 2002). However, the source-sink model (model 4) predicts also that genetic diversity will be

lower in the sink subpopulations relative to the source patch (Gaggiotti and Smouse 1996).

We study here the genetic structure of the largest remaining population of *A. fulva* var. *pendulina* at Liminka Bay. The aim of this study is to examine the level of genetic diversity in *A. fulva* var. *pendulina* population, and the distribution of genetic variation among and within subpopulations. We also tested the suitability of the various theoretical models (see above) to explain the pattern of genetic variation within and among the subpopulations to provide guidelines for conservation strategies and management.

Materials and methods

Study site and sampling design

Shoot material was collected from six of the existing seven *A. fulva* subpopulations belonging to the same population in Liminka Bay area in years 2000 and 2001 (Figure 1; Table 1). The seventh subpopulation south of the main study area is an isolated one and consists of only one small patch (5 m² in 2000). The small size of this population did not allow adequate sampling for population genetic analysis. Each studied subpopulations consists of several patches, and the number of patches varies yearly (Rautiainen et al. 2004). In year 2000, when most of the samples were collected, the total number of patches was 103. Five of the studied subpopulations are scattered in a stepping-stone fashion close to each other in the estuary of the Temmesjoki river (see Figure 1), and the sixth one (Kempele) is located approximately 2 km north of the others. Among other subpopulations the distance to the nearest neighbouring subpopulation varies between 80 and 250 metres, and no *A. fulva* shoots have been found growing between the subpopulations. In each subpopulation 23–37 shoot samples were collected (c.f. Krauss 2000; Nybom 2004). When the area of the patch exceeded 30 m² we sampled one individual at each node of a regular 4 × 4 m grid. In smaller patches the samples were collected along a transect including both the middle and edges of the patch. Sampled shoots were stored at –70 °C prior to DNA extraction. A total of 197 *A. fulva* shoots sampled from 81 different patches

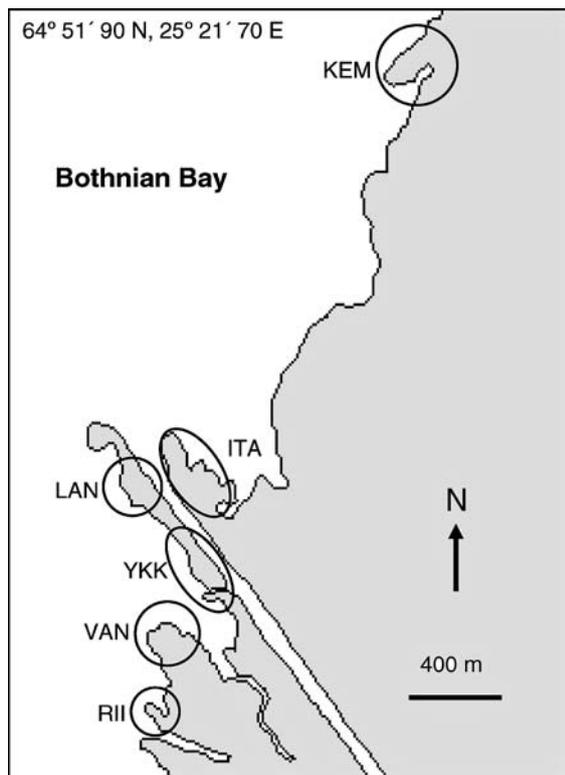


Figure 1. Schematic map of the six subpopulations of *Arctophila fulva* in the Liminka Bay population. The abbreviations of the subpopulation names refer to Table 2. The latitude and longitude given refers to the upper left corner of the map.

Table 1. The four primer combinations used in the AFLP-analysis, the number of bands found and the degree of polymorphism

Primer pairs	Total bands	Polymorphic bands	% polymorphism
E-ACA/M-CTC	51	44	86.3
E-ACT/M-CTA	64	57	89.1
E-AAG/M-CTC	62	59	95.2
E-ACA/M-CTG	51	47	92.2
Total	228	207	90.8

(c. 79% of the patches existing in 2000) were subjected to AFLP analysis.

DNA extraction and AFLP analysis

DNA was extracted using the modified CTAB method of Rogers and Bendich (1985) using approximately 100 mg of *A. fulva* shoots ground in liquid nitrogen as a starting material.

We chose to use AFLP technique (Vos et al. 1995) for genetic analysis, because AFLPs require no prior sequence knowledge, their repeatability is generally good, quantity and quality requirements of DNA are small, and the resulting DNA fingerprints provide a large number of genetic markers. The drawback is that the markers are dominant and therefore heterozygotes cannot be distinguished from homozygotes. However, a recent review by Nybom (2004) indicates that AFLP markers may be as effective as codominant markers in estimation of intraspecific genetic diversity in plants, given that sufficiently large amount of loci are scored.

AFLP reactions were conducted using the AFLP™ Plant Mapping kit supplied by Perkin-Elmer Applied Biosystems (CA). We used a kit optimised for a regular plant genome (genome size of 500 to 6000 Mb) according to the manufacturer's instructions with small modifications. All the amplifications were carried out in a Perkin Elmer GeneAmp PCR System 9700 thermocycler. Sixteen of the 64 possible primer combinations were screened, and the four first primer pairs that amplified a reasonable number of bands in *A. fulva* were selected for use. The selective nucleotides of the primer pairs are given in Table 1. When screening for the selective amplification primer pairs, the reactions were made according to the manufacturer's instructions. However, after the primer pairs were chosen the total volume of the selective amplification reactions was reduced to a half of the manufacturer's instructions. The samples were analysed with an automated DNA sequencer ABI prism® 377 (Applied Biosystems, CA).

Deionized formamide (1.25 µl) was mixed with blue dextran in 25 mM EDTA loading solution (0.25 µl) and 0.5 µl of a Gene Scan 500 ROX internal lane standard (Applied Biosystems) was added. 1.2 µl of this solution was mixed with 1 µl of selective amplification product. Samples were denatured at 95 °C for 5 min and analysed on an automated DNA sequencer (ABI Prism® 377). AFLP electropherograms were analysed using GENE SCAN software (Applied Biosystems, CA). AFLP genotypes were scored for presence or absence of certain fragments between 50 and 500 bp using GENOTYPER software (Applied Biosystems). Each band in the AFLP profile was treated as an independent locus with two alleles,

and a binary matrix based on the bands was thus generated.

Genetic diversity and linkage disequilibrium

Genetic diversity was quantified as (i) the percentage of within-population polymorphic loci, and Nei's unbiased expected gene diversity. Because no prior information of the mating system of *A. fulva* was available, we estimated gene diversity assuming (ii) Hardy–Weinberg equilibrium (i.e. $F_{IS}=0$), and (iii) total inbreeding (i.e. $F_{IS}=1$). The genetic diversity indices were estimated separately for each locus and averaged using the POPGENE-program (Yeh et al. 1997). Often the presence of particular multilocus genotypes in great excess is the most robust and significant evidence of clonal reproduction (e.g. Gregorius 2005). Therefore, for each subpopulation AFLP multilocus data was used to determine the number of multilocus genotypes and the genotypic diversity, G_o .

Non-random association of alleles has also been used to infer whether organisms recombine (e.g. Gregorius 2005). Non-random association may be analysed statistically by calculating linkage disequilibrium between loci. Linkage equilibrium analysis for each subpopulation was conducted using LIAN software version 3.1 (Hauboldt and Hudson 2000). Tests for independent assortment were conducted first computing the number of loci at which each pair of taxa differs. From the distribution of mismatch values a variance (V_D) was calculated. This was compared to the variance expected (V_E) for linkage equilibrium. The significance of the ratio was tested by Monte Carlo simulation test with 10000 random resamplings (see Hauboldt and Hudson 2000; Hauboldt 2001). LIAN software was also used to estimate the standardized index of association (I_A) for each subpopulation. This index is comparable between studies as long as it can be assumed that the neutral mutation parameter is constant.

Population structure

To reject the possibility that the subpopulations of *A. fulva* form a single panmictic population (model 1), we analysed the presence of genetic structure in the total population using Bayesian method of Holsinger et al. (2002; see also

Holsinger and Wallace 2004). The method allows direct estimates of F_{ST} from dominant markers without assuming previous knowledge of the degree of within-subpopulation inbreeding, or that genotypes within subpopulations are in Hardy–Weinberg proportions. We used the program HICKORY version 0.7 (Holsinger and Lewis 2002) to estimate θ_B (estimate of F_{ST}). Because estimates of f (estimate of F_{IS}) given by the program may be unreliable (see Holsinger and Lewis 2002), we used the “free model” option of the program in which f is not estimated. Instead the program will choose values of f at random from its prior distribution while estimating other parameters during the MCMC run. Estimates of θ_B obtained in this way incorporate all of the uncertainty in f (Holsinger and Lewis 2002). We conducted several runs using non-informative priors for f and θ_B and with default sampling parameters (burnin = 50000, sample = 250000, thin = 50) to ensure that the results are repeatable.

The distribution of genetic variation on subpopulation and regional levels (predicted by our models 2–5) was investigated by using Analysis of Molecular Variance (AMOVA; Excoffier et al. 1992), which is essentially based on a hierarchical variance of gene frequencies. However, in the case of dominant markers AMOVA partitions the genotypic variance, and not the variance of allele frequencies. When using AMOVA with dominant markers it has to be assumed that the mating pattern is the same in all of the subpopulations. In our AMOVA model the hierarchical analysis of variance partitions the total genotypic variance into covariance components due to inter-individual, inter-patch and inter-subpopulation differences. We used program ARLEQUIN version 2.00 (Schneider et al. 2000) to perform the AMOVA analysis.

Even though one could find significant substructuring within population, the subpopulations may still represent only fragments of a formerly continuous population (model 2) without any present-day migration between them. In order to analyse whether or not there has been any gene flow between the subpopulations, we used the program 2MOD (Ciofi et al. 1999) to estimate the relative likelihoods of drift vs. immigration models since a certain time. In the drift model (corresponding to our model 2) it is assumed that an ancestral panmictic population is separated into

several independent units which start diverging purely by genetic drift, whereas the gene-flow model assumes that the gene frequencies within subpopulations are determined by a balance between genetic drift and immigration (corresponding to models 3–5). The program uses a coalescent theory and a Markov's chain Monte Carlo simulation approach with Metropolis–Hastings sampling to explore the alternative models, as described in Ciofi et al. (1999). Two independent simulations with 100 000 iterations were carried out to check the convergence of the posterior probabilities of the models. We discarded the first 10% of points in both outputs to avoid dependence on initial starting values, and reported the results for the combined output.

Different theoretical models of population structuring give different predictions on the migration pattern between subpopulations (see Introduction). When immigration between subpopulations is sufficiently low, assignment analysis may be used to estimate dispersal pattern between subpopulations by identifying the individuals that are possible immigrants (Cornuet et al. 1999; Cain et al. 2000). We used the computer program AFLPOP (Duchesne and Bernatchez 2002; see also Cambell et al. 2003) to find out to which subpopulation each individual AFLP genotype was most likely to belong on the basis of its multilocus genotype. AFLPOP performs population assignment using a modification of the allocation method of Paetkau et al. (1995). Before the assignment analysis we used the programs property to find clusters of loci that show identical scores among all specimens across all subpopulations. These loci are redundant in information content and probably linked. In this filtering phase 27 redundant loci were removed and the assignment was based on 201 loci.

Even though the assignment analysis may provide information on migration patterns, the information given is only for present day migration, and it does not necessary tell very much about long term equilibrium migration patterns. However, in some cases genetic relatedness between populations may provide information on population structure. In the stepping-stone model (model 3) at equilibrium, genetic distance between subpopulations should increase monotonically with increasing geographic distance (Kimura and Weiss 1964; Hutchinson and Templeton 1999). We tested the assumptions on association between genetic and

geographic distances using pairwise F_{ST} -values as genetic distances between subpopulations. They were estimated between each subpopulation pair using the approach of Holsinger et al. (2002). Linearized pairwise F_{ST} -values i.e. $F_{ST}/(1-F_{ST})$ (see Rousset 1997) and geographic distances were used to construct scatterplots of $F_{ST}/(1-F_{ST})$ on geographic distances and to calculate the correlation coefficient describing the relationship between them. Because pairwise elements of distance matrices are not independent and thus violate the basic assumptions associated with standard tests of significance, a Mantel test (e.g. Manly 1985) was used to assign an estimate of the 95% upper tail probabilities for each matrix correlation coefficient (10000 replicates). To determine whether the degree of scatter increased with the geographic distance as expected under the null hypothesis of equilibrium (Hutchison and Templeton 1999), the residual obtained from a standard linear regression of F_{ST} -value on geographic distances was correlated with geographic distance. We used linear distances i.e. we assumed one-dimensional stepping-stone model populations because the long axis of *A. fulva* subpopulations in the Liminka Bay area is much greater than their extent perpendicular to the shore. Accordingly, logarithmic transformation of distances, which corresponds with a two-dimensional stepping-stone model (Rousset 1997), yielded much poorer fit as compared to the linear model (data not shown).

Results

Genetic diversity and linkage disequilibrium

The primer pairs (only the selective nucleotides are shown) used in the AFLP analysis and the degree of polymorphism for each primer pair are given in Table 1. The number of unambiguous bands amplified by different primers ranged from 51 to 64. The percentage of polymorphic bands between primer pairs varied from 86.3% to 95.2%. The total number of bands was 228, of which 207 were polymorphic (90.8%).

Each examined shoot exhibited a unique AFLP pattern i.e. the genotypic diversity (G_O) in the sample equalled the sample size. The proportion of polymorphic loci (P) in different subpopulations varied between 65% and 82% (Table 2). The mean

Table 2. Genetic diversity within subpopulations of *Arctophila fulva*

Subpopulation	Abbr.	Area	N	<i>P</i> (%)	He_N	He_I	I_A
Kempele	KEM	26	23	66.7	0.265	0.200	0.063
Itäpuoli	ITA	474	37	73.9	0.273	0.173	0.036
Länsipuoli	LAN	1938	38	81.6	0.316	0.238	0.048
Ykkösväylä	YKK	721	28	69.6	0.257	0.209	0.032
Vanhaväylä	VAN	1162	36	65.2	0.247	0.183	0.065
Riitasaari	RII	532	35	65.2	0.244	0.180	0.035
Mean		808.8	32.8	70.4	0.267	0.197	0.047

Area is the population area in square metres, *N* is the number of individuals analysed, *P* is the percentage of polymorphic loci, He_N is Nei's gene diversity assuming H–W equilibrium ($F_{IS} = 0$), He_I is the gene diversity assuming total inbreeding ($F_{IS} = 1$), and I_A is the index of association between loci.

genetic diversity within each subpopulation assuming Hardy–Weinberg equilibrium (He_N) was 0.267; the estimate assuming total inbreeding (He_I) was slightly lower (0.197). Correlation between these estimates among subpopulations was high ($r=0.76$, $N=6$) even though not significant ($P=0.080$) suggesting that the assumed mating model did not affect very much the estimates of gene diversities within subpopulations. Both estimates varied only a little between subpopulations. The range of estimates was between 0.244 and 0.316 for the estimate assuming H–W, and between 0.173 and 0.238 for the estimate assuming total inbreeding.

One prediction of the stepping-stone model (model 3) is that the genetic diversity is expected to be higher near the centre of the habitat than near margins (Wilkins and Wakeley 2002). Average values of *P*, He_N and He_I appeared to be higher in the central LAN and YKK (means 75.6%, 0.287 and 0.223, respectively) than in the other more peripheral subpopulations (means 67.8%, 0.257 and 0.184, respectively). However, this difference was significant only for He_I ($t=-3.17$, $df=4$, $P=0.034$).

No significant association was detected between the estimated subpopulation area and genetic variation, suggesting that there is no equilibrium between drift and mutation within subpopulations as predicted by the equilibrium models (models 1–3) but not the metapopulation models (models 4–5). The regression coefficient between the estimated subpopulation size and percentage of polymorphic loci was very low ($b=0.006$) and statistically not significant ($t=1.75$, $df=5$, $P=0.155$). There was no significant asso-

ciation between subpopulation size and Nei's gene diversity when assuming H–W equilibrium ($b=0.00003$, $t=1.68$, $df=5$, $P=0.181$) or total inbreeding ($b=0.00002$, $t=1.64$, $P=0.177$).

Significant linkage disequilibrium between loci was found at each subpopulation ($P<0.001$). The standardized index of association was rather similar in all six subpopulations (Table 2) ranging from 0.032 (in YKK) to 0.065 (in VAN).

Population structure

In the Bayesian analysis of population structure using the free model the posterior mean estimate of $F_{ST}(\theta_B)$ was 0.071 ± 0.006 (95% credible intervals 0.061, 0.083), and there was very strong evidence that the estimate was higher than 0. Significant F_{ST} estimate suggested that the populations did not form a single panmictic unit (model 1) and that there are significant genetic differences between the subpopulations (i.e. supporting our models 2–5), even though the low value of the estimate proposes that the levels of differentiation are not very high.

A summary of the AMOVA analysis is shown in Table 3. The variance components were highly significant at all hierarchical levels ($P<0.001$ at each level; 1023 permutations). Most of the variation (76.0%) appeared to be within patches. The variation between subpopulations explained only 4.6% of the total variation (even though the differences among them were statistically significant; Table 3), and small Φ_{ST} estimate (0.046) between subpopulations also suggested low amount of genotypic differentiation between subpopulations. However, all the pairwise exact tests between

Table 3. The analysis of molecular variance (AMOVA) of 197 *Arctophila fulva* individuals

Source of variation	df	Sum of squares	Variance components	Percentage of variation	$P <$
Among subpopulations	5	365.15	1.031	4.57	0.001
Among patches within subpopulations	75	2026.10	4.378	19.42	0.001
Within patches	116	1987.82	17.136	76.01	0.001
Total	196	4370.07	22.545		

subpopulations were significant at level $P < 0.001$, suggesting that the genotype frequencies in different subpopulations were dissimilar. There was much more variation among patches within subpopulations (19.4%), and the reasonably large estimate of differentiation between patches ($\Phi_{PT} = 0.203$) also suggested significant genotypic substructuring within subpopulations.

In the 2MOD simulations the likelihood of the pure drift model (corresponding to model 2) was 0, and gene-flow model (corresponding to our models 3–5) was 1 i.e. none of the simulations supported the drift model. This result indicates that the subpopulations of *A. fulva* are not totally isolated units as suggested by the fragmentation model (model 2), but that there is some migration between the subpopulations (supporting our models 3–5).

In the assignment analysis to reveal the migration pattern the total assignment success was low suggesting that migration between the subpopulations is high, and/or that the subpopulations are so similar that the resolving power of the analysis is not very high. Only 75 of the total of 197 (38.1%) individuals were assigned to the subpopulation from which they were sampled (Table 4). Assignment success was over 50% only

in the most isolated KEM (56.5%) subpopulation. It was lowest in RII (22.9%), VAN (33.3%) and LAN (36.8%) subpopulations, and only slightly higher in YKK (46.4%) and ITA (40.5%) subpopulations. There was a lot of mixing between adjacent YKK and VAN subpopulations supporting the stepping-stone model (model 3). Interestingly, a much higher proportion of individuals from VAN subpopulation (10 of 36; 27.8%) was assigned to YKK subpopulation than vice versa (5 of 28; 17.9%) suggesting that migration between these subpopulations may still not be symmetric probably because of the prevailing wind direction. However, all the mixing was not between adjacent populations. In RII subpopulation 14 of 35 (40.0%) individuals were assigned to the ITA subpopulation and comprehensively 10 of the 37 (27.0%) individuals from ITA subpopulation were assigned to the RII subpopulation, even though these populations are not especially close to each other (see Figure 1).

The scatterplot (Figure 2) and the Mantel correlation analysis suggested relatively strong association between pairwise $F_{ST}/(1-F_{ST})$ values and linear geographical distance between subpopulations ($r_M = 0.513$; $P = 0.046$, 10000 permutations) also supporting the stepping-stone model (model 3). There was also indication that the degree of scatter was associated with geographic distance ($r_M = 0.286$), even though the increase in scatter was significant only at level $P = 0.070$ (10000 permutations). Under the assumption that subpopulation differentiation reflects genetic isolation, the inverse of the slope of the graph could be used as an estimator of “neighbourhood size, $4D\delta^2$ (Rousset 1997), where D is the subpopulation density and δ^2 is the variance of axial dispersal distances between generations. Given the slope of $b = 1.3340 \times 10^{-5}$ between linear distances and genetic differentiation our estimate of $4D\delta^2$ was 74635.

Table 4. Results of the assignment analysis of 197 *Arctophila fulva* individuals. The number of individuals assigned correctly are given in bold

Given subpopulation	Inferred subpopulation						Total
	KEM	ITA	LAN	YKK	VAN	RII	
KEM	13	0	5	3	1	1	23
ITA	2	15	1	8	1	10	37
LAN	6	5	14	10	2	1	38
YKK	3	5	2	13	5	0	28
VAN	11	1	0	10	12	2	36
RII	5	14	0	8	0	8	35

Discussion

Genetic diversity and clonality

Relatively high amount of variation was found in the Liminka Bay population of *A. fulva* as the mean Nei's expected heterozygosity within subpopulations assuming random mating (He_N) and total inbreeding (He_I) scored 0.267 and 0.197, respectively. Nybom (2004) reviewed studies using nuclear DNA markers for assessment of among and within-population diversity in wild plants. She showed that the levels of genetic diversity estimates derived using dominant markers (RAPD, AFLP, ISSR) are comparable among species. When comparing the present results to her review, *A. fulva* seems to exhibit high level of genetic diversity, since the grand mean of He_N within populations in AFLP based studies in the review was 0.23. The level of diversity in *A. fulva* seems to be typical for outcrossing species with 0.24 as mean He_N in dominant marker based studies.

According to Balloux et al. (2003) dominant genetic markers do not properly allow for the disentanglement between genetic variation within loci and within genotypes. Their theoretical analysis suggests that genetic diversity (the sum of allelic and genotypic variability) does not provide any clear prediction on the rate of clonal

reproduction. In order to obtain evidence of asexual reproduction, the genotypic diversity in a sample can be compared to the genotypic diversity expected under random mating, G_E (Stoddart 1983; Hoffman 1986; Gregorius 2005). When a large number of variable marker loci are used to identify genotypes, nearly every individual in a sample will have a unique genotype if the population is fully sexual, panmictic, and freely recombining. Thus, G_E may be assumed to equal N , the sample size (e.g. Ceplitis 2001). Each examined shoot of *A. fulva* exhibited a unique AFLP pattern i.e. the observed genotypic diversity (G_O) equalled the sample size, suggesting that sexual reproduction occurs in this species despite conflicting field observations. The existence of unique AFLP genotypes for each sample suggests that the effect of clonal growth contributes little to within-subpopulation diversity, or that effect occurs over a spatial scale less than our sampling scheme (4 m in most patches and even 20 cm in the smallest ones). An alternative explanation is that the clones are so long-lived that somatic mutations are accumulating to different parts of the clone thus confusing the clonal origin of shoots (but see below). It is unlikely that mis-scoring of phenotypes led to the false interpretation that there are no clones as any ambiguous loci were excluded from the analysis. Still another possible explana-

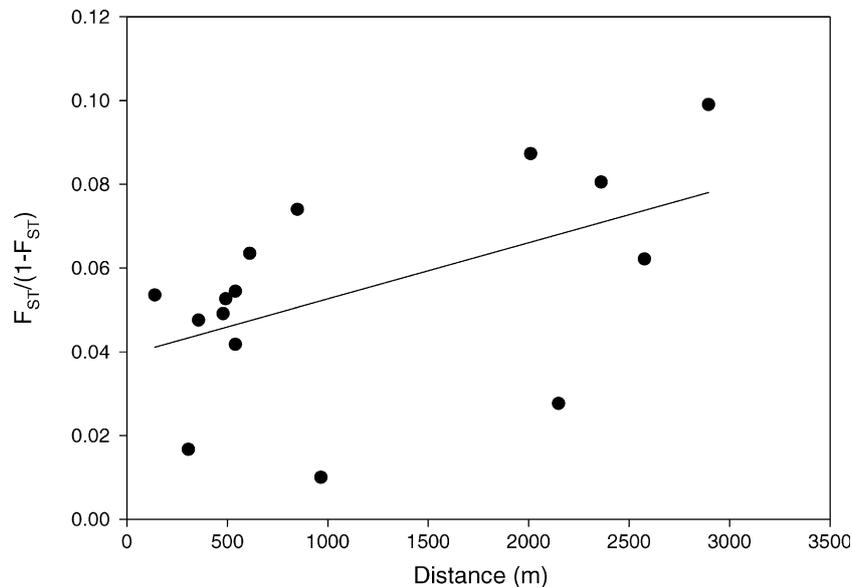


Figure 2. Pairwise genetic ($F_{ST}/(1-F_{ST})$) and geographical distances (meters) between the six *Arctophila fulva* subpopulations.

tion for the lack of evidence for clonal reproduction is that clonality may be a recent phenomenon. Missing evidence for clonality due to recent origin in this case would require very recent cessation of sexual reproduction and inefficient vegetative expansion. *A. fulva* seems, however, to spread effectively through perennial underground rhizomes. Because it is not known whether this species is reproducing sexually in any part of its distribution range, it is difficult to evaluate how recently clonal reproduction has arisen.

In some cases, high levels of clonal or genotypic variation have been found in clonal species with low levels of seedling recruitment (e.g. Jonsson et al. 1996; Auge et al. 2001; Lundqvist and Andersson 2001; Persson and Gustavsson 2001; Hangelbroek et al. 2002). For example, in *Carex bigelowii* no seed-derived plantlets have been observed in natural stands, even though the species is genotypically highly variable (Jonsson et al. 1996). The high genotypic diversity in *A. fulva* can be taken as evidence for effective sexual reproduction at some time during the history of the population. The model of Bengtsson (2003) has shown that a small number of sexual individuals per generation is sufficient to make an apparently asexual population genotypically highly variable.

On the other hand, the observed significant linkage between loci in each subpopulation suggested non-random association of alleles, which may suggest a loss of recombination in *A. fulva* (there are, however, other possible reasons for linkage disequilibrium related to population structure; see below). The models of Bengtsson (2003) show that because of the “memory-effect” a population which was started by a small number of sexually derived propagules may retain its initial genotypic variation for a very long time, even if it later reproduces almost exclusively asexually. A reasonably large population size is sufficient to ensure this effect. According to Bengtsson (2003) “there is thus rarely any reason to invoke selection, somatic mutations, or any other specific mechanism to explain the presence of genotypic variations in organisms that are phenotypically highly asexual but retain the capacity to produce sexual offspring”. This may also apply to *A. fulva*. However, the relative proportions of sexual and asexual reproduction in *A. fulva* are not fully resolved, and this question needs further analysis.

Population structure

We found significant differences in genotype and allele frequencies between subpopulations. The AMOVA estimate of genotypic differentiation ($\Phi_{ST}=0.046$) was very similar to the posterior Bayesian mean estimates of genetic differentiation ($F_{ST}=0.071$), both suggesting a very low amount of differentiation (even though significant) among subpopulations. Interestingly, very low pairwise Φ_{ST} - and F_{ST} -values have been reported in species occupying a similar niche as *A. fulva*. Bockelmann et al. (2003) reported low Φ_{ST} -, F_{ST} - or R_{ST} -values among closely located populations of a grass species, *Elymys athericus*, in European salt marshes. In another marine flowering plant, *Zostera maritima*, Reusch et al. (2000) reported R_{ST} -values of 0.029–0.053 in the Baltic and in Nova Scotia populations at the scales of 15–35 km.

Significant linkage between loci among subpopulations and the significant patch level component in AMOVA analysis suggested hierarchical structuring within subpopulations. There was much more variation among patches within subpopulations (19.4% of the total variation) than between subpopulations (4.6%), and the estimate of differentiation at patch level ($\Phi_{ST}=0.203$) was much higher than at subpopulation level ($\Phi_{ST}=0.046$). A very similar pattern of hierarchical population structure has been described in *Silene dioica*. In this species the estimate of differentiation between populations (islands) is much lower ($F_{IL}=0.035$) than among patches within islands ($F_{PL}=0.080$) (Giles et al. 1998; Ingvarsson and Giles 1999). It has been suggested that in *S. dioica* the small-scale structuring occurs during population expansion, and that the observed patterns of genetic differentiation can be attributed to the population being substructured into family groups (Ingvarsson and Giles 1999). Same kind of mechanism may also be behind the hierarchical population structuring observed in *A. fulva*.

Our analysis clearly show that the subpopulations in *A. fulva* were not genetically uniform over all spatial scales and therefore do not form a single panmictic population (model 1). The results did not either support the fragmentation model (model 2). Significant differentiation between the subpopulations and the results of 2MOD analysis indicated at least some migration between the

subpopulations and hence they cannot be regarded as fragments of a formerly continuous population.

Our results supported some predictions of the stepping-stone model (model 3). Genetic diversity within subpopulations appeared to be highest near the centre of the habitat and there was correlation between geographical and genetic distances as expected in the stepping-stone model (Hutchison and Templeton 1999; Wilkins and Wakeley 2002). Genetic distance between subpopulations increased monotonically with increasing geographical distance between the subpopulations ($r_M = 0.513$; $P = 0.046$). However, the pattern of the two distances (Figure 2) suggests that the significant correlation is only due to pairwise distances between the most isolated KEM and the other subpopulations, and in fact there is not association between geographic and genotypic correlations when the distance is less than 1000 m. Isolation-by-distance has been reported in other coastal and marine species. Bockelmann et al. (2003) found a weak ($r = 0.308$) but significant correlation between pairwise Φ_{ST} -values and geographical distances in a grass species, *Elymys athericus*. In *Zostera maritima* a strong correlation between pairwise distances among eight European populations was revealed by Reusch et al. (2000), even though no such pattern was found among Canadian populations. Isolation-by-distance in all these species having rather similar habitat requirements suggests that water currents and waves may effectively disperse their seeds or other propagules along shoreline, even though this hydrochory seems to be effective only in a limited range. Reusch et al. (2000) reported two estimates (2440 and 5000) of $4D\delta^2$ for *Zostera maritima* in Europe based on two different estimates of population differentiation. These estimates appeared to be about a one tenth of that for *A. fulva* suggesting that dispersal in *Zostera* species is not as efficient as in our study species. Reusch et al. (2000) suggest that the major mode of dispersal in eelgrass is rafting of the fruit-bearing plants, which probably are rafting less efficiently than the smaller rhizomes of *A. fulva*.

Not all the evidence seems to support the stepping-stone model of population structure (model 3). The assignment analysis suggested that all the migration events were not only between the adjacent populations. Moreover, the absence of an inverse relationship between subpopulation area

and genetic diversity suggests that there was no equilibrium in subpopulations between drift and migration as expected in this model (e.g. Nei 1987).

The absence of an inverse relationship in all of our study subpopulations seems to indicate metapopulation-type dynamics (i.e. models 4 and 5) with recent colonizations and extinctions (Ohta 1982). Even though this result strongly suggests that *A. fulva* population at the Liminka Bay has some characters of metapopulation models, the evidence does not seem to support the source-sink model (model 4). Although the level of genetic diversity was high in the central LAN and YKK subpopulations implying a structure of few source subpopulations with high level and several sink subpopulations with low levels of genetic diversity (cf. Gaggiotti and Smouse 1996), migration between subpopulations in the assignment analysis was not mainly unidirectional from these central populations to the more peripheral ones as expected in the source-sink model. Consequently, the *A. fulva* population structure resembled more the classical metapopulation model (model 5) than the source-sink model (model 4).

It is not surprising that the genetic population structure of *A. fulva* seems to have some characters of metapopulations. These results agree with those of Rautiainen et al. (2004) over the subpopulation dynamics of *A. fulva* at patch level at the Liminka Bay, showing concurrent extinctions and formation of new patches. Because some subpopulations consist only of few patches, these dynamics may be expected to work also at a higher hierarchical level causing extinctions and colonizations of subpopulations.

In conclusion, the overall pattern of distribution of genetic variation and linkage disequilibrium within and between subpopulations did not give full support to any of the five theoretical models presented in the Introduction, and the true model describing *A. fulva* population genetics appears to contain elements from more than one of them. The migration pattern and distribution of genetic diversity among the subpopulations in *A. fulva* was mainly similar to the stepping-stone model (model 3). However, the population did not seem to be in drift-migration equilibrium, manifesting classical metapopulation type population structure (model 5) in *A. fulva*.

Conservation implications

One primary objective of nature conservation is the maintenance of genetic diversity. Empirical assessment of genetic variability is therefore essential for successful management of endangered plants (e.g. Escudero et al. 2003). Our analysis of genetic variation within and between subpopulations in *A. fulva* indicates that despite the evident clonal reproduction and recent decrease in the number of populations, the species has maintained a high level of genetic and genotypic diversity and large effective neighbourhood size at the Liminka Bay area. However, our analysis did not allow estimation of the amount of inbreeding within subpopulations. Slate et al. (2004) have recently shown that multilocus heterozygosity seems to be only weakly correlated with inbreeding coefficient. Thus, even though the genetic diversity in *A. fulva* is not especially low, it does not imply that there could not be inbreeding and inbreeding depression in the study population. Moreover, given the observed reasonably large neighbourhood size, the “memory-effect” (see Bengtsson 2003) may have retained the initial genotypic variation of the original sexually reproducing *A. fulva* population for a very long time, even though the species may at present reproduce mainly asexually. It is possible that the genetic diversity of *A. fulva* may be decreasing because of possible inbreeding and clonality (especially if clonality is a recent phenomenon), which may expose the population to extinction because of genetic degeneration. Accordingly, the genetic diversity of the species should be carefully monitored also in the future.

In our present analysis some characters of the *A. fulva* population structure seem to follow the classical metapopulation model. This result, as well as the analysis of patch dynamics (Rautiainen et al. 2004), suggests that the subpopulations are more or less ephemeral. Accordingly, the conservation and management effort in this species should be targeted to conservation of the required habitat of the species instead of extant subpopulations. This may be achieved by keeping the meadows open by cattle grazing or hay making.

Our results indicate that there are no specific immediate genetic threats to *A. fulva* in Liminka Bay area. However, the other populations of *A. fulva* are much smaller and may be more vulnerable to extinction (see eg. Matthies et al. 2004

for a review of empirical evidence in plants). Long term survival of these populations may require active translocation of individuals from the Liminka Bay population to supplement or reinforce the existing populations. Since our results did not rule out sexual reproduction in *A. fulva*, it is possible that the individuals of Liminka Bay population may be used in “genetic rescue” (e.g. Tallmon et al. 2004) of the more threatened populations. Moreover, because some formerly known populations of *A. fulva* have gone extinct, individuals of Liminka Bay population may be used for reintroductions or attempts to establish the species in formerly inhabited sites.

In general, the individuals chosen for introduction to small inbred populations, for recovery of fitness and genetic diversity, should be genetically differentiated from the population to which they are being introduced (e.g. Frankham et al. 2002). Given that there is no local adaptation or co-adapted gene complexes within populations, the best selection strategy for reintroductions to formerly inhabited sites is often to choose the most unrelated individuals from the source population (e.g. Vergeer et al. 2004).

Accordingly, the genotypes of the individuals chosen for introduction, and in the case of augmentation, the genetic composition of the potential recipient populations should also be studied before translocations. If this is not possible in practice, our recent results provide a rough guideline for selection of individuals for such translocation purposes. First, because there was no association between subpopulation area and genetic diversity, it is not necessary to choose the individuals for rescuing or reintroducing from the largest subpopulations (cf. Vergeer et al. 2004). Second, we found large amount of genotypic variation among patches within subpopulations, which suggests that one should collect individuals for translocation from several patches within subpopulations in aim to augment existing subpopulations or to establish new ones with high level of genetic diversity. Finally, in this study we found isolation-by-distance between subpopulations suggesting that collecting individuals as far apart as possible would decrease the relatedness between the chosen individuals. However, plants may also be adapted to local conditions or there may be co-adapted gene complexes within populations. Input of new genes in this case may dis-

rupt local adaptations or lead to break-up of favourable gene combinations (see e.g. Vergeer et al. 2004). Unfortunately, determination of local adaptation or existence of co-adapted gene-complexes is not easily done, in contrast to assessing differentiation using neutral genetic markers. Because we did not have this kind of information on *A. fulva*, introduction or reinforcement in this case could be best achieved through material from the closest subpopulation (c.f. Vergeer et al. 2004) in aim to avoid disruption of local adaptation or coadapted gene complexes, and sampling as many patches as possible to maximise diversity.

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References

- Auge H, Neuffer B, Erlinghagen F, Grupe R, Brandl R (2001) Demographic and random amplified polymorphic DNA analyses reveal high levels of genetic diversity in a clonal violet. *Mol. Ecol.*, **10**, 1811–1819.
- Balloux F, Lehmann F, de Meeüs T (2003) The population genetics of clonal and partially clonal diploids. *Genetics*, **164**, 1635–1644.
- Balloux F, Lugon-Moulin N (2002) The estimation of population differentiation with microsatellite markers. *Molecular Ecology*, **11**, 155–165.
- Bengtsson BO (2003) Genetic variation in organisms with sexual and asexual reproduction. *J. Evol. Biol.*, **16**, 189–199.
- Bockelmann AC, Reusch TBH, Bijlsma J, Bakker JP (2003) Habitat differentiation vs. isolation-by-distance: The genetic population structure of *Elymus athericus* in European salt marshes. *Mol. Ecol.*, **12**, 505–515.
- Cain ML, Milligan BG, Strand AE (2000) Long-distance seed dispersal in plant populations. *Am. J. Bot.*, **87**, 1217–1227.
- Cambell D, Duchnese P, Bernatchez L (2003) AFLP utility for population assignment studies: Analytical investigation and empirical comparison with microsatellites. *Mol. Ecol.*, **12**, 1979–1991.
- Ceplitis A (2001) The importance of sexual and asexual reproduction in the recent evolution of *Allium vineale*. *Evolution*, **55**, 1581–1591.
- Ciofi C, Beaumont MA, Swingland IR, Bruford MW (1999) Genetic divergence and units for conservation in the Komodo dragon *Varanus komodoensis*. *Proc. R. Soc. Lond. B*, **266**, 2269–2274.
- Cornuet J-M, Piry S, Luikart G, Estoup A, Solignac M (1999) New methods employing multilocus genotypes to select or exclude populations as origins of individuals. *Genetics*, **153**, 1989–2000.
- Dobson JL (1989) *Autecology of aquatic and terrestrial growth forms of Arctophila fulva, an arctic tundra grass of northern Alaska*. Ph. D. Thesis. University of Idaho, Moscow, ID. US.
- Dudash MR, Fenster CB (2000) Inbreeding and outbreeding depression in fragmented populations. In: *Genetics, Demography and Viability of Fragmented Populations* (eds. Young AG, Clarke GM), pp. 35–53. Cambridge University Press, Cambridge.
- Duchesne P, Bernatchez L (2002) AFLPOP: A computer program for simulated and real population allocation based on AFLP data. *Mol. Ecol. Notes*, **3**, 380–383.
- Escudero A, Iriondo JM, Torres ME (2003) Spatial genetic analysis of genetic diversity as a tool for plant conservation. *Biol. Cons.*, **113**, 351–365.
- Ericson L, Wallentinus HG (1979) Sea-shore vegetation around the Gulf of Bothnia. Guide for the International Society of Vegetation Science, July–August 1977. *Wahlenbergia*, **5**, 1–142.
- Excoffier L, Smouse PE, Quattro JM (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: Application to human mitochondrial DNA restriction data. *Genetics*, **131**, 479–491.
- Frankham R, Ballou JD, Briscoe DA (2002) *Introduction to Conservation Genetics*, Cambridge University Press, Cambridge.
- Gaggiotti OE, Smouse PE (1996) Stochastic migration and maintenance of genetic variation in sink populations. *Am. Nat.*, **147**, 919–945.
- Giles BE, Lundqvist E, Goudet J (1998) Restricted gene flow and subpopulation differentiation in *Silene dioica*. *Heredity*, **80**, 715–723.
- Gregorius H-R (2005) Testing for clonal propagation. *Heredity*, **94**, 173–180.
- Hangelbroek HH, Ouborg NJ, Santamaria L, Schwenk I (2002) Clonal diversity and structure within a population of the pondweed *Potamogeton pectinatus* foraged by Bewick's swans. *Mol. Ecol.*, **11**, 2137–2150.
- Haubold B (2001) Documentation for LIAN 3.1 http://kiwi.i-ce.rnpg.de/lian/documentation/lian_3.1_doc.pdf.
- Haubold B, Hudson RR (2000) Lian, version 3.0: Detecting linkage disequilibrium in multilocus data. *Bioinformatics*, **16**, 847–848.
- Hoffman RJ (1986) Variation in contributions of asexual reproduction to the genetic structure of populations of the sea anemone *Metridium senile*. *Evolution*, **40**, 357–365.
- Holsinger KE, Lewis PO (2002) *Hickory: A Package for Analysis of Population Genetic Data v 0.7*, Department of Ecology & Evolutionary Biology, University of Connecticut.
- Holsinger KE, Lewis PO, Dipak KD (2002) A Bayesian approach to inferring population structure from dominant markers. *Mol. Ecol.*, **11**, 1157–1164.
- Holsinger KE, Wallace LE (2004) Bayesian approaches for the analysis of population genetic: An example from *Plantanthera leucopaea* (Orchidaceae). *Mol. Ecol.*, **13**, 887–894.
- Hutchison DW, Templeton AR (1999) Correlation of pairwise genetic and geographic distance measures: Inferring the relative influences of gene flow and drift on the distribution of genetic variability. *Evolution*, **53**, 1898–1914.

- Ingvarsson PK, Giles BE (1999) Kin-structured colonization and small-scale genetic differentiation in *Silene dioica*. *Evolution*, **53**, 605–611.
- Kimura M, Weiss GH (1964) The stepping-stone model of population structure and the decrease of genetic correlation with distance. *Genetics*, **49**, 561–576.
- Krauss SK (2000) Accurate genetic diversity estimates from amplified fragment length polymorphism. *Mol. Ecol.*, **9**, 1241–1245.
- Jonsson BO, Jonsdottir IS, Cronberg N (1996) Clonal diversity and allozyme variation in populations of the arctic sedge *Carex bigelowii* (Cyperaceae). *J. Ecol.*, **84**, 449–459.
- Lundqvist E, Andersson E (2001) Genetic diversity in populations of plants with different breeding and dispersal strategies in a free-flowing boreal river system. *Hereditas*, **135**, 75–83.
- Manly BJF (1985) *The Statistics of Natural Selection on Animal Populations*, Chapman & Hall, London.
- Markkola J (1993) Perämeren niityt ja niiden suojele In: *Avoimet perinneympäristöt osana suomalaista luontoa, hoito ja suojele* (eds. Marttila O), pp. 12–15. Etelä-Karjalan allergia- ja ympäristöinstituutti, Lappeenranta.
- Matthies D, Braüer I, Maibom W, Tschardt T (2004) Population size and the risk of local extinction: Empirical evidence from rare plants. *Oikos*, **105**, 481–488.
- McVean GAT (2002) A genealogical interpretation of linkage disequilibrium. *Genetics*, **162**, 987–991.
- Nei M (1987) *Molecular Evolutionary Genetics*, Columbia University Press, New York.
- Nyblom H (2004) Comparison of different nuclear DNA markers for estimating intraspecific genetic diversity in plants. *Mol. Ecol.*, **13**, 1143–1155.
- Ohta T (1982) Linkage disequilibrium with the island model. *Genetics*, **101**, 139–155.
- Paetkau D, Calvert W, Stirling I, Strobeck C (1995) Microsatellite analysis of population structure in Canadian polar bears. *Mol. Ecol.*, **4**, 347–354.
- Persson HA, Gustavsson BA (2001) The extent of clonality and genetic diversity in lingonberry (*Vaccinium vitis-idaea* L.) revealed by RAPDs and leaf-shape analysis. *Mol. Ecol.*, **10**, 1358–1397.
- Rassi P, Alanen A, Kanerva T, Mannerkoski I (2001) *Suomen lajien uhanalaisuus 2000*, Ympäristöministeriö & Suomen ympäristökeskus, Helsinki.
- Rautiainen P, Laine A-L, Aikio S, Aspi J, Siira J, Hyvärinen M (2004) Seashore disturbance and the management of the clonal *Arctophila fulva*: Modelling patch dynamics. *Appl. Veget. Sci.*, **7**, 221–228.
- Reusch TBH, Stam WT, Olsen JL (2000) A microsatellite-based estimation of clonal diversity and population subdivision in *Zostera marina*, a marine flowering plant. *Mol. Ecol.*, **9**, 127–140.
- Rogers SO, Bendich AJ (1985) Extraction of DNA from milligram amounts of fresh, herbarium and mummified plant tissues. *Plant Mol. Biol.*, **5**, 69–76.
- Rousset F (1997) Genetic differentiation and estimation of gene flow from *F*-statistics under isolation by distance. *Genetics*, **145**, 1219–1228.
- Schneider S, Roessli D, Excoffier L (2000) *ARLEQUIN: A Software for Population Genetics Data Analysis. Version 2.000*, Department of Anthropology, University of Geneva, Genetics and Biometry Laboratory.
- Siira J (1994) The occurrences and ecology of *Arctophila fulva* (Poaceae) on the Liminka Bay (The Gulf of Bothnia). *Aquilo Ser. Bot.*, **33**, 107–120.
- Slate J, David P, Dodds KG, Veenliet BA, Glass BC, Broad TE, McEwan JC (2004) Understanding the relationship between the inbreeding coefficient and multilocus heterozygosity: Theoretical expectations and empirical data. *Hereditas*, **93**, 255–265.
- Sork VL, Nason J, Campbell DR, Fernandez JF (1999) Landscape approaches to historical and contemporary gene flow in plants. *Trends Ecol. Evol.*, **14**, 219–224.
- Stoddart JA (1983) A genotypic diversity measure. *J. Hered.*, **74**, 489–490.
- Tallmon DA, Luikart G, Waples RS (2004) The alluring simplicity and complex reality of genetic rescue. *Trends Ecol. Evol.*, **19**, 489–496.
- Tero N, Aspi J, Siikamäki P, Jäkäläniemi A, Tuomi J (2003) Genetic structure and gene flow in a metapopulation of an endangered plant species, *Silene tatarica*. *Mol. Ecol.*, **12**, 2073–2085.
- Wilkins JF, Wakeley J (2002) The coalescent in a continuous, finite, linear population. *Genetics*, **161**, 873–888.
- Vergeer P, Sonderer E, Ouborg NJ (2004) Introduction strategies put to test: Local adaptation versus heterosis. *Cons. Biol.*, **18**, 812–821.
- Vos P, Hogers R, Bleeker M, Reijans M, van de Lee T, Hornes M, Frijters A, Pot J, Peleman J, Kuiper M, Zabeau M (1995) AFLP: A new technique for DNA fingerprinting. *Nucleic Acids Res.*, **23**, 4407–4414.
- Yeh FC, Yang R-C, Boyle TBJ, Ye Z-H, Mao JX (1997) *POPGENE, the User-Friendly Shareware for Population Genetic Analysis*, Molecular Biology and Biotechnology, Centre University of Alberta, Canada.
- Young A, Boyle T, Brown T (1996) The population genetic consequences of habitat fragmentation for plants. *Trends Ecol. Evol.*, **11**, 413–418.