Evolutionary Processes and Spatial Genetic Variation in *Euphrasia stricta* on the Baltic Island of Gotland

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Abstract

The identification of processes governing genetic structure at different spatial scales remains a major challenge in evolutionary biology and is of considerable applied interest in conservation biology. In Euphrasia stricta five varieties have been identified (brevipila, gotlandica, stricta, suecica and tenuis) based on differences in habitat, phenology and morphology. In this thesis, I examined genetic variation at AFLP and microsatellite marker loci in relation to variation in habitat and morphology within and among varieties of E. stricta on the island Gotland in the Baltic Sea. The results are discussed in relation to evolutionary processes acting within this species complex.

In a study conducted at the regional scale, the two early-flowering varieties suecica and tenuis each formed a genetically distinct group, while the three late-flowering varieties brevipila, gotlandica and stricta formed a third group. The results suggest that suecica and tenuis have ancient origins since they are genetically different both from the brevipila/gotlandica/stricta group and from each other despite their similar habitat preferences. This pattern was obtained using both marker systems. Discrepancies between AFLP and microsatellites were found in patterns of isolation by distance and in estimates of expected heterozygosity, $H_e$.

Focusing on the mixed genetic group brevipila/gotlandica/stricta and the causes behind their clustering together despite differences in morphology and habitat preferences, I performed a study at a smaller geographic scale. Studying a population of E. stricta I found that, although gene flow within the population was strong, it had not prevented the formation of genetic groups associated with micro-habitat properties.

An important result for conservation of the rare variety suecica is its distinct genetic separation from variety tenuis. If the aim of conservation is to preserve the uniqueness of suecica, the two varieties should be treated as separated entities.

Keywords: genetic differentiation, regional processes, conservation genetics, AFLP, microsatellites, gene flow, local adaptation, shape analysis

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urn:nbn:se:uu:diva-8536 (http://urn.kb.se/resolve?urn=urn:nbn:se:uu:diva-8536)
Ode till ullah kardborre

O, min stilige hjälte, så ståtlig som en fur,
så eldig som en tjärugadd – vem kan begripa hur
det kom sig att du föll så tvärt med bingel, brak och dån.
I stora tjocka Mossberg bland poppel, pil och slån
en liten blyg viol bland alla träden hade tur!

Vem kan väl konsten att som du bemästra gamla ånglok,
köra följespot och sjunga utantill ur spexets sångbok?
Sprida ut hemtrevnad och limma ihop en trasig stol.
En sälls synt hybridisering av fura och viol.
Två små rara sticklingar nässlat sig in hos oss,
fast senaste säsongen har varit tuff förstås!
Vi jagats utav vilda får och ögontrösten härjar svår,
men tiden som doktorand har tack och lov ett slut.
Jag älskar dig för alltid, till dess vi blommar ut.
The thesis is based on the following papers, which will be referred to in the text by their Roman numerals.


Paper I and IV are printed with permission from the publisher.

For all papers I planned the studies, and performed the laboratory work and the statistical analyses of the data. I also had the main responsibility for writing the manuscripts.
Introduction

Genetic variation between individuals in a population is one of the cornerstones in evolution. Using molecular marker variation it is possible to gain information about evolutionary processes that have occurred in the past, and hence increase knowledge about the evolution of populations and species. Detectable processes are changes in population size and number of populations, dispersal and gene flow patterns, and impact of natural selection and genetic drift within species and populations.

Genetic diversity increases the viability and evolvability of populations and species. Literature reviews indicate that genetically more variable populations are more viable than populations with a lower degree of variation (Reed and Frankham 2003; Leimu et al. 2006). Genetic diversity can also be of importance at the ecosystem level. In an experimental study of the eel grass *Zostera marina*, Reusch et al. (2005) showed that more genetically variable plots in the study system could withstand environmental changes better than the plots being less genetically variable, and were associated with a higher abundance of individuals of other species or taxonomic groups associated to eel grass. Two recent studies (Booth and Grime 2003; Prentice et al. 2006) indicate that genetic composition of a population influence succession and interspecific interactions. The traditional ecological view recognizes interspecific interactions between individuals at ecosystem level rather than interactions between interspecific genotypes. By setting up plots containing microcosms mimicking a natural grassland where each species consisted of individuals having the same genotype, four different genotypes or each individual being a unique genotype, Booth and Grime (2003) showed that genetically more diverse microcosms had a faster and directionally stable convergence of species composition than less genetically diverse microcosms. An application of their finding would for example be that having genetically diverse material when restoring vegetation in meadows may increase the success of the restoration by facilitating the convergence towards the original species composition. One should also not forget the important fact that genetic diversity within species and populations today are the material for evolution tomorrow and constitutes the evolutionary potential within populations and species.
Patterns of genetic diversity

Factors influencing levels of genetic diversity and shaping genetic structure at the species level are breeding system, seed dispersal mechanism, life form (trees, leguminous herbs etc.), taxonomic status, and geographic range (Hamrick and Godt 1996). In their study of how life history traits affect genetic diversity in plant species, Hamrick and Godt (1996) conclude that outcrossing species, regardless of their other traits, have significantly less genetic diversity among populations than species with other types of breeding systems (mixed mating and selfing). Also, species having less interpopulation genetic differentiation tend to have more overall genetic diversity. This implies for example that selfing, annual species with poor seed dispersal generally have low within population genetic diversity, but high genetic differentiation.

Phylogeography

By plotting genetic variation on a geographic map, a pattern emerges that may give valuable information about past evolutionary processes creating current spatial genetic structure (Avise et al. 1987). Since the first paper dealing with phylogeography (Avise et al. 1987) many studies have followed. Focus was at first on animals, mostly due to the lack of variable molecular markers in plants (Schaal et al. 1998). Today the phylogeographic approach is common also in plant studies, e.g., when studying refuges during, and migration routes after, the last glacial period (Mátyás 1996; Tremblay and Schoen 1999; Lumaret et al. 2002; Pimentel et al. 2007) and when exploring evolutionary processes shaping spatial genetic structure (Olsen 2002; Rivera-Ocasio et al. 2002; Schönswetter et al. 2006). A phylogeographic approach is, however, less common in studying intraspecific phylogenetic diversification, which is one of the aims of this thesis.

Local adaptation

Because most plants are sessile, adaptation to local environmental conditions are of crucial importance for survival and reproductive output. Plants have adapted to a range of different environments, either natural such as altitudinal gradients (Galen et al. 1991), or along gradients of water availability (Rice and Mack 1991; Dudley 1996), but also to man-made semi-natural disturbances such as mowing and live-stock grazing in pastures (Zopfi 1997; 1998b). Contaminated areas around mines may inhabit plant species adapted to soils with, e.g., high levels of copper, nickel, lead and
zinc (Gregory and Bradshaw 1965). A given environmental condition does not necessarily result in the same type of adaptation. For example, adaptation to water availability can be achieved either by flowering before water becomes limiting (Rice and Mack 1991), or by increased water use efficiency during the whole growing season (Dudley 1996).

The stability and strength of the selection pressure (Linhart and Grant 1996), and the amount of gene flow all affect local adaptation (Slatkin 1987; Linhart and Grant 1996; Lenormand 2002). This is illustrated by a study on the grass *Bromus tectorum* (Rice and Mack 1991), where adaptation to local conditions were found in the two extremes of the studied habitats. In one of these, characterised by being arid and saline, high incoming gene flow was counteracted by strong selection leading to local adaptation. In the other extreme habitat, characterised by cool and mesic conditions, only weak selection pressure was needed. This was due to the habitat's isolated location, i.e., isolation resulted in low input of less adapted genes breaking up the local gene pool. In the grass *Bouteloua rigidiseta*, adaptive differentiation between a population on a homogenous and constant microhabitat characterised by rocky and shallow soil, and a population in a semi-natural grassland site with a more heterogeneous micro-environment, was attributed to stable differences in selection pressure between the two populations (Miller and Fowler 1994). Van Tienderen (1992) found no local adaptation in a *Plantago lanceolata* population when comparing four different sites situated at a riverbed. This was suggested to be due to a high frequency of dispersal events between the sites. Local adaptation can also be affected by the amount of variability in the character that selection works upon (Dudley 1996). If the variation in a trait important for local adaptation is limited, the possibility of local adaptation will as a consequence also be limited.

Population genetic differentiation is affected by selection, but also by gene flow, genetic drift and mutations (Slatkin 1987). For a number of species, selection has led to genetic differentiation between populations, with local adaptation as a consequence. This have resulted in a phylogenetic diversification in terms of, e.g., ecotypes and subspecies (Turesson 1922; Zopfi 1998a; Gustafsson and Lönn 2003). In many species population genetic structure also show the signature of past demographic events such as genetic bottlenecks and founder events (Gaudeul et al. 2000; Despres et al. 2002).
Aims of the thesis

Both Karlsson (1986) and Yeo (1968) emphasize habitat specialization as an important factor in speciation within the genus *Euphrasia*. They argue that high variability and hybridization provide opportunities for the evolution of habitat specialization. In the *Euphrasia stricta* species complex, different habitat preferences have been documented. The study of neutral genetic variation within the *E. stricta* species complex may provide information about the relationship between genetic structure and evolutionary processes shaping the intraspecific ecological differentiation within this species. The aims of my thesis were to:

1. Reveal whether the varieties within *Euphrasia stricta* are locally adapted ecotypes originating at several occasions or if they are distinct units over Gotland, implying historical evolutionary explanations (I, II).
2. Test the evolutionary relationships between varieties suggested by Karlsson (1976; 1984) based on morphology and habitat (I, II).
3. Explore associations between habitat and fitness variables, and local spatial genetic structure and fine-scale habitat association within a population of *E. stricta* (III).
4. Determining conservation units for the rare variety *E. stricta* var. *suecica* and the declining *E. stricta* var. *tenuis* on Gotland (IV).
Material and Methods

Study species

The species Euphrasia stricta Wolff ex Lehm contains five varieties according to current taxonomy (Krok and Almquist 2001). All varieties occur on the Baltic Island of Gotland, where fieldwork was conducted. The five varieties overlap to some extent in morphology, phenology and habitat preference. Of the two early-flowering varieties, suecica ((Murb. & Wettst. ex Wettst.) Karlsson) grows only in the wooded hay-meadows on Gotland, and is red listed as critically endangered (CR) (Gärdenfors 2005). Tenuis ((Brenner) Wettst.) is declining in abundance and has a northerly distribution in Sweden. They flower from the end of June to middle of July, which is presumed to represent adaptations to flowering before mowing (Karlsson 1984). The most reliable morphological character separating these varieties is that tenuis produces glandular hair underneath the leaves, which suecica does not.

The other three varieties, brevipila ((Burnat & Gremli) Hartl), gotlandica (Ahlfv.) and stricta, flower later in the season, from August and until September. Within these three varieties, brevipila and stricta overlap in habitat preference. Both varieties grow along road verges, pastures and beaches, i.e., in areas with a short grass turf. The variety brevipila has colonised the whole of Sweden, while stricta is restricted to calcareous bedrock areas, mainly Skåne and Gotland (Karlsson 1984, 1986). Brevipila and stricta are easy to separate morphologically, brevipila is hairy, while stricta is glabrous.

The fifth variety gotlandica has a distinct habitat choice, temporary pools, an environment linked to the alvar areas on Öland and Gotland in Sweden. It is difficult to separate gotlandica and stricta morphologically, since only colour and number of branches distinguishes the two varieties, gotlandica has a reddish colour and few branches.

Karlsson (1984) proposes that the meadow varieties tenuis and suecica have evolved from brevipila and stricta respectively, all four varieties being adapted to semi-natural habitats and evolving parallel with the development of farming (Karlsson 1976). The proposed phylogenetic relationships were based on morphological characters such as number of nodes below the first flower, flower size, and the density of glandular hairs. The average fertility when crossing stricta and brevipila is not lower compared to crosses within
Molecular markers

To quantify genetic differentiation and diversity, I have used two different molecular marker systems, Amplified Fragment Length Polymorphism (AFLP) (I, III, IV) and microsatellites (also known as SSR and VNTRs) (II), of which both are assumed to reflect neutral genetic variation. The two markers differ, however, in their properties. AFLP display presence or absence of restriction fragments, while microsatellites are tandem repeats of short sequences (one to six nucleotides) (Allendorf and Luikart 2007). Primers for AFLP are easier and less expensive to develop compared to microsatellites. Once up and running, both marker systems are, however, robust and show high variability of alleles (Allendorf and Luikart 2007). An important difference is that AFLP generate dominant markers (Mueller and Wolfenbarger 1999; Savelkoul et al. 1999) as compared to the codominant microsatellites. Dominance means that it is not possible to recognize heterozygotes and certain assumptions must be addressed when analyzing the data generated (Lynch and Milligan 1994). First, it is assumed that there is no occurrence of comigration of markers, i.e., markers of the same length has the same nucleotide sequence. Secondly, it is assumed that only one of the alleles per locus in a two-allele system is amplified in the PCR. The first assumption also concerns microsatellites, and is if it not full-filled called homoplasy. Finally, the two markers are assumed to differ in their mutation rate. Microsatellites have a higher mutation rate ($10^{-3}$ to $10^{-4}$ mutations per generation) than the average gene (Allendorf and Luikart 2007), while the mutation rate for AFLP is not thoroughly investigated (Bonin et al. 2007).

Quantifying genetic diversity and differentiation

In my studies I have used three common ways of estimating genetic diversity within and between populations. The estimates are expected heterozygosity ($H_e$, equivalent to Nei’s gene diversity (Nei 1978)) (I, II, IV), Wright’s $F_{ST}$ (Wright 1951; Lynch and Milligan 1994) (I, IV) and hierarchical gene diversity analysis (Chakraborty et al. 1982) (I, IV). The measure of within population gene diversity, $H_e$, estimates the probability that two randomly
chosen alleles will be different (Charlesworth and Pannell 2001). Hence, large values of $H_e$ means a high genetic diversity within a population. $F_{ST}$ and hierarchical gene diversity analysis are two equivalent measures. The first measures the amount of genetic variation between populations compared to the total genetic diversity of the sampled populations. The latter analysis is based on an analysis of molecular variance, AMOVA, between specified hierarchies. For example, in paper I the hierarchies are variety, populations and individuals and the proportion of genetic differentiation between varieties, populations and individuals were calculated. Carefulness should be applied when comparing $F_{ST}$ between studies and species, since microsatellites are highly variable because of their high mutation rate (Allendorf and Luikart 2007). The high mutation rate may lead to underestimation of the populations true genetic differentiation since allele identity is not recognized in $F_{ST}$ calculations (Hedrick 1999).

In addition, I have used constrained principal coordinates analysis of genetic data to test for effects of variety identity, and population and habitat variables on genetic variation in multidimensional space (I, II, III, IV). This analysis arranges and visualizes variation in the data multidimensionally, and can also statistically test for associations between specified variables and the genetic data.

For detecting spatial patterns in the genetic variation I have applied Mantel tests (Mantel 1967) on some of my data (I, II, III). A Mantel test is an analysis searching for correlation between two matrices, e.g., a genetic distance matrix and a geographic distance matrix are used as input data when looking for isolation by distance in neutral genetic variation. A significant positive isolation by distance implies a stepping stone dispersal pattern (Slatkin 1993), i.e., the further away populations are the more dissimilar they are. In paper IV, I used the Mantel test for correlating a morphological distance matrix both to a genetic and a geographic distance matrix.

**Sampling**

*Genetic diversity and differentiation on Gotland (I, II)*

A geographic mosaic sampling of populations for the genetic analysis was performed. This sampling enables me to remove spatial variation in the sample when testing the effect of varieties (see for example Prentice et al. 2006). Effort was put on sampling as many populations as possible, making few sampled individuals per population an intentional trade-off. In July 2002 and August 2003, populations from the five varieties were sampled all over Gotland, resulting in 43 sampled populations and 290 individuals (Figure 1).

The study of regional genetic variation was performed using both marker systems presented above, AFLP (I) and microsatellites (II), applied on the
same plant material. One primer combination resulted in 192 polymorphic AFLP loci from 290 individuals, and the five microsatellites amplified resulted in five to 15 alleles per locus from 289 individuals.

Figure 1. Geographic location of the 43 studied populations of *Euphrasia stricta* on the Baltic island of Gotland, Sweden. B1 – B2 are populations of the variety *brevipila*, G1 – G2 are populations of the variety *gotlandica*, ST1 – ST28 are populations of the variety *stricta*, SU1 – SU6 are populations of the variety *suecica* and T1 – T5 are populations of the variety *tenuis*. Figure reprinted from Kolseth and Lönn (2005) with permission from the publisher.
Local genetic structure and fine-scale adaptation (III)

In June 2005, I sampled a population of *Euphrasia stricta* on northern Gotland. The population is situated on calcareous bedrock with a thin soil layer (so called alvar) surrounded by Scots pine forest, *Pinus sylvestris*. The area inhabited by *Euphrasia*, approximately 50 times 30 m, was sampled according to a regular grid pattern with grid points separated by two m, with circular plots with a radius of 15 cm positioned at the grid points. Each plot was sampled for the presence/absence of surrounding species and percent coverage of herbs, graminoids, lichens, mosses and bare soil. In addition, soil depth was measured. Data of species presence/absence, vegetation cover and soil depth were subsequently collected for grid points containing and lacking *Euphrasia*. By using this systematic and repetitive sampling strategy a general habitat matrix of the area habituated by *E. stricta* was established, including plots from a range of available micro-environments. In addition, to habitat data, the survival of seedlings during the summer was noted, and the performance (weight and number of seed capsules) of individuals surviving the whole growing season, from May/June to the beginning of October, was measured. With these data I analyzed the probability of finding a *Euphrasia* seedling in different micro-habitats, and studied the association between habitat and survival of seedlings. In addition, by connecting genotypes to habitat and fitness variables, I explored questions on fine-scale spatial genetic structure, local environmental selective processes, and extent of adaptation to the local environment.

Conservation units (IV)

When sampling on Gotland in 2001, more individuals were sampled from populations of the two varieties *suecica* and *tenuis* than from populations of *brevipila*, *gotlandica* and *stricta* with the aim of studying the structure of within genetic diversity. Leaf material for DNA extractions were obtained from four to ten individuals from five *suecica* and seven to 15 individuals from six *tenuis* populations (Figure 1).

Morphological survey (IV)

Material for morphological measurements was collected from all populations of *suecica* and *tenuis*. The leaves situated below the growing seasons first flower and the lower flower lip of this flower were collected from 3 – 11 (leaf) or 3 – 7 (flower) individuals in each of the studied populations. The morphological material was digitally photographed and the shape of each leaf and flower lip was digitized and used in a linear discriminant analysis to assign individuals to the two varieties. The results were evaluated by reassignment of individuals into varieties and populations. Distance matrices of morphological traits from the linear discriminant analysis were used to
study associations between genetic and morphological variation, and between geographic location and morphological variation.
Results and Discussion

Genetic diversity and differentiation on Gotland (I, II)
The partitioning of genetic diversity between varieties within *Euphrasia stricta* expressed as $F_{ST}$ is 0.14. This means that 86 percent of the genetic diversity is found within varieties (I). Between 0.26 (*brevipila*) and 0.6 (*suecica*) percent of the total genetic diversity observed within varieties could be attributed to differences among populations. These relatively high values indicate restricted gene flow (Wright 1943) both between varieties, and between populations within varieties (I). The hierarchical gene diversity analysis partitioned genetic diversity between varieties and populations in a similar way (I).

When testing for genetic differentiation between varieties using a constrained principal coordinates analysis, the two different marker systems showed the same pattern but with different resolution (Figure 2). AFLP gave a much more distinct and significant pattern of three genetic groups consisting of *suecica*, *tenuis* and a mixed group of *brevipila/gotlandica/stricta* (Figure 2a) (I). Comparing this with Figure 2b it can be seen that the borders between the three groups are much more ambiguous using microsatellites, even though there is a significant separation (II). The distinct groups in Figure 2a imply that the meadow varieties *suecica* and *tenuis* have an ancient origin as proposed by Karlsson (1984). The inability to separate the mixed *brevipila/gotlandica/stricta* group into three distinct groups implies that *gotlandica* and *brevipila* has a more recent origin on Gotland, perhaps caused by repeated emergence through local adaptation. As already mentioned, Karlsson (1986) argued that *brevipila* and *stricta* are close relatives since they show high inter-fertility.
a) First constrained principle coordinates axis

b) First constrained principle coordinates axis
Figure 2. A constrained principal coordinates analysis of genetic diversity in 43 populations of *Euphrasia stricta* on Gotland, Sweden. Genetic differentiation is expressed between the five varieties within the species. a) Genetic diversity displayed by AFLP. b) Genetic diversity displayed by microsatellites. Open circles: *E. stricta* var. *brevipila*, open diamonds: *E. stricta* var. *stricta*, open squares: *E. stricta* var. *suecica*, closed circles: *E. stricta* var. *tenuis* and closed diamonds: *E. stricta* var. *gotlandica*. Figure a is reprinted from Kolseth and Lönn (2005) with permission from the publisher.

The geographic pattern of the genetic diversity differs quite remarkably between the two marker systems in the three investigated varieties *stricta*, *suecica* and *tenuis*. The two remaining varieties *brevipila* and *gotlandica* had too few sampled populations for making a biologically relevant analysis. When applying a Mantel test on AFLP data isolation by distance was found in *suecica* and *tenuis*, but not in *stricta*. Microsatellite data show the opposite results with a significant isolation by distance for *stricta*, but not for *suecica* and *tenuis*. Despite incongruent results from the different molecular markers the analysis implies spatial structure in the data. This may be caused by gene flow dependent on a historical pattern of land use in *suecica* and *tenuis* and a more recent gene flow in *stricta*. Discrepancies between molecular marker systems may be explained by an ability of AFLP to identify more ancient evolutionary processes compared to microsatellites. It may be assumed that recent gene flow is detected by fast-mutating microsatellites, but not by AFLP. The difference in resolution in the constrained ordination may depend on recent mutations in microsatellites separating populations and varieties in random directions, but may also depend on low variability in microsatellite loci. In a study of microsatellite variation in *Poa alpina*, Rudmann-Maurer et al. (2007) found distinct genetic separation between populations using the same number of microsatellite loci as in my study, but the loci they screened had a higher allele richness, other parameters being similar.

Both marker systems showed considerable variation in expected heterozygosity, $H_e$, and the values obtained from AFLP and microsatellites were correlated. When comparing $H_e$ between varieties for both marker systems, *suecica* had a significantly lower $H_e$ than the other varieties. The reason for this may be that this variety at some point has gone through a bottle-neck (Nei et al. 1975).

Local genetic structure and fine-scale adaptations (III)

The density of *Euphrasia stricta* seedlings varied among micro-habitats. The probability of finding a *E. stricta* seedling was related to local species composition, vegetation cover and mean soil depth. Of the habitat factors
quantified, only vegetation cover had a significant impact on *Euphrasia*

A genetic clustering of individuals was performed using a Bayesian assignment software, Structure 2.2 (Pritchard et al. 2000). This software groups individuals together based on their genetic similarities. Two clusters of genetically similar individuals were identified, A and B (Figure 3). In addition, five individuals were impossible to assign to either of the two clusters. Because of this I have let these individuals constitute a cluster of their own, cluster C (Figure 3). From the visualization of the genetic variation it can be seen how cluster A and B are genetically dissimilar, with cluster C being genetically situated in between but closer to cluster A. The clusters were included as factor when studying the impact of micro-habitat on established individuals. The clusters were significantly associated with cover of lichens. Cluster A individuals inhabited plots with less cover of lichens than cluster C individuals. No isolation by distance was detected in the population.

The clustering of established individuals and the principal coordinates analysis (Figure 3) together imply a genetic substructure within the population. This substructure was possible to connect to micro-habitat differences. There was a difference in vegetation cover between genetic clusters which could reflect local adaptation to the micro-habitat. Lack of isolation by distance indicates strong gene flow while the genetic substructuring implies that either the gene flow is low enough for genetic differentiation to occur at a small scale or selection pressure strong enough to overcome the gene flow, allowing genetic differentiation. A future study designed to sample a larger geographic area, or more populations, increasing the sample of each micro-habitat, may enhance the possibility to find evidence of local adaptation.

![Graph showing genetic clusters A, B, and C with principal coordinates analysis](image-url)
Conservation units and values (IV)

I found significant genetic differentiation among the two varieties *Euphrasia stricta* var. *suecica* and var. *tenuis* (as in paper I), among populations within varieties and among subpopulations within populations. These results were obtained analyzing genetic differentiation both by $F_{ST}$ and constrained principal coordinates analysis. Both *suecica* and *tenuis* are on Gotland confined to wooded hay-meadows. Karlsson (1984) proposed that separation between the two varieties is facilitated by subtle differences in habitat optima, e.g., in relation to soil moisture. Flower and leaf shape may be used as discriminating characters between the varieties, they can discriminate both between varieties and populations based on the consistent reassigning using linear discriminant analysis. The local spatial genetic substructure within meadows is probably due to restricted pollen and seed flow (Fischer and Matthies 1998; Fischer et al. 2000; Lennartsson 2002), different degrees of selfing (Levin 1981; Karlsson 1986) or local patchiness with local adaptation (Lönn 1993). Recognizing the partitioning of genetic diversity between varieties and populations is of importance for conservation purposes. First, both varieties have a conservational value since they are two clearly distinct genetic units. Secondly, it is desirable that as many populations as possible are preserved to maximize the amount of genetic diversity that is possible to protect. Thirdly, since much of the variation lies between populations, I recommend that seeds from different populations are not mixed in re-introduction trials to avoid outbreeding depression and genetic swamping (Ellstrand and Elam 1993; Hufford and Mazer 2003), since the effect of outbreeding depression and genetic swamping in *Euphrasia* is unknown.

Conclusions

The aim of this thesis was to reveal the relationship of intraspecific ecological and genetic diversity within *Euphrasia stricta*. The exploration of assumed neutral genetic variation at different spatial scales has resulted in novel information about the evolutionary processes acting within this species.

Studying *E. stricta* I have found support for Karlsson’s (1986) and Yeo’s (1968) suggestion that habitat specialization may have been important for the speciation in the genus *Euphrasia*. Three distinct genetic groups have
been detected within *E. stricta*, where the differentiation can be associated to the varieties and, hence, their ecological and morphological characteristics. The two meadow varieties constitute two distinct genetic groups while the third group consisted of an aggregate of the three other varieties *brevipila/gotlandica/stricta*, having another habitat optimum. The genetic differentiation of the varieties implies that *suecica* and *tenuis* have a more ancient origin than *gotlandica* since they are genetically different both from the *brevipila/gotlandica/stricta* group but also from each other, despite their similar habitat preference. The *brevipila* and *gotlandica* varieties, on the other hand, may have a recent origin on Gotland because they are genetically similar to *stricta*. My results confirm to some extent the proposed evolutionary relationship between the varieties defined by Karlsson (1976; 1984), identifying *suecica* and *tenuis* as their own genetic entities in comparison to *brevipila*, *gotlandica* and *stricta* in this study.

The result of my study of *suecica* and *tenuis* has provided the protection action plan of the endangered variety *suecica* confined to Gotland (Johansson 2007) with information of the genetic status of *suecica* compared to *tenuis* on Gotland and genetic pitfalls when reintroducing *suecica* to the wooded hay-meadows where it has been lost. I have shown that *suecica* and *tenuis* are two genetically different units and should be treated accordingly in conservation. A large part of the genetic diversity lies between populations making each population left today an important conservation unit if the aim of protection is to conserve as much genetic diversity for the future as possible. Care should be taken not to mix seeds from different *suecica* populations when reintroducing *suecica* to meadows where it historically has been reported. This would be to avoid outbreeding depression and genetic swamping between locally adapted source populations. For the same reasons sowing of externally produced seeds should only be done into sites where the variety is missing.
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Being affiliated to the department of plant ecology at Uppsala University during my graduate studies did not only give me a second supervisor. It has also given me a whole department focusing on plants and ecology, giving me a chance to deepen my knowledge in this subject through seminars and a graduate student trip to Spain. Thank you for taking me on as a PhD candidate.

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Hard work demands time and places to recover in order to keep up motivation, creativity and energy. For this time and space I’m indebted to my friends and family.

Linda, Rosie and Joffi – we’ve known each other more than half of our lives, scattered around nationally and internationally you’re no more than an email, phone call or flight away.

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Svensk sammanfattning

Evolutionära processer och genetisk variation hos gotländska ögontröstar

Vikten av variation

Att anpassa sig till sin omgivning


Ögontröst

förekommande på Gotland och sällsynt på fastlandet. Den femte varieteten, vätögontröst (*E. stricta* var. *gotlandica*), är knuten till väter på de ölandska och gotländska alvarmarkerna. Väter är områden på alvaret som blir översvämmade om våren och torkar ut under sommaren, de har mycket tunna jordar och sparsam växtlighet.

Svensk och späd ögontröst blommar tidigt om sommaren, från juni och framåt, för att hinna sätta från innan slättern, medan vanlig ögontröst, uddögontröst och vätögontröst inte börjar blomma förrän i augusti. Inte bara blommningstiden utan också utseendet är resultat av anpassningar till de olika habitattyperna. Ångsvarieteterna är långa och slanka och når upp till ljuset där de växer omgivna av andra ängsarter. Svensk och späd ögontröst är sinsemellan mycket lika, med små skillnader i bl.a. antal glandelhår (körtelhår) och bladform. Vanlig ögontröst, uddögontröst och vätögontröst förekommer i kortare gräs och blir kortare och kraftigare med många grenar.


Genetiska skillnader och likheter

Lokal anpassning – finns det hos ögontröst?
Den senblommande gruppen med vätögontröst, vanlig ögontröst och uddögontröst som var genetiskt lika fångade mitt intresse. Varför var denna

**Bevarande av svensk ögontröst**

Svensk ögontröst är den mest sällsynta av ögontröstvarieteterna. Med min studie har jag kunnat visa att den är tydligt genetiskt olik späd ögontröst, även om de ser lika ut och båda växer i ängar. Om målet är att bevara det unika hos svensk ögontröst måste vi därför bevara svensk och späd ögontröst som två olika varieteter motsvarande deras likheter. Det är också viktigt att bevara så många populationer som möjligt av svensk ögontröst. I och med att en stor del av den genetiska variationen finns fördelad mellan populationerna blir varje enskild population viktig för att bevara så mycket som möjligt av den genetiska variationen. Förutsättningarna för att bevara svensk ögontröst hänger ihop med ett fortsatt brukande av ängar på traditionellt vis. Det innebär att räfsa i ängarna om våren för att ta bort kvistar och löv, s.k. fagning, att anpassa slätten till frösättningen, att inte tillföra gödsel samt att beskära, hamla, träden i ängarna för att få tillräckligt ljusinsläpp. Då kan framtidens generationer njuta av svensk ögontröst och många andra hotade arter som är unika i de svenska ängarna.


A doctoral dissertation from the Faculty of Science and Technology, Uppsala University, is usually a summary of a number of papers. A few copies of the complete dissertation are kept at major Swedish research libraries, while the summary alone is distributed internationally through the series Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Science and Technology. (Prior to January, 2005, the series was published under the title “Comprehensive Summaries of Uppsala Dissertations from the Faculty of Science and Technology”.)

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