Sverre Lundemo

Molecular studies of genetic structuring and demography in *Arabidopsis* from Northern Europe

Thesis for the degree of Philosophiae Doctor

Trondheim, May 2010

Norwegian University of Science and Technology
Faculty of Natural Sciences and Technology
Department of Biology

NTNU
Norwegian University of Science and Technology

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ISBN 978-82-471-2152-8 (printed ver.)
ISBN 978-82-471-2153-5 (electronic ver.)
ISSN 1503-8181

Doctoral theses at NTNU, 2010:95

Printed by NTNU-trykk

PREFACE

This PhD study was carried out at the Department of Biology, and was financed by the Faculty of Natural Sciences and Technology, Norwegian University of Science and Technology (NTNU).

I would first like to thank my main supervisor, Professor Hans K. Stenøien at the Museum of Natural History and Archaeology (NTNU), for guidance, enduring all my questions, my troublesome habit of trying to explore new ideas when I should be working on something completely else, and in general involving myself too much in other things. I would also like to thank my co-supervisors; Professor Outi Savolainen at the University of Oulu – especially for helping me out during my research stays in Finland, and Associate Professor Bård Pedersen at my own department.

I would also like to thank Mohsen Falahati-Anbaran for all the numerous talks and discussions we have had in our office, making the process towards results much easier.

The following people helped out in different aspects of my PhD, be it field work, green house work, lab work, analyses or comments – for which I am very grateful: Johanna Leppälä, Meeri Otsukka, Guri F. Hansen, Kirsti Stengrundet, Nina Sletvold, Mariann Nilsen, Marianne D. Hansen, Per Toräng, Line Johansen.

The working environment at the department has been really nice, thanks to all of you for making my time here memorable and enjoyable.

Finally, I would like to thank my family for supporting me and trying to understand what my thesis was actually about, and Marte, for showing an admirable patience all the times I had the need to discuss matters related to both my own research, as well as research education in general.

Synopsis

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Synopsis

LIST OF PAPERS

This thesis is based on four papers, which will be referred to in the text by their Roman numerals.

I) Lundemo S, Falahati-Anbaran M, Stenøien HK (2009) Seed banks cause elevated generation times and effective population sizes of *Arabidopsis thaliana* in Northern Europe. Molecular Ecology, 18, 2798-2811

II) Falahati-Anbaran M, Lundemo S, Ågren J, Stenøien HK. Genetic consequences of seed banks in the perennial plant *Arabidopsis lyrata* ssp. *petraea* (Brassicaceae). (submitted to Molecular Ecology)

III) Lundemo S, Stenøien HK, Savolainen O. Investigating the effects of topography and clonality on genetic structuring within a large Norwegian population of *Arabidopsis lyrata*. (Annals of Botany, in press)

IV) Lundemo S. Flower visitation has small effects on outcrossing levels in a natural population of *Arabidopsis thaliana*. (manuscript)

Declaration of contributions

In paper I) Stenøien initiated the project and planned the field work, whereas Lundemo and Falahati-Anbaran did the analysis, and all authors did field work and wrote the paper. In paper II) Stenøien initiated the project, Lundemo planned and did the field work, Falahati-Anbaran did the analysis, and Falahati-Anbaran and Lundemo wrote the paper with contributions from the other authors. In paper III) Savolainen initiated the project, all authors planned the field work, while Lundemo did the field work, the analysis, and wrote the paper with contributions from the other authors.

Synopsis

SUMMARY

The seed bank is an important part of plant population dynamics, capable of storing genotypes accumulated over several seasons, thereby creating a genetic reservoir which can resupply the above ground population regularly or after disturbance events. Thus, the seed bank can function to increase genetic diversity and effective population size in the population as a whole. Additionally, the seed bank can restore the above ground population if it is reduced or even completely destroyed. Consequently, seed banks may have an impact on both the ecology and evolution of species.

In this thesis, I have mainly investigated effective population size, generation time and geographic patterns of structuring in two closely related crucifers, *Arabidopsis thaliana* and *A. lyrata*, especially how this is impacted by the presence of a seed bank. They are both naturally occurring in Norway, inhabiting disturbed habitats with low levels of competition. It has been suggested that the species diverged from each other approximately 5-9 million years ago. Due to the availability of molecular tools for this genus, they are widely used in research focusing on e.g. variation in physiological and morphological traits and how this may affect evolutionary processes such as local adaptations to environmental conditions. Both *A. thaliana* and *A. lyrata* harbour seed banks, although the density of seedlings and the impact of this cohort on effective population size (N_e) seem to be different. *A. thaliana* have a higher seedling density as seen from soil sample germination, and its seed bank cohort contribute more to N_e, compared to in *A. lyrata*. Both species show a geographically structured pattern throughout the Norwegian distribution range, although differentiation between populations is much higher in *A. thaliana*. This indicates that populations of *A. thaliana* are more isolated, and this may be due to differences in mating system and habitat characteristics.

A. thaliana is an annual species, and population dynamics in this species should therefore be more dependent upon the seed bank than in *A. lyrata*, which is perennial and therefore should exhibit more stable population size and composition. The latter is also capable of vegetative spread by sending out lateral stolons, thus producing new rosettes genetically identical with the mother plant and further reducing the need for high annual seedling recruitment. The amount of clonal genets, as well as the size of these genets, vary within a population, and are more likely related to resource availability than plant density. Clonality is found to cause fine scale genetic structure in a large population of *A. lyrata* at Spiterstulen, Norway. However, there was no overall population structure, and topographic features believed to constitute barriers for gene flow did not seem to cause genetic

substructures. Still, it is not yet clear how seed flow and pollen flow act separately on spatial genetic structure in the population.

Whereas *A. lyrata* is strictly outcrossing throughout most of its distribution range, *A. thaliana* is almost exclusively self-pollinating, and plant-pollinator interactions have not been explored thoroughly in this species. It is shown that insect visitation rates in a Central Norwegian population is about ten times higher than a similar study from Germany, but this did apparently not result in outcrossing events during the observation season. However, evaluation of genotypic data from other seasons in this population show that outcrossing events do occur, and this highlights the importance of a more comprehensive sampling regime when trying to describe population genetic structure.

GENERAL INTRODUCTION

Understanding causes and consequences of population genetic structure is an important part of biological research. By evaluating the composition of plant populations, how this varies and interacts with abiotic and biotic factors, one can obtain information on questions related to ecology, evolution and conservation. E.g., by investigating whether isolation by distance is present in a population or a group of populations, one can evaluate patterns of subdivision and which factors are causing these patterns. Furthermore, being self-pollinating or self-incompatible is likely to be associated with selective constraints on other traits, like reproduction and floral morphology (see e.g. Armbruster et al. 2002 and references therein). Thus, type of reproductive system has the potential to influence population structure. In addition, using plant species with a common evolutionary history may benefit studies on the evolution of genes, traits, adaptation and speciation (Clauss and Koch 2006). One group well suited for such purposes is the genus *Arabidopsis* (Brassicaceae).

Arabidopsis thaliana (L.) Heynhold (Fig. 1) is the plant equivalent of the common fruit fly (*Drosophila melanogaster*), having been applied in numerous studies within molecular and cell biology since Laibach (1943) first introduced the species as suitable for this purpose more than 60 years ago. Its relative *A. lyrata* (L.) O'Kane and Al-Shehbaz (Fig. 1) is not as commonly used, but due to the close relationship with *A. thaliana*, it is fast becoming a study object of high interest (Clauss and Koch 2006). *A. thaliana* became the first plant to have its full genome sequenced in 2000 (AGI 2000), whereas the process of sequencing the genome of *A. lyrata* was finished in 2009, and a paper on the results is now in preparation (D. Weigel, personal communication). Both species are small plants, rarely exceeding 20-30 cm in height of main inflorescence, growing in rosettes, and exhibiting small, white flowers with four petals forming a cross (petal length 2-4 mm in *A. thaliana*, 3-7 mm in *A. lyrata*; Fig. 2) (Lid and Lid 2005). However, there are some clear differences in life history traits between these species, in particular concerning reproductive system and plant longevity.

Fig.1: *A. thaliana* growing at Lauset, Trondheim (left), and *A. lyrata* from Kamtjern, Oppdal (right). Both photos: S. Lundemo.

Fig. 2: Inflorescences of *A. thaliana* (left) and *A. lyrata* (right), showing the evident difference in flower size between the species. Both photos: S. Lundemo.

Seed bank – function and dynamics

After seed shedding from a mother plant, viable seeds being deposited on suitable growth substrates can either germinate or enter the seed bank. A seed bank is the deposition and storage of seeds for longer or shorter periods of time, usually in or on top of soil (Fenner and Thompson 2005). However, in some plant species seeds are retained on the plant, thus constituting some kind of aerial seed bank (see e.g. Barrett et al. 2005). A seed bank can be viewed as a part of a bet-hedging strategy, through which the plant can ensure some of its offspring may survive in the case of stochastic disturbance events that destroy or severely weaken the above ground population. Another effect of the seed bank is that seeds can stay

dormant in the soil when conditions are not optimal for growth and reproduction, potentially making the population appear and disappear between seasons. This is seen in many desert species that germinate, flower and reproduce only after heavy rain (see e.g. Epling et al. 1960). The advantage of this is also evident in *A. thaliana*, where a population can exhibit a high census size one season, and then be completely gone the next (Bergelson et al. 1998; Jørgensen and Mauricio 2004).

In temperate climates, which have been studied most extensively, soil seed banks are often split into transient seed banks, where seeds are present in the soil for less than one year, and persistent seed banks, where seeds are present in the seed bank for more than one year. The latter category can be further split into short-term persistent, where seeds persist in the soil for more than one year, but less than five years, and long-term persistent, where seeds persist for at least five years (Thompson et al. 1997). Some seeds have been shown to be extremely persistent. Seeds of sacred lotus (*Nelumbo nucifera*), can survive for up to 1,200 years in lake bed depositions (Shen-Miller et al. 1995), and seeds of date (*Phoenix dactylifera*) can persist up to 2,000 years with dry room storage (Sallon et al. 2008). It is expected that persistent seed banks will be more common when suitable habitats are fragmented and/or small, and the dispersal rate is low (Thompson 2000). Annual species should thus be more dependent upon their seed bank than perennials, as the latter have more stable above ground populations. There is a trend for woodlands, arctic and alpine communities to have small seed banks, whereas disturbed habitats like heath lands and wetlands can contain thousands of seeds per m^2. This can be related to the stability of the communities, as communities stable in time and space are expected to have smaller seed banks than those opposite to this (Fenner and Thompson 2005). However, this pattern is not absolute, and because seeds in a seed bank seem to persist longer at higher altitudes, they can accumulate sizable seed banks in such habitats as well. In a study by Cummins and Miller (2002), seed density in the soil increased with altitude, while the seed rain, the amount of seeds being shed during a season, decreased. Morphology is important for the duration of seeds in the soil as well, small seeds tend to persist for longer periods than large seeds, mostly because they get buried more easily (Thompson et al. 1993).

It was previously assumed that any viable, but ungerminated seed was inactive and dormant, although this has later proved to be incorrect (Fenner and Thompson 2005). The dispute arose partly from the lack of a common definition of the term. As a consequence, Vleeshouwers et al. (1995) therefore defined seed dormancy as being a characteristic of a seed, the degree of which can vary depending on which conditions are necessary to make it

germinate. They stressed that is important to note that seeds are not inactive when dormant, and can adjust their level of dormancy to changes in the environment. From this, it follows that dormancy is flexible and can be viewed as the conditions necessary for germination at any given time. In addition, being in a seed bank does not necessarily imply dormancy (Thompson et al. 2003).

Seed banks can reduce the effect of genetic drift in populations in partly the same way as through immigration, by adding new genotypes or extra copies of existing genotypes into a population's gene pool. Thus, the probability of a given genotype of being eliminated from a population due to stochastic events is lowered. For a species with seed bank, this can also result in the differentiation between populations being lower when examining the seed bank population as compared to the above ground population (Nunney 2002; Vitalis et al. 2004; Honnay et al. 2008). In species with persistent seed banks, new seeds are recruited over time, causing an accumulation of a diverse seed bank pool, in which different genotypes can be added in different seasons. This has lead to a discussion of whether the level of genetic diversity is higher in the seed bank than in the above ground population, and whether one should expect a differentiation between the seed bank and the above ground population. Several studies have shown higher genetic diversity in the seed bank (McCue and Holtsford 1998; Aparicio et al. 2002), the opposite (Alvarez-Buylla et al. 1996; Uesugi et al. 2007), or no significant difference between the cohorts at all (Tonsor et al 1993; Cabin 1996; Mahy et al. 1999; Uchiyama et al. 2006). Honnay et al. (2008) showed in a meta-analysis that genetic similarity was high between seed bank and above ground populations, that genetic variation was not higher in the seed bank, and if so was the case, it was the result of the occurrence of rare alleles. This could be due to a sieving effect acting from the seed bank to the above ground population through removal of inbred and less adapted genotypes, as was described for *Atriplex tatarica* (Mandák et al. 2006).

To conclude, seed banks are present in many plant species, although the importance and extent of the seed bank can show large differences due to e.g. mating system, seed characteristics, topography and climate. Examining the seed bank is therefore crucial when studying population dynamics, especially for conservation purposes, as it can be vital when describing gene flow patterns and estimating effective population sizes.

Arabidopsis thaliana – Thale cress

A. thaliana is native to most of Europe, Northern Africa and eastwards to Central Asia. In addition it has been naturalised in East Asia, Africa south of Sahara, Australia, South America, as well as North America (Al-Shehbaz and O'Kane 2002; Hoffmann 2002). The species is thought to have originated in eastern parts of the Mediterranean, possibly in the area from Turkey to Iran, as many of the species in the genus are endemic to Europe (see e.g. Hoffmann 2002; Beck et al. 2008; Couvreur et al. 2010 and references therein). In Norway (Fig. 3), *A. thaliana* occurs in a range of habitat types with various levels of disturbance such as roadsides, rocky outcrops or boulders, steep slopes, and sandy beaches. Typical for more or less all these habitats is a thin or almost absent soil layer which dries out easily and is not stable. It can also grow as a pioneer species on newly disturbed soil, but as time since disturbance increases, the chances for it being displaced by other species due to its low competitive ability becomes higher as well.

Several different estimates have been published for time since divergence between *A. thaliana* and *A. lyrata*. According to Koch et al. (2000), the split between the species may have taken place approximately 5 million years ago, whereas Ossowski et al. (2010) argue that the mutation rate used in the previous study is too high, and that 8.7 million years since divergence is a more likely estimate. The diploid genome (2n=10) of *A. thaliana* has been reduced compared to the ancestral state, with a genome size as low as 0.18 pg (Oyama et al. 2008). The reason for this reduction is most likely illegitimate recombination (recombination between DNA sequences with few identical nucleotides) which has been shown to be an important factor in removing noncoding DNA in this species (Devos et al. 2002).

As an annual species, with local populations fluctuating greatly in size, the seed bank is expected to be an important part of its population dynamics. Some have also proposed that it has extensive metapopulation dynamics, with regular extinctions and recolonizations among interacting populations (Le Corre 2005; He et al. 2007). The life cycle of *A. thaliana* is variable, with populations often being divided into summer/autumn annual and winter annual accessions (see e.g. Shindo et al. 2007; Donohue 2009). Spring and summer annuals germinate in spring, summer or early autumn, flower, set seed and then die. Their seeds go dormant after seed shedding and remain in this state until next season. Individuals exhibiting spring or summer annuality are thus often referred to as "rapid cyclers" as they complete the life cycle from germination to death within one growth season. Winter annuals, which are supposed to constitute most of Scandinavian populations (Kuittinen et al. 1997; Koornneef et al. 2004), germinate in the autumn and establish small rosettes which survive the winter until

next spring, when they flower, set seed and die in quick succession. As germination conditions are rarely favourable during summer, the seeds stay dormant until autumn. During this period, the seeds go through physiological changes collectively termed after-ripening, in which dormancy is gradually lost (Baskin and Baskin 1972). A cold spell can break this dormancy, and seeds that are to be used in experiments are often given a short cold treatment, stratification, before they are exposed to conditions optimal for germination. To avoid initiating flowering in late autumn and risk not being able to mature the seeds produced, rosettes require winter chilling, also known as vernalization, in order to bolt and flower. This response is dependent upon genetic pathways, and populations harbouring non-functional versions of the alleles involved have been shown in nature (see e.g. Napp-Zinn 1987).

The reproductive system of *A. thaliana*

A. thaliana has evolved self-compatibility since it separated from *A. lyrata*, and close to all reproduction occur through self pollination events (see e.g. Abbott and Gomes 1989; Bergelson et al. 1998; Stenøien et al. 2005). However, insect visitation and its potential implications for population dynamics in *A. thaliana* has largely been ignored so far, with population studies merely referring to the species as being visited by flies or bees (e.g. Clapham et al. 1962; Snape and Lawrence 1971; Lawrence 1976; Bergelson et al. 1998).To my knowledge, only one study has so far evaluated flower visitation rates and pollinator assemblage in *A. thaliana*, using a semi-natural population in Germany (Hoffmann et al. 2003). Here it was found that insects in the orders Hymenoptera, Diptera and Thysanoptera were the most important visitors. Hence, the impact of flower visitation on outcrossing in this species is largely unknown, although authors have argued that it can be of importance in some populations (Stenøien et al. 2005).

***Arabidopsis lyrata* – Northern rock-cress**

A. lyrata occurs as two different subspecies, ssp. *petraea* in Central and Northern Europe, and ssp. *lyrata* in central and eastern parts of North America (Clauss and Koch 2006). Until recently, a third subspecies, ssp. *kamchatica*, occurring in eastern Asia and north-western North America, was recognized as well, but it is now treated as a separate species, *A. kamchatica* (Shimizu et al. 2005). In Norway (Fig. 3), the distribution of the species is limited to the south-western parts of the country, where it typically grows on river banks, old river

beds and moraine ridges, occurring almost all the way from the sea level up to alpine environments (Lid and Lid 2005). *A. lyrata* is mainly diploid (2n=16), but tetraploid populations (2n=4x=32) occur in Central Europe (Dart et al. 2004).

The reproductive system of *A. lyrata*

In contrast to *A. thaliana*, *A. lyrata* exhibits a strong self-incompatibility (SI) system, in which an allele system with alleles of varying dominance levels control the growth of pollen tubes positioned on the style (van Treuren et al. 1997; Schierup 1998; Charlesworth et al. 2000). However, self-compatible individuals of *A. lyrata* ssp. *lyrata* have been found in several North American populations, although the mechanisms leading to the loss of SI is not yet known for this subspecies (Mable et al. 2005; Mable and Adam 2007). Being a self-incompatible species, it is dependent upon insects for pollen transport, and flower visitors include Diptera and Hymenoptera (Clauss and Mitchell-Olds 2006; Sandring and Ågren 2009). *A. lyrata* is perennial and clonal, although the level of clonality varies among populations in Scandinavia (Gaudeul et al. 2007), and has not been detected at all in Central Europe (Clauss and Mitchell-Olds 2006). Due to the clonality, one cannot assume that all rosettes within a population are of independent origin unless you investigate it mechanically through excavating plants or by using molecular techniques. Such genetically unique individuals can be termed genets, whereas rosettes belonging to the same genet (having identical multilocus genotypes) are called ramets. In populations exhibiting high levels of clonal growth one can expect this to influence spatial genetic structure, SGS (cf. *A. halleri*; van Rossum et al. 2004).

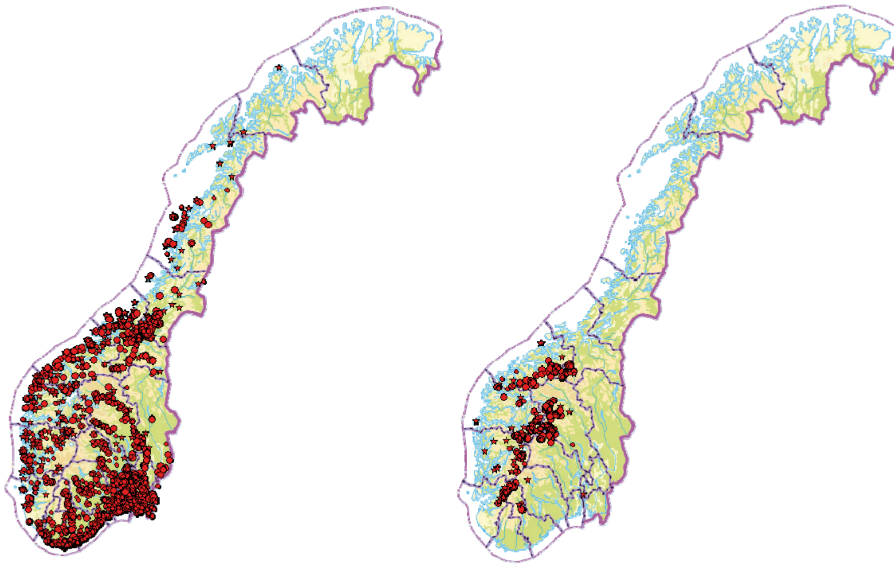

Fig. 3: Distribution of *Arabidopsis thaliana* (left), and *A. lyrata* (right) in Norway, illustrated through herbarium registrations. Each red dot represents a registration, while stars are used to denote geographically uncertain registrations. Data sets made available by Artsdatabanken, downloaded from http://artskart.artsdatabanken.no/, 2010-02-01.

AIMS

The purpose of my studies has been to apply genetic analyses to understand processes causing geographical patterns of genetic variation in *Arabidopsis thaliana* and *A. lyrata*. In particular, this has been addressed through studies of genetic variation in seed banks and above ground plants, and differentiation between these cohorts. Additionally, consequences for population dynamics due to characteristics of the species' reproductive system have been examined.

The specific aims outlined in the various papers were:

- To investigate the presence of seed banks in Norwegian populations of the annual *A. thaliana*, and evaluate the contribution of the seed bank cohort on generation time and effective population sizes compared to the above ground population (paper I).
- To investigate the presence of seed banks in Norwegian populations of the perennial and clonal *A. lyrata*, and examine how seed banks influence effective population sizes using two different approaches (paper II).
- Examine at which distance levels spatial genetic structure can be observed, and how this is influenced by topography and clonality in a large population of *A. lyrata* (paper III).
- Record insect visitation rates, identify potential pollinators and examine potential effects of flower visitation on outcrossing levels and genetic diversity in a natural population of *A. thaliana* (paper IV).

METHODS

Paper I Seed bank in *A. thaliana*

The experiment was initialized in 2005, when soil samples from 26 Norwegian populations were collected. Throughout each population, 10 soil samples (approximately 10 cm×10 cm×10 cm) were sampled. In addition, siliques were sampled from most populations in 2005 and 2006, and seeds from these siliques were grown to obtain seedlings from as many maternal families as possible. Soil samples were sown out in a thin layer (about 1 cm) atop ordinary plant soil in a green house to facilitate germination of as many seedlings as possible and grown for at least 6 months until no more seedlings appeared. Up to 20 seedlings were sampled from the seed bank of each population, and the total number of seedlings per population registered. Leaf tissue was sampled and subsequently dried before DNA extraction, and the DNA samples from totally 27 populations were screened for 96 single nucleotide polymorphisms (SNPs).

Paper II Seed bank in *A. lyrata*

In 2006, soil samples were collected from 14 Norwegian populations, following the same procedure as for *A. thaliana*. Populations were sampled in the south-western parts of Norway, covering the entire distribution range of the species in Norway (Fig. 3). Population structure of above ground individuals from the same populations has been described in Gaudeul et al. (2007), and data from this study was included for comparison with the seed bank data. Soil samples were sown out in a thin layer atop ordinary plant soil and grown in the green house until no more seedlings emerged. DNA from leaves of germinating plants was extracted and subsequently genotyped, using 15 microsatellite markers from Gaudeul et al. (2007). Effective population size using the parameter theta (θ) was estimated both with a contemporary approach using a linkage disequilibrium (LD) method, and a historical approach using maximum likelihood (ML) based on coalescent theory.

Paper III Spatial genetic structure in *A. lyrata*

Using a large population of *A. lyrata* at Spiterstulen (N61°37 E008°24; 1,104 m a.s.l.), Lom, Norway, 17 squares, each 10m×10 m (Fig. 4 and 5) were created along both sides of the river Visa in 2006. Within each plot, the position of 50 *A. lyrata* rosettes was registered to the nearest cm, and 2-3 rosette leaves from totally 850 individual rosettes were sampled and

dried. Care was taken to sample individuals throughout the entire plot, as well as including rosettes close to each other, to detect any signs of clonality. In addition, census size, i.e. total rosette number, of *A. lyrata* was registered in all squares. Leaf samples were dried, and DNA extracted for microsatellite analysis. In total 14 microsatellite markers were used to screen the samples.

Paper IV Flower visitation in *A. thaliana*

In May 2007, number and type of insects visiting a dense patch of *A. thaliana* plants was registered at Lauset, Trondheim, Norway (N63˚24.466, E10˚04.878; 28 m a.s.l). The observations were done throughout the entire flowering period in the patch. In addition, a number of insect were caught for subsequent identification. In total 255 individuals of flower visitors were registered and classified to group (solitary bees, other Hymenopterans, Syrphids, other Dipterans, ants, beetles, and others) during the observation period, and 68 of them were captured for identification. As solitary bees and Syrphids were expected to be the most important flower visitors at this site, they were identified to species level, whereas other insects and arachnids were identified to family or superfamily.

In relation to the seed bank study (paper I), SNP data from the same locality based on *A. thaliana* samples from 2006, 2007, and 2008 were included to investigate whether the visits had any effect on outcrossing rates. In 2008, plant tissue for DNA extraction was sampled as three different batches (one sampling only in the observation patch, one population-wide sample, and finally, the seed bank).

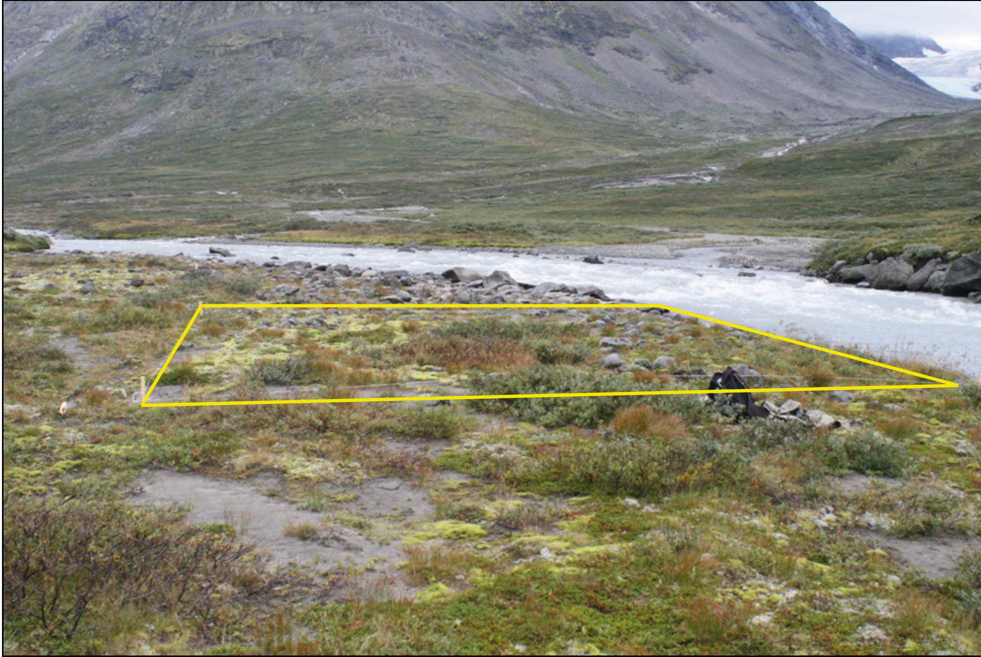

Fig. 4: A 10m×10m plot (marked by wooden poles and white string - highlighted in yellow) next to the river Visa, at Spiterstulen, Lom. Photo: S. Lundemo.

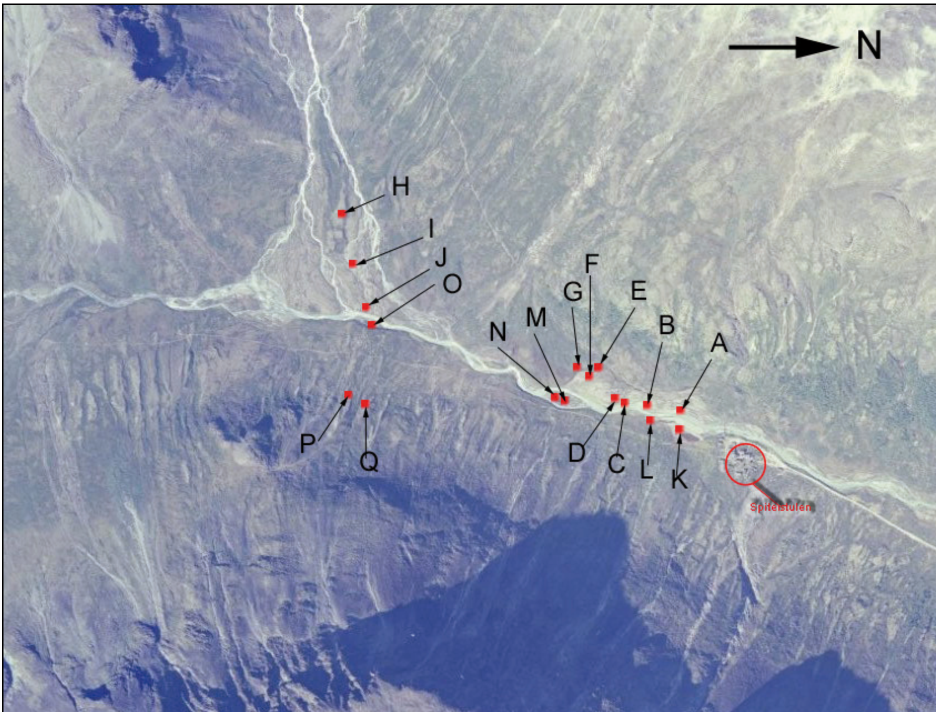

Fig. 5: Aerial photo showing the 17 squares sampled in the *A. lyrata* population at Spiterstulen, Lom. Source: Norge i bilder (http://norgeibilder.no/).

Statistical analyses

Analyses of regional population genetic structure (paper I, II) were done using Structure (Pritchard et al. 2000; Falush et al. 2003) and BAPS (Corander et al. 2003; 2004). Historical effective population size (N_e), using a Markov Chain Monte Carlo (MCMC) approach, was estimated using Migrate (Beerli and Felsenstein 1999; 2001) (paper I, II). Contemporary N_e was estimated with NeEstimator (Peel et al. 2004) (paper II). Population differentiation and differentiation between cohorts (paper I, II, III, IV) were estimated based on pairwise comparisons of F_{ST} (Weir and Cockerham 1984) in Arlequin (Excoffier et al. 2005). Patterns of spatial genetic structure within squares, i.e. spatial autocorrelation analysis, as well as neighbourhood sizes, were estimated in SPAGeDi 1.2 (Hardy and Vekemans 2002) (paper III). Isolation by distance (paper I, III) was tested for using a Mantel test (Mantel 1967) as implemented in GenAlex (Peakall and Smouse 2006). AMOVA (analysis of molecular variance) was performed in Arlequin (Excoffier et al. 2005) (paper II, III). Correlations tests (Spearman rank correlation with Bonferroni or stepwise Bonferroni) and comparisons between data sets (paired t-tests, pairwise Wilcoxon tests) was done in SPSS (SPSS Inc.) (paper I, II, III, IV). Arlequin (Excoffier et al. 2005), GenePop (Rousset 2008), GenAlex (Peakall and Smouse 2006) and FSTAT (Goudet 2002) were used to compute various population genetic parameters and test for Hardy-Weinberg equilibrium (paper I, II, III, IV).

RESULTS AND DISCUSSION

The demographic and evolutionary consequences of the seed bank (paper I, II)

Seed banks were registered in both *Arabidopsis* species, although the seedling density was of one magnitude larger in *A. thaliana* than in *A. lyrata*. The results also indicate that the effect of the seed bank cohort on the effective population size is higher for *A. thaliana* than *A. lyrata*.

In *A. thaliana*, seedlings were registered in soil samples from all populations, but the size of the seed bank varied greatly between populations (mean=94.2, range=3.8-265.6 seedlings/dm³). There was, however, no correlation between seedling density and census sizes of the populations in 2005 or 2006 (P=0.332 and 0.876, respectively). In total 1035 individuals were successfully genotyped, and average (±SE) genetic diversity was 0.051±0.007. There was positive and significant isolation by distance for all three cohorts (*r*=0.64, 0.68 and 0.65 for seed bank, 2005 above ground and 2006 above ground, respectively; P<0.01), possibly reflecting a stepwise colonization followed by subsequent isolation. Pairwise F_{ST} (mean±SE) between populations was as high as 0.83±0.01, indicating a high level of differentiation. As *A. thaliana* is a highly selfing species, populations are more susceptible to random loss of genetic variation through genetic drift. Together with limited gene flow, this seems to be the reasons for the high differentiation values found in this study. Based on an admixture analysis in Structure (Pritchard et al. 2000; Falush et al. 2003), the most likely number of clusters of genetically similar groups were found to be 15. Two main clusters were detected, one including populations from south-eastern and southern parts of Norway, whereas the other included populations from Central and Northern Norway. Thus, the results from Structure support the isolation by distance pattern already described. Estimates of the diversity parameter theta (θ) showed a higher value for SNP data based on above ground and seed bank individuals combined compared to individuals from only one of the cohorts alone. Effective population size based on θ was calculated with a subset of populations for which microsatellite data was available (from Stenøien et al. 2005), and this value was higher than the census size for 4 out of 6 populations. This gave an average generation time of approximately four, indicating longevity of up to four years for a cohort. This is of course a crude and uncertain estimate, but it shows that due to the presence of a seed bank, the efficiency of genetic drift is reduced, and effective population size and effective generation time are elevated compared to when only examining above ground

populations. This can be viewed in two different ways; firstly, a single seed can potentially be stored for years before germinating, hence its effective longevity will be more than the time where it exists as a plant. Secondly, if looking at a cohort of seeds produced during a single season, the cohort may subsequently germinate at different times, creating an overlap of generations and contributing to a prolongation in the life of any given cohort.

In *A. lyrata*, seedlings germinated from soil samples from 12 of the 14 populations. Average (±SD) density was 7.9±9.4 seedlings/dm^3, considerably lower than for *A. thaliana*. There was negligible differentiation between the cohorts within all populations, and population genetic parameters were very similar. Average genetic diversity was 0.297 and 0.296 for seed bank and above ground cohorts, respectively. Although each cohort had some private alleles, these were mostly present at very low frequencies. Based on an analysis of molecular variance (AMOVA), approximately 29% of the total genetic variation was due to variation among populations, and 71% due to variation within populations. Differentiation (F_{ST}) between populations was on overall 0.29. Based on Structure analyses of seed bank and above ground data, the 14 populations were clustered into 5 groups. Effective population size (N_e) based on both cohorts was significantly higher than based on either of the cohorts alone. Contemporary and historical N_e based on combined data from both cohorts was 40.7 and 332.2, respectively. Thus, this study shows that the presence of the seed bank increases effective population size in *A. lyrata*, although it may seem that the effect is lower than what was found in *A. thaliana*. Still, it can be argued that the seed bank is of high importance for population dynamics in the species, as it can reduce the effect of genetic drift.

Together, these studies show that the seed bank has a clear influence on population demography and dynamics in the two *Arabidopsis* species. The seed bank can directly affect effective population size both by harbouring genotypes accumulated during previous seasons but no longer present in the above ground cohort, and by adding to a greater pool of genotypes already present above ground (Nunney 2002; Vitalis et al. 2004). Especially the latter will be important in reducing the effect of genetic drift in a population, as genotypes being removed from the above ground cohort through stochastic events can subsequently be replaced by the seed bank. A seed bank can contribute to recovery of the population in case of it being reduced or destroyed (see e.g. Kalamees and Zobel 2002; for a review, see Bossuyt and Honnay 2008), a property of high relevance for the *Arabidopsis* species as they both typically occur on unstable soil. In addition, *A. thaliana* often grows on exposed soil in the lowland, and its habitats may be susceptible to anthropogenic disturbance, which can possibly contribute to the species' dispersal. The seeds of *A. thaliana* are considerably smaller than for

A. lyrata, and they may therefore be susceptible to human-mediated dispersal as they can e.g. attach to the footwear of people walking through localities (Jørgensen and Mauricio 2004; Beck et al. 2008). Still, the effect of this can not be seen in the Hvaler populations, wherein plants from four different localities not more than 500 meters apart from each other, in an area much used for recreational purposes, were significantly different (4 out of 6 pairwise comparisons significantly different; Global differentiation test, $P<0.05$). Thus, human-mediated dispersal seems to be of little importance in this area. This is also in contrast to theoretical predictions, that seed banks should reduce differentiation between populations (Nunney 2002; Vitalis et al. 2004). The reason for this may be limited gene flow for several generations.

Spatial genetic structure (paper III)

Clonal growth is likely a main factor in creating the fine scale spatial genetic structure (SGS) seen in this study. In contrast, SGS across the population as a whole is absent, indicating a random pattern of mating and/or seed dispersal. The river does not seem to cause differentiation within the population through inhibiting pollen transport across it, and may possibly be linked to seed dispersal through water transport instead.

Of the 850 *A. lyrata* individuals sampled at Spiterstulen, 827 were successfully genotyped. All groups of identical multilocus genotypes were determined to be ramets of the same genet based on probability analyses. The whole sample consisted of 546 genets, of which 415 were present in a single ramet only. A subset of this sample (n=423 genets) was used when examining genet size and shape in the population. Genetic diversity (±SD) based on genet data was 0.480±0.031 (range 0.424-0.522). Clonality could possibly affect level of genetic diversity in the plots, but no significant difference was found (pairwise Wilcoxon tests; $P=0.29$ for expected and $P=0.98$ for observed diversity, respectively). On fine spatial scales clonality was widespread, but size and shape of genets varied throughout the population (mean genet size±SD=6.4±22.5 cm; range 0-267.2 cm). In some of the squares, the total density of *A. lyrata* rosettes was lower, and it was thus possible to sample most of them (>80%), which should provide a better estimate of genet size. However, when only considering data from these squares (n=5), genet size was on average (±SD) 10.4±33.4 cm (range 0-267.2 cm). Thus, mean size of genets did increase in squares where sampling coverage was very high, but so did the variation in genet size. Likewise, the proportion of unique genotypes, i.e. number of genets divided by the number of ramets, varied greatly

between the squares (population mean=0.66, range=0.22-0.85). However, there does not seem to be a clear pattern of genet size in relation to e.g. plant density in the population. This can be seen in the lack of correlation between clonality levels and census size that was found (P=0.52), hence clonal growth seems to be dependent upon other factors. It may be that microsite variation in growth conditions, like e.g. nutrient availability, is more important in shaping the pattern and level of clonality within the population (Gaudeul et al. 2007; Gonzales et al. 2008). Neighbourhood size, the effective number of individuals in a random breeding neighbourhood (Wright, 1946), was expected to be negatively correlated with proportion of genets, but although a trend for such a relationship was shown it was not statistically significant. Furthermore, there was no uniform growth pattern, i.e. neither the guerrilla or phalanx growth strategy was dominant in the population as a whole. A previous study by Gaudeul et al. (2007) found no evidence for clonality in this population, but this was likely due to a sampling artefact. As they sampled rosettes with 50 cm distance in between, this would not detect most clonal individuals found in my study. In a study from Central Europe, fine scale sampling did not result in detection of any clonality (Clauss and Mitchell-Olds 2006). However, this deviation in life history between central and northern populations could be due to reduced levels of sexual reproduction in the harsher environments found in range margins, as was shown for the tree species *Sorbus torminalis* in Denmark (Rasmussen and Kollmann 2008).

Prior to the sampling, it was expected that the river, due to turbulent winds, could possibly provide a barrier for gene flow by restricting pollinator movement across it. If there was such an effect, it should be possible to detect differentiation between squares based on their location. There was, however, no effect of the river on genetic differentiation within the population, and almost all genetic variation (95.3%) was due to variation within squares. A bit unexpectedly, no sign of isolation by distance was found for the population as a whole. This indicates that despite its size, the Spiterstulen population has a random mating structure. It is difficult to say what causes this lack of structure, as one can not distinguish the contribution of pollen flow from seed flow in our data set. Still, the river may influence population genetic structure at Spiterstulen in other ways. Dispersal of seeds can in many terrestrial plants be facilitated by water (see e.g. Walker et al. 2003; Hampe 2004; Walker et al. 2009), a process known as hydrochory. In Norway, many of the populations are situated on the banks of rivers and streams, and these are often subject to periodic flooding. Hence, it is possible that some seeds will be transported by water, which can lead to subsequent establishment on new sites, as well as immigration of new genotypes to existing localities downstream of the origin.

Flower visitation (paper IV)

Flower visitation in the observation patch was much higher than anticipated, but it did not result in any outcrossing events. However, heterozygous individuals were sampled from the population during other years, suggesting that outcrossing does occur but can be infrequent.

As close to all reproduction in *A. thaliana* is due to selfing (Abbott and Gomes 1989; Bergelson et al. 1998; Stenøien et al. 2005), I expected very low visitation rates and low pollen transfer success at the locality. However, flower visitation rates were quite high, and it was estimated that close to 7% of all flowers received a visit. This visitation rate was about 10 times higher than the only previous study comparable (Hoffmann et al. 2003). However, no outcrossing events were registered based on single nucleotide polymorphism (SNP) data from plants sampled this season. Still, heterozygous individuals were registered among seedlings germinated from seeds sampled in both 2006 and 2008, showing that genetic diversity and outcrossing rates seem to vary between years. This was also shown through a differentiation test, in which the population-wide sample in 2008 was significantly differentiated from the other sampling batches sampled in 2007 and 2008, whereas no other comparisons were significantly different. This indicates that pollen transfer efficiency is very low for insects visiting flowers in *A. thaliana*, but that such events occur, and they are thus important for the population dynamics in the species. Low pollen transfer efficiency could be due to both low numbers of pollen grains on the stamens (Pylatuik et al. 1998), efficient autogamy mechanisms (Jones 1971), or inefficient pollinators (Schemske and Horvitz 1984). In advance, it was expected that the number of species visiting *A. thaliana* flowers at this latitude would be lower than what was found in the study by Hoffmann et al. (2003), due to a global trend of decreasing species richness at higher latitudes (see e.g. Hillebrand 2004). Even though this was also the case for Hymenopterans, and especially for Thysanopterans, in our study, the number of Syrphid species was more or less the same. The expectation of one or a few species dominating flower visitation was confirmed as the solitary bee *Lasioglossum fratinellum*, the most common visitor, contributed with more than two-thirds of all visits.

The between-season genetic variation and its possible de-coupling from flower visitation rates found in this study illustrate the importance of proper sampling. One cannot necessarily assume a priori that sampling only a part of the population, or only sampling one season is enough to capture all or most genetic variation. Even for a species renowned for

being almost completely selfing, this may be crucial to gain insight into population diversity and dynamics.

CONCLUSIONS AND FURTHER STUDIES

The studies in my thesis focused on evaluating genetic structure in relation to life history traits in plant populations, using *A. thaliana* and *A. lyrata* as focal species. Despite their genetic relationship and similar habitat types, they differ in characteristics like reproductive system and plant longevity. *A. thaliana* is annual, mainly self-pollinating, and produces a lot of seeds. In contrast, *A. lyrata* is perennial, produces fewer seeds, is strictly outcrossing, and have the ability of clonal propagation. Together with an ever-increasing availability of molecular tools, and the fact that some of these tools are transferable between species, the *Arabidopsis* genus is therefore well suited for comparative studies.

My studies have provided insight in several aspects concerning population dynamics. Both *Arabidopsis* species have extensive seed banks, and this may increase effective population size and generation time, thus reducing the effect of genetic drift, one of the major evolutionary forces affecting genetic composition of populations. Furthermore, clonal growth has been shown to affect fine scale genetic structure in *A. lyrata*, but whether these results also holds for lowland populations and small and/or isolated populations needs to be investigated. In the final study, insect visitation rate was found to be higher than expected in *A. thaliana*, but this did not result in any apparent outcrossing events. However, pollinator visitation studies should ideally be run over several seasons and/or in several populations simultaneously to give more consistent results, and my study has merely scratched the surface. In *A. lyrata*, some studies on plant-pollinator interactions have been performed already, although how sexual reproduction is related to e.g. altitude, and how reproductive rate in terms of seed production vs. ramet production varies among populations and years has yet to be addressed. Finally, studies of demography in natural populations of *A. thaliana* would be worth pursuing further. Through registrations of recruitment and mortality, combined with the evaluation of genotype frequencies, it is possible to gain insight in how selection and drift influence populations. This may uncover whether populations of annuals like *A. thaliana* can be static for several generations, or if they are rather dynamic, frequently changing in genetic composition and fine scale distribution of genotypes.

Thus, despite the extensive knowledge that already exists about plant population dynamics, much is still to be learnt about life history traits and how these interact with the environment. Molecular tools are here essential to get a thorough insight into these processes.

REFERENCES

The Arabidopsis Genome Initiative (2000) Analysis of the genome sequence of the flowering plant *Arabidopsis thaliana*. Nature, 408, 796-815.

Abbott RJ, Gomes MF (1989) Population genetic structure and outcrossing rate of *Arabidopsis thaliana* (L.) Heynh. Heredity, 62, 411-418.

Al-Shehbaz IA, O'Kane SL. (2002) Taxonomy and phylogeny of *Arabidopsis* (Brassicaceae). In: *The Arabidopsis Book*. American Society of Plant Biologists, Rockville, MD. doi: 10.1199/tab.0001, http://www.aspb.org/publications/arabidopsis/.

Alvarez-Buylla ER, Chaos A, Pinero D, Garay AA (1996) Demographic genetics of a pioneer tropical tree species: Patch dynamics, seed dispersal and seed banks. Evolution, 50, 1155-1166.

Aparicio A, Albaladejo RG, Ceballos GL (2002) Genetic differentiation in silicicolous *Echinospartum* (Leguminosae) indicated by allozyme variability. Plant Systematics and Evolution, 230, 189-201.

Armbruster WS, Mulder CPH, Baldwin BG, et al. (2002) Comparative analysis of late floral development and mating-system evolution in tribe Collinsieae (Scrophulariaceae s.l.). American Journal of Botany, 89, 37-49.

Barrett LG, He T, Lamont BB, Krauss SL (2005) Temporal patterns of genetic variation across a 9-year-old aerial seed bank of the shrub *Banksia hookeriana* (Proteaceae). Molecular Ecology, 14, 4169-4179.

Baskin JM, Baskin CC (1972) Ecological life cycle and physiological ecology of seed germination of *Arabidopsis thaliana*. Canadian Journal of Botany, 50, 353-360.

Beck JB, Schmuths H, Schaal BA (2008) Native range genetic variation in *Arabidopsis thaliana* is strongly geographically structured and reflects Pleistocene glacial dynamics. Molecular Ecology, 17, 902-915.

Beerli P, Felsenstein J (1999) Maximum likelihood estimation of migration rates and effective population numbers of two populations using a coalescent approach. Genetics, 152, 763–773.

Beerli P, Felsenstein J (2001) Maximum likelihood estimation of a migration matrix and effective population sizes in *n* subpopulations by using a coalescent approach. Proceedings of the National Academy of Sciences USA, 98, 4563–4568.

Bergelson J, Stahl E, Dudek S, Kreitman M (1998) Genetic variation within and among populations of *Arabidopsis thaliana*. Genetics, 148, 1311-1323.

Bossuyt B, Honnay O (2008) Can the seed bank be used for ecological restoration? An overview of seed bank characteristics in European communities. Journal of Vegetation Science, 19, 875-884.

Cabin RJ (1996) Genetic comparisons of seed bank and seedling populations of a perennial desert mustard, *Lesquerella fendleri*. Evolution, 50, 1830-1841.

Charlesworth D, Awadalla P, Mable BK, Schierup MH (2000) Population-level studies of multiallelic self-incompatibility loci, with particular reference to Brassicaceae. Annals of Botany, 85 (Supplement A), 227-239.

Clapham AR, Tutin TG, Warburg EF (1962) *Flora of the British Isles* (2nd ed). Cambridge University Press, Cambridge, UK.

Clauss MJ, Koch MA. (2006) Poorly known relatives of *Arabidopsis thaliana*. Trends in Plant Science, 11, 449-459.

Clauss MJ, Mitchell-Olds T (2006) Population genetic structure of *Arabidopsis lyrata* in Europe. Molecular Ecology, 15, 2753-2766.

Corander J, Waldmann P, Marttinen P, Sillanpää MJ (2004) BAPS 2: enhanced possibilities for the analysis of genetic population structure. Bioinformatics, 20, 2363-2369.

Corander J, Waldmann P, Sillanpää MJ (2003) Bayesian analysis of genetic differentiation between populations. Genetics, 163, 367-374.

Couvreur TLP, Franzke A, Al-Shehbaz IA, et al. (2010) Molecular phylogenetics, temporal diversification, and principles of evolution in the Mustard family (Brassicaceae). Molecular Biology and Evolution, 27, 55-71.

Cummins RP, Miller GR (2002) Altitudinal gradients in seed dynamics of *Calluna vulgaris* in eastern Scotland. Journal of Vegetation Science, 13, 859-866.

Dart S, Kron P, Mable BK (2004) Characterizing polyploidy in *Arabidopsis lyrata* using chromosome counts and flow cytometry. Canadian Journal of Botany, 82, 185-197.

Devos KM, Brown JKM, Bennetzen JL (2002) Genome size reduction through illegitimate recombination counteracts genome expansion in *Arabidopsis*. Genome Research, 12, 1075-1079.

Donohue K (2009) Completing the cycle: maternal effects as the missing link in plant life histories. Philosophical Transactions of the Royal Society B – Biological Sciences, 364, 1059-1074.

Epling C, Lewis H, Ball FM (1960) The breeding group and seed storage: a study in population dynamics. Evolution, 14, 238-255.

Excoffier L, Laval G, Schneider S (2005) Arlequin ver. 3.0: An integrated software package for population genetics data analysis. Evolutionary Bioinformatics Online, 1, 47-50.

Falush D, Stephens M, Pritchard JK (2003) Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. Genetics, 164, 1567-1587.

Fenner M, Thompson K (2005) *The ecology of seeds*. Cambridge University Press, Cambridge, UK.

Gaudeul M, Stenøien HK, Ågren J (2007) Landscape structure, clonal propagation, and genetic diversity in Scandinavian populations of *Arabidopsis lyrata* (Brassicaceae). American Journal of Botany, 94, 1146-1155.

Gonzales E, Hamrick JL, Smouse PE (2008) Comparison of clonal diversity in mountain and Piedmont populations of *Trillium cuneatum* (Melanthiaceae-Trilliaceae), a forest understorey species. American Journal of Botany, 95, 1254-1261.

Goudet J (2002) FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9.3). Available from http://www.unil.ch/izea/softwares/fstat.html. Updated from Goudet (1995).

Hampe A (2004) Extensive hydrochory uncouples spatiotemporal patterns of seedfall and seedling recruitment in a “bird-dispersed” riparian tree. Journal of Ecology, 92, 797-807.

Hardy OJ, Vekemans X (2002) SPAGeDi: a versatile computer program to analyse spatial genetic structure at the individual or population levels. Molecular Ecology Notes, 2, 618-620.

He F, Kang D, Ren Y, et al. (2007) Genetic diversity of the natural populations of *Arabidopsis thaliana* in China. Heredity, 99, 423-431.

Hillebrand H (2004) On the generality of the latitudinal diversity gradient. The American Naturalist, 163, 192-211.

Hoffmann MH (2002) Biogeography of *Arabidopsis thaliana* (L.) Heynh. (Brassicaceae). Journal of Biogeography, 29, 125-134.

Hoffmann MH, Bremer M, Schneider K, et al. (2003) Flower visitors in a natural population of *Arabidopsis thaliana*. Plant Biology, 5, 491-494.

Honnay O, Bossuyt B, Jacquemyn H, Shimono A, Uchiyama K (2008) Can a seed bank maintain the genetic variation in the above ground population? Oikos, 117, 1-5.

Jones ME (1971) The population genetics of *Arabidopsis thaliana*. I. The breeding system. Heredity, 27, 39-50.

Jørgensen S, Mauricio R (2004) Neutral genetic variation among wild North American populations of the weedy plant *Arabidopsis thaliana* is not geographically structured. Molecular Ecology, 13, 3403-3413.

Kalamees R, Zobel M (2002) The role of the seed bank in gap regeneration in a calcareous grassland community. Ecology, 83, 1017-1025.

Koch MA, Haubold B, Mitchell-Olds T (2000) Comparative evolutionary analysis of chalcone synthase and alcohol dehydrogenase loci in *Arabidopsis*, *Arabis*, and related genera. Molecular Biology and Evolution, 17, 1483-1498.

Koornneef M, Alonso-Blanco C, Vreugdenhil D (2004) Naturally occurring genetic variation in *Arabidopsis thaliana*. Annual Review of Plant Biology, 55, 141-172.

Kuittinen H, Mattila A, Savolainen O (1997) Genetic variation at marker loci and in quantitative traits in natural populations of *Arabidopsis thaliana*. Heredity, 79, 144-152.

Laibach F (1943) *Arabidopsis thaliana* (L.) Heynh. als Object für genetische und entwicklungs-physiologische Untersuchungen. Botanisches Archiv, 44, 439-455.

Lawrence MJ (1976) Variations in natural populations of Arabidopsis thaliana (L.) Heynh. In: *The biology and chemistry of the Cruciferae* (eds. Vaughan JG, Macleod AJ, Jones BMG), pp 167-190. Academic Press, London, UK.

Le Corre V (2005) Variation at two flowering time genes within and among populations of *Arabidopsis thaliana*: comparison with markers and traits. Molecular Ecology, 14, 4181-4192.

Lid J, Lid DT (2005) *Norsk flora*, 5th ed. Det Norske Samlaget, Oslo.

Mable BK, Adam A (2007) Patterns of genetic diversity in outcrossing and selfing populations of *Arabidopsis lyrata*. Molecular Ecology, 16, 3565-3580.

Mable BK, Robertson AV, Dart S, Di Berardo C, Whitham L (2005) Breakdown of self-incompatibility in the perennial *Arabidopsis lyrata* (Brassicaceae) and its genetic consequences. *Evolution* 59, 1437-1448.

Mandák B, Bímová K, Mahelka V, Plačková I (2006) How much genetic variation is stored in the seed bank? A study of *Atriplex tatarica* (Chenopodiaceae). Molecular Ecology, 15, 2653-2663.

Mantel N (1967) The detection of disease clustering and a generalized regression approach. Cancer research, 27, 209-220.

Mahy G, Vekemans X, Jacquemart A-L (1999) Patterns of allozymic variation within *Calluna vulgaris* populations at seed bank and adult stages. Heredity, 82, 432-440.

McCue KA, Holtsford TP (1998) Seed bank influences on genetic diversity in the rare annual *Clarkia springvillensis* (Onagraceae). American Journal of Botany, 85, 30-36.

Napp-Zinn K (1987) Vernalization – Environmental and genetic regulation. In: *Manipulation of flowering* (ed. Atherton JG), pp. 123-132. Butterworths, London, UK.

Nunney L (2002) The effective size of annual plant populations: The interaction of a seed bank with fluctuating population size in maintaining genetic variation. The American Naturalist, 160, 195-204.

Ossowski S, Schneeberger K, Lucas-Lledó JI, et al. (2010) The rate and molecular spectrum of spontaneous mutations in *Arabidopsis thaliana*. Science, 327, 92-94.

Oyama RK, Clauss MJ, Formanová N, et al. (2008) The shrunken genome of *Arabidopsis thaliana*. Plant Systematics and Evolution, 273, 257-271.

Peakall R, Smouse PE (2006) GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. Molecular Ecology Notes, 6, 288-295.

Peel D, Ovenden JR, Peel SL (2004) NeEstimator: software for estimating effective population size, Version 1.3. Queensland Government, Department of Primary Industries and Fisheries.

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. Genetics, 155, 945-959.

Pylatuik JD, Bonham-Smith PC, Davis AR (1998) The additional stamens of *flo*10-1 mutants of *Arabidopsis thaliana* are compromised in production and viability of pollen. Canadian Journal of Botany, 76, 1733-7142.

Rasmussen KK, Kollmann J (2008) Low genetic diversity in small peripheral populations of a rare European tree (*Sorbus torminalis*) dominated by clonal reproduction. Conservation Genetics, 9, 1533-1539.

Rousset F (2008) GENEPOP ' 007: a complete re-implementation of the GENEPOP software for Windows and Linux. Molecular Ecology Resources, 8, 103-106.

Sallon S, Solowey E, Cohen Y, et al. (2008) Germination, genetics, and growth of an ancient date seed. Science, 320, 1464.

Sandring S, Ågren J (2009) Pollinator-mediated selection on floral display and flowering time in the perennial herb *Arabidopsis lyrata*. Evolution, 63, 1292-1300.

Schemske DW, Horvitz CC (1984) Variation among floral visitors in pollination ability: A precondition for mutualism specialization. Science, 225, 519-521.

Schierup MH (1998) The effect of enzyme heterozygosity on growth in a strictly outcrossing species, the self-incompatible *Arabis petraea* (Brassicaceae). Hereditas, 128, 21-31.

Shen-Miller J, Mudgett MB, Schopf JW, Clarke S, Berger R (1995) Exceptional seed longevity and robust growth: ancient sacred lotus from China. American Journal of Botany, 82, 1367-1380.

Shimizu KK, Fujii S, Marhold K, Watanabe K, Kudoh H (2005) *Arabidopsis kamchatica* (Fish. ex DC.) K. Shimizu & Kudoh and *A. kamchatica* subs. *kawasakiana* (Makino) K. Shimizu & Kudoh, new combinations. Acta Phytotaxonomica et Geobotanica, 56, 163-172.

Shindo C, Bernasconi G, Hardtke CS (2007) Natural genetic variation in *Arabidopsis*: Tools, traits and prospects for evolutionary ecology. Annals of Botany, 99, 1043-1054.

Snape JW, Lawrence MJ (1971) The breeding system of *Arabidopsis thaliana*. Heredity, 27, 299-302.

Stenøien HK, Fenster CB, Tonteri A, Savolainen O (2005) Genetic variability in natural populations of *Arabidopsis thaliana* in Northern Europe. Molecular Ecology, 14, 137-148.

Thompson K (2000) The functional ecology of soil seed banks. In: *Seeds – The ecology of regeneration in plant communities* (ed. Fenner M), pp. 215-236. CABI Publishing, Wallingford, UK.

Thompson K, Bakker JP, Bekker RM (1997) The soil seed banks of North West Europe: Methodology, density and longevity. Cambridge University Press, Cambridge, UK.

Thompson K, Band SR, Hodgson JG (1993) Seed size and shape predict persistence in soil. Functional Ecology, 7, 236-241.

Thompson K, Ceriani RM, Bakker JP, Bekker RM (2003) Are seed dormancy and persistence in soil related? Seed Science Research, 13, 97-100.

Tonsor SJ, Kalisz S, Fisher J, Holtsford TP (1993) A life-history based study of population genetic structure: Seed bank to adults in *Plantago lanceolata*. Evolution, 47, 833-843.

Uchiyama K, Goto S, Tsuda Y, Takahashi Y, Ide Y (2006) Genetic diversity and genetic structure of adult and buried seed populations of *Betula maximowicziana* in mixed and post-fire stands. Forest Ecology and Management, 237, 119-126.

Uesugi R, Nishihiro J, Tsumura Y, Washitani I (2007) Restoration of genetic diversity from soil seed banks in a threatened aquatic plant, *Nymphoides peltata*. Conservation Genetics, 8, 111-121.

van Rossum F, Bonnin I, Fénart S, et al. (2004) Spatial genetic structure within a metallicolous population of *Arabidopsis halleri*, a clonal, self-incompatible and heavy metal tolerant species. Molecular Ecology, 13, 2959-2967.

van Treuren R, Kuittinen H, Kärkkäinen K, Baena-Gonzalez E, Savolainen O (1997) Evolution of microsatellites in *Arabis petraea* and *Arabis lyrata*, outcrossing relatives of *Arabidopsis thaliana*. Molecular Biology and Evolution, 14, 220-229.

Vitalis R, Glémin S, Olivieri I (2004) When genes go to sleep: the population genetic consequences of seed dormancy and monocarpic perenniality. The American Naturalist, 163, 295-311.

Vleeshouwers LM, Bouwmeester HJ, Karssen CM (1995) Redefining seed dormancy: An attempt to integrate physiology and ecology. Journal of Ecology, 83, 1031-1037.

Walker NF, Hulme PE, Hoelzel AR (2003) Population genetics of an invasive species, *Heracleum mantegazzianum*: implications for the role of life history, demographics and independent introductions. Molecular Ecology, 12, 1747-1756.

Walker NF, Hulme PE, Hoelzel AR (2009) Population genetics of an invasive riparian species, *Impatiens glandulifera*. Plant Ecology, 203, 243-252.

Weir BS, Cockerham CC (1984) Estimating F-statistics for the analysis of population structure. Evolution, 38, 1358-1370.

Wright S (1946) Isolation by distance under diverse systems of mating. Genetics, 31, 39-59.

ERRATA LIST

Changes from the original text are indicated by underlining.

Synopsis, page 3

II) Falahati-Anbaran M, Lundemo S, Ågren J, Stenøien HK. Genetic consequences of seed banks in the perennial plant *Arabidopsis lyrata* ssp. *petraea* (Brassicaceae). (submitted to Molecular Ecology)

III) Lundemo S, Stenøien HK, Savolainen O. Investigating the effects of topography and clonality on genetic structuring within a large Norwegian population of *Arabidopsis lyrata*. (Annals of Botany, in press)

Synopsis, page 17

Fig. 4: A 10m×10m plot (marked by wooden poles and white string - highlighted in yellow) next to the river Visa, at Spiterstulen, Lom. Photo: S. Lundemo.

Paper I

Supplementary information has been added after the article itself.

Paper I

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Paper II

Genetic consequences of seed banks in the perennial plant *Arabidopsis lyrata* ssp. *petraea* (Brassicaceae)

Mohsen Falahati-Anbaran[1], Sverre Lundemo[1], Jon Ågren[2], Hans K. Stenøien [3,*]

[1]Department of Biology, Norwegian University of Science and Technology, N-7491 Trondheim, Norway.

[2]Department of Ecology and Evolution, Evolutionary Biology Centre, Uppsala University, Villavägen 14, SE-752 36 Uppsala, Sweden.

[3]Systematics and Evolution Group, Section of Natural History, Museum of Natural History and Archaeology, Norwegian University of Science and Technology, N-7491 Trondheim.

[*]Corresponding author: telephone +47 735 92284, E-mail: stenoien@ntnu.no

Abstract

Seed banks may increase effective population sizes of plants because of elevated coalescence times for alleles residing in the populations. This has recently been demonstrated empirically for natural populations of the selfing, annual herb *Arabidopsis thaliana*, whereas comparable data for perennial species are currently lacking. We studied the contribution of seed banks on effective sizes of natural populations of a close relative, the self-incompatible, perennial *Arabidopsis lyrata* ssp. *petraea*. Fourteen populations collected throughout the Norwegian distribution range were analyzed using microsatellite markers. The genetic composition of seed bank and above ground cohorts was similar, but the proportion of private alleles was higher in the above ground cohort than in the seed bank. We found negligible within-population differentiation between cohorts in most of the populations. The historical effective population size (N_e), estimated by a maximum likelihood approach based on coalescent theory, was considerably higher than contemporary N_e, obtained using a linkage disequilibrium method. The seed bank significantly increased total effective population sizes, but the contribution from the seed bank to total N_e was lower than from the above ground cohort in most populations. Based on the historical effective

population size approach, N_e was found to be larger than census sizes in most of the studied populations, but this was not the case for any population when considering contemporary effective population sizes. The results indicate that seed banks contribute substantially less to the effective population size in the perennial *A. lyrata* compared to in the annual *A. thaliana*.

Introduction

Theory predicts that seed banks may buffer the effects of stochastic processes on the genetic diversity of plant populations because they increase the effective population size (Nunney 2002; Vitalis et al. 2004). While this has been confirmed in empirical studies of annual plants (Del Castillo 1994; Lundemo et al. 2009), little is known about the importance of seed banks for the structuring of genetic variation in perennial species. Natural populations of annual plant species often exhibit substantial variation in census sizes between years (Del Castillo 1994; Husband and Barrett 1998; Williams 2000; Nunney 2002; Lundemo et al. 2009). By comparison, perennial plant species should exhibit less temporal variation in the number of established plants since life span and population stability tend to be correlated (García et al. 2008), cf. classical *r*- and *K*-selection theory (MacArthur and Wilson 1967). Hence, populations of annual species may suffer more from loss of genetic variation every generation due to stochastic genetic drift since effective population size, N_e, is highly influenced by fluctuations in population size across generations (Crow and Kimura 1970; Ellstrand and Elam 1993; Frankham 1996). However, plants have means to counteract stochastic environmental effects. The seed bank is in most cases an accumulation of ungerminated but viable seeds buried in the soil, on the soil surface or in the litter layer, potentially capable of replacing adult plants (Baker 1989; Garwood 1989; Simpson et al. 1989). It has recently been shown that the presence of soil seed banks increases total N_e in natural populations of the annual *Arabidopsis thaliana* (Lundemo et al. 2009). Seed banks thus seem to buffer against detrimental census size changes between years in this species. It is of interest to determine to what extent seed banks play a similar role for perennial species where the census population sizes may vary less between years.

Genetic variation in plant populations harboring seed banks is the result of mutual gene flow between the seed bank and the above ground individuals. Firstly, seeds from a given cohort can germinate either directly or after a period of after-ripening, or they can enter the seed bank.

Furthermore, the proportion of seeds geminating and entering the seed bank can be expected to vary according to e.g. environmental conditions and maternal effects (Lundemo et al. 2009). Correspondingly, the number of seedlings being recruited from the seed bank to the above ground vegetation may vary between years. Therefore, we can expect that most genotypes from the seed bank containing rare and common alleles are also present in the above ground cohort. Dormant seeds produced by outcrossing of different genotypes can accumulate in the soil over long periods, creating a diverse gene pool. Consequently, in perennials, both seed bank and above ground cohorts contain an overlap of genotypes between generations (Templeton and Levin 1979). Persistent seed banks may have an important influence on population dynamics by maintaining genetic variability within populations and preventing the extinction of small and isolated populations (Honnay et al. 2008). This is due to the interdependency between the seed bank and the above ground population, wherein each of them directly affects the composition and density of the other (Villiers et al. 2003; Amiaud and Touzard 2004; Caballero et al. 2008; for a review, see Hopfensperger 2007). However, natural and anthropogenic disturbances may have great influence on seed banks in most ecosystems, e.g. the temporal fluctuation of water flow in riparian habitats may alter the composition of the seeds that have accumulated in the soil (Goodson et al. 2001). Natural populations inhabiting such habitats are therefore more prone to genetic erosion due to stochastic events (Young et al. 1996; Aguilar et al. 2008).

Even though stochastic factors have a major influence on N_e, theoretical expectations and experimental studies suggest that seed banks may moderate the effect of genetic drift through buffering against changes in census sizes in above ground cohorts (Epling 1960; Templeton and Levin 1979; Nunney 2002; Vitalis et al. 2004; Honnay et al. 2008; Lundemo et al. 2009). The rate of loss of genetic variation per generation due to drift is inversely related to effective population size and can be determined by $1/(2N_e)$ (Fisher 1930; Wright 1931). Estimated effective population sizes should thus be associated with the rate at which populations lose genetic variation due to stochastic processes. N_e have been estimated by a number of different approaches, based on both genetic and demographic models (Nunney and Elam 1994; Frankham 1996; Schwartz et al. 1998; Leberg 2005; Wang 2005). Classical approaches to infer N_e include estimates based on linkage disequilibrium (Hill 1981), heterozygosity excess (Pudovkin et al. 1996), and temporal changes in allele frequencies (Waples 1989). Coalescent theory may also be

used to estimate historical N_e (Beerli and Felsenstein 2001), either directly from the $\theta=4N_e\mu$ parameter, where μ is the mutation rate, or as a relative measure between populations or cohorts by dividing different θ values and assuming similar mutation rates (Lundemo et al. 2009).

It is known that seed banks may influence effective sizes of annuals, like it was shown for *Phacelia dubia* (Del Castillo 1994) and *A. thaliana* (Lundemo et al. 2009), but it is not known to what extent such an effect is found in perennials. *Arabidopsis lyrata* (L.) O'Kane and Al-Shebaz is a close relative of *A. thaliana*, capable of clonal growth, and inhabiting disturbed habitats like river banks throughout most of its distribution range in Norway. As a perennial plant, it is expected to experience less variation in census sizes due to gradual regeneration from the seed bank. However, due to its habitat preferences, the level of genetic variation in natural populations may change due to stochastic genetic drift caused by disturbance events such as flooding. In this study, we aimed at examining the existence of soil seed banks and the contribution of the seed bank in maintaining genetic diversity in the clonal and perennial *A. lyrata*. We compared genetic composition and structure of the seed bank cohort relative to the above ground cohort in natural populations of *A. lyrata* throughout its Norwegian distribution range. The following questions were addressed: 1) to what extent do natural populations of *A. lyrata* establish soil seed banks, 2) how much genetic variation exists in the soil seed bank, 3) how does the genetic contribution from the seed bank affect total effective population size in natural populations, and 4) what is the historical versus contemporary effective population size in this species.

Materials and methods

Sampling and germination

Soil samples from 14 Norwegian *A. lyrata* populations, in which the above ground cohort had previously been genetically analyzed (Gaudeul et al. 2007), were collected before seed maturation in July 2006. Ten random soil samples (10×10 cm wide, and up to 5 cm deep) were taken from throughout each population. The soil samples were spread out in a thin layer (1-2 cm) on top of ordinary plant soil in 10×10 cm pots to stimulate germination of as many seeds as possible. The pots were put in the greenhouse under 16 hour day length for at least 10 months until no more seedlings emerged. Leaf tissue from emerged seedlings at the rosette stage was

collected and dried for 24 hours at 45°C. The total number of seedlings emerging from the soil samples was 243, varying from 5 seedlings in populations N8 and N16 to 58 seedlings in N13. There was no germination in N2 (Sunndalsøra) and N15 (Roaldkvam), and we excluded these populations from further analyses. The mean density (±SD) of viable seeds per dm^3 of soil in the twelve populations harboring seed banks was 7.9±9.4.

Microsatellite analysis

Genomic DNA was extracted from dried leaf tissue of 191 seedlings using the E.Z.N.A. ™ SP Plant DNA Kit (Omega Bio-Tek. Inc., Norcross, GA). We used 15 microsatellite markers, originally described in Clauss et al. (2002) and later used by Gaudeul et al. (2007) to analyse the above ground cohort, for genetic analyses. Microsatellites were amplified using multiplex PCR in two separate reactions (Table 1). PCR were performed in a 10 µL volume containing 10 ng DNA template, 1 x PCR Buffer containing 1.5 mM $MgCl_2$, 0.2mM of each dNTP, 1.5 U Taq Polymerase (all from Qiagen, Hilden, Germany), and varying concentrations of primers (determined to optimize the intensity of each microsatellite band). For each locus, forward primer was labeled with a fluorescent dye at the 5′ end (Invitrogen for HEX- and 6-FAM-labeled primers; Applied Biosystems for NED-labeled primers). The following thermal cycling conditions were used: 5 min at 95°C, followed by the first 10 cycles as touchdown composed of 30 s denaturation at 94°C, 45 s annealing at 60°C to 50°C, 45 s extension at 72°C and 25 cycles composed of 30 s denaturation at 94°C, 45 s annealing at 50°C, 45 s extension at 72°C, followed by 15 min final extension at 72°C. PCR products for each sample were diluted (1:30 ddH_2O) and mixed with 0.15 µL GeneScan- 600 LIZ size standard and 8.85 µL Hi-Di™ formamide (both from Applied Biosystems, Foster City, CA). Fragment analysis was performed on a 3130xl ABI Genetic analyzer, and fragment detection and determination of fragment sizes were performed with the GeneMapper ver. 4.0 software (Applied Biosystems, Foster City, CA).

Statistical analysis

We compared genetic diversity parameters from the seed bank cohorts with results for above ground cohorts from Gaudeul et al. (2007). Genetic diversity parameters, i.e. observed heterozygosity (H_O), expected heterozygosity (H_E; Nei 1987) and the inbreeding coefficient (F_{IS}) were calculated for each cohort in all populations using *Arlequin 3.11* (Excoffier et al. 2005).

Significant deviations from Hardy-Weinberg expectations were tested following the procedure described in Guo and Thomson (1992) performing 160,000 random permutations. Proportion of polymorphic loci (PPL) was calculated by dividing the number of polymorphic loci by the total number of loci for each population. For each population, allelic richness, the number of alleles in a sample corrected for sample size with rarefaction, was calculated based on a refraction method implemented in *FSTAT* 2.9.3.2 (Goudet 2002). This measure considers the number of alleles independent of sample size, thus allowing comparison of this quantity between samples of different sizes. The percentage of loci in significant linkage disequilibrium in relation to the maximum possible number of loci in linkage disequilibrium, P_D, (Stenøien and Såstad 1999) was calculated for each population. Differences in genetic composition between seed bank and above ground cohorts were compared using allelic richness, H_E, H_O, F_{IS}, PPL, P_D and N_e (see below). All comparisons between cohorts were made using Wilcoxon signed-rank test. The correlation between effective population size and within-population genetic variation for the above ground cohort and seed bank cohort was investigated using Spearman's ρ. We tested for a difference in allele frequencies between the cohorts using a two-tailed t-test. Finally, we tested for a possible difference between *A. lyrata* and *A. thaliana* for differentiation between cohorts. This was tested for by comparing pairwise F_{ST} values (t-test, unequal variances), using *A. thaliana* data from Lundemo et al. (2009).

Population genetic structure was investigated using hierarchical analysis of molecular variance, AMOVA (Weir and Cockerham 1984; Excoffier et al. 1992), to partition total genetic variance into variance among cohorts (between seed bank and above ground), among populations within cohorts, among individuals within populations, and within individuals. AMOVA was also performed to partition total genetic variation into its components for each cohort separately. In addition, the global F_{ST} was calculated among populations by including both seed bank and above ground cohorts. Pairwise F_{ST} between all pairs of populations was estimated using the methods of Weir and Cockerham (1984). Pairwise population differentiation was performed using 10,000 permutations as implemented in *FSTAT*, and the exact significance level was estimated after Bonferroni correction (Goudet 2002). Proportion of significant pairwise F_{ST} was calculated by dividing the number of significant pairwise F_{ST} by total number of pairwise F_{ST} comparisions for each cohort. We tested whether the genetic differentiation (pairwise F_{ST}) among populations

within the seed bank cohort was correlated with that found among populations within the above ground cohort using non-parametric correlation analysis (Spearman's ρ). To investigate whether including seed banks reduced differentiation among populations, we tested for a differentiation between above ground data and combined data (both above ground and seed bank together) using a paired t-test. All comparison and correlation analyses were performed in *SPSS* ver. 16 (SPSS Inc., Chicago, IL).

Genetic structure was also inferred with Bayesian clustering using *Structure* (Pritchard et al. 2000; Falush et al. 2003; 2007) and *BAPS* (Corander et al. 2003; 2004; Corander and Marttinen 2006). In *Structure*, we used the admixture model, in which each individual has inherited some fraction of its genome from K ancestral populations, and we examined models with both independent and correlated allele frequencies. *Structure* runs used 200,000 Markov chain Monte Carlo (MCMC) iterations after a burn-in length of 100,000 iterations for inference of each of K populations. Fifteen runs were considered for each K value, where K is the number of genetic clusters to be inferred, for K ranging from 1 to 15. The most probable number of clusters was inferred by calculating an *ad hoc* statistic ΔK based on rate of change in the log probability of data between successive K clusters (Evanno et al. 2005). We used the *Distruct* program (Rosenberg 2004) to display graphically the results produced by *Structure*. In *BAPS,* posterior probabilities are created for the desired range of possible number of source populations in the form of likelihood estimates. The best optimal partition is found for the highest log marginal likelihood (Corander and Marttinen 2006; Corander et al. 2008). A neighbor-joining tree was constructed based on a Nei divergence matrix obtained by *BAPS*.

To infer contemporary and historical N_e we estimated effective population size, N_e, by linkage disequilibrium (Hill 1981) and maximum likelihood (Beerli and Felsenstein 2001) approaches, respectively. Estimates were obtained separately for the seed bank cohort (S), the above ground cohort (A), and for the combined seed bank and above ground cohorts (T). N_e based on linkage disequilibrium was estimated as in Bartley et al. (1992) with a method implemented in *NeEstimator* (Peel et al. 2004). Most approaches to estimate contemporary N_e are based on certain assumptions, which exclude the effect of migration, mutation and selection in changing the allelic frequency of populations. The maximum likelihood approach is based on coalescent

theory and implemented in *Migrate 3.0* (Beerli and Felsenstein 1999; 2001). We used a Brownian motion approximation to the ladder model, similar to the stepwise mutation model, SMM, which is appropriate for microsatellites. Although *Migrate* assumes a constant mutation rate for all loci or sites to estimate θ, the mutation rate is incorporated in the formula to calculate N_e. The program uses an MCMC approach based on an expansion of the coalescence model (Beerli 2004) to estimate θ, which then can be used to calculate effective population size from the relationship between θ, N_e and the mutation rate, μ: θ=4 $N_e\mu$. Each MCMC run consisted of 10 short chains (sampling 10,000 trees) and three long chains (sampling 100,000 trees), with a burn-in period of 10,000 trees. Because the result of each run varies with respect to different chain lengths, Beerli and Felsenstein (2001) suggested that summarizing over different runs or different chains would improve the results. Thus, in each analysis all runs were repeated five times to verify consistency of results and an average of the five runs over all loci was calculated in *Migrate*. In addition, we replicated each analysis three times and calculated the mean. Finding the appropriate microsatellite mutation rate can be a challenge. In plants, rates of 1×10^{-2} and 3.9×10^{-3} in chickpeas (Udupa and Baum 2001), 2.4×10^{-4} in durum wheat (Thuillet et al. 2002), and 7.7×10^{-4} in maize (Vigouroux et al. 2002) have been reported. Thus, it is obvious that any estimate of N_e is prone to a very large variation (spanning more than two orders of magnitude) depending on which mutation rate is used. We used a mutation rate of 4×10^{-4} that was estimated for microsatellite markers in transgenic lines of *A. thaliana* (Depeiges et al. 2004) to calculate N_e in our populations. The same estimate was also used in Lundemo et al. (2009) for similar calculations in *A. thaliana* populations. A relative ratio measure can be used to represent the contribution of seed bank and above ground cohorts to total N_e using the N_e(T)/N_e(A) and N_e(T)/N_e(S) ratio, respectively (Lundemo et al. 2009). The N_e/N ratio, where N is the census size of population reported by Gaudeul et al. (2007), was calculated based on both N_e estimation methods.

Results

Genetic diversity

Of 15 microsatellite markers, *ICE12* was monomorphic in all populations and cohorts and therefore excluded from further analyses. *F19K23* was polymorphic in two of the seed bank

populations, while *ICE5* and *nga162* were polymorphic in one of the above ground populations. The marker *AthGAPAb* amplified two microsatellite loci differing in size range, hence the results from this marker are represented as two different loci; *AthGAPAb* and *AthGAPAb´* (Table 2). In population N9, the same identical multilocus genotype was found in two samples from the seed bank, and one of the samples was excluded from further analysis. In total 62 alleles were detected in the polymorphic loci. Of these, 14 (23%) and 6 (10%) were private alleles in the above ground and seed bank cohorts, respectively. The average (±SE) frequency of private alleles was 0.006±0.001 in above ground cohorts and 0.011±0.006 in seed bank cohorts. The average allele frequency did not differ between the seed bank and the above ground cohorts (two-tailed t-test; P=0.99; Table 2).

Within-population genetic diversity quantified as H_E, H_O, F_{IS} and PPL did not differ between seed bank and aboveground cohorts (Wilcoxon signed-rank test, P>0.05), whereas P_D was significantly different (P=0.023; Table 3). Average gene diversity (±SE) in the seed bank populations (0.297±0.017) did not differ from that in the above ground populations (0.296±0.013; P=0.75). Average observed heterozygosity of seed bank populations was also very similar to that of above ground populations (0.298±0.019 vs. 0.298±0.011). The average inbreeding coefficient (F_{IS}) was -0.01 and 0 for seed bank and above ground cohorts, respectively, and F_{IS} was not significantly different from zero for any seed bank or above ground population (P<0.05). Average (±SE) allelic richness in seed bank and above ground populations was 1.82±0.05 and 1.84±0.05, respectively. The mean proportion of polymorphic loci, PPL, (±SE) was 0.70±0.03 and 0.71±0.02 for seed bank and above ground cohorts, respectively. Low levels of linkage disequilibrium, P_D, were observed in both the seed bank (0.041±0.010) and the above ground (0.076±0.012) cohorts.

Genetic structure

The global F_{ST} among all populations based on both seed bank and above ground cohorts was 0.29 (AMOVA; P<0.01). The pattern of partitioning of total genetic variation into among populations and within populations (29% and 71%, respectively) was similar for both seed bank and above ground cohorts. Average F_{ST} (±SE) among above ground cohorts was similar to among seed bank cohorts (0.28±0.01), but slightly higher than and significantly different from F_{ST}

among combined cohorts (seed bank and above ground together; 0.25±0.01, paired t-test; P<0.001). Pairwise F_{ST} among populations estimated based on seed bank and above ground cohorts were positively correlated (Spearman's ρ=0.70, P<0.001). Mean F_{ST} between cohorts in *A. lyrata* was 0.084 (range 0.019-0.254). Pairwise F_{ST} indicated significant differentiation between above ground and seed bank cohorts in three populations only (N7, N10 and N14; P<0.01). In these three populations, estimates of pairwise F_{ST} ranged from 0.095 to 0.254. We observed a higher proportion of significant pairwise F_{ST} comparisons in above ground (0.97) than in seed bank (0.67) cohorts. The frequency distribution of pairwise F_{ST} among populations within seed bank and above ground cohorts showed a similar pattern (Figure S1).

Bayesian inference of genetic structure in *BAPS* and *Structure* revealed similar patterns of structuring among populations, and consistent with pairwise F_{ST} results. In *BAPS*, an admixture analysis based on individual (n=406) and population level (n=24) clustered samples into 19 and 13 groups, respectively. *BAPS* clustered all seed bank and above ground cohorts belonging to each population together except for population N7 and N10, in which seed bank and above ground separated to different clusters (Figure 1). In *Structure*, posterior probabilities of the data increased with increasing subdivision or population stratification; the lowest ln likelihood of data occurred in K=1 and the values increased with increasing number of Ks (Figure 2a). The true number of clusters determined by plotting ΔK across different Ks showed an abrupt change after K=5 (Figure 2b). Thus, based on mean values of probability of data and ΔK, we chose K=5 as an optimal number of cluster. The highest posterior probability across different runs for K=5 was used to illustrate the origin of individuals in the various populations (Figures 3 and S2).

Effective population size

The estimated effective population size using a maximum likelihood approach revealed a clear pattern among cohorts (N_e(T)>N_e(A)>N_e(S); Table 4 and Figure 4). N_e(T) was significantly different from N_e(A) and N_e(S) based on a Wilcoxon signed-rank test (P<0.01, two-tailed). However, N_e(S) and N_e(A) were not significantly different from each other (P>0.01, two-tailed). The arithmetic average (±SE) of historical N_e estimated from θ, assuming a mutation rate of 4×10^{-4}, was 332±23, 233±18 and 202±17 in total (combined seed bank and above ground cohorts), above ground and seed bank cohorts, respectively. Although contemporary N_e estimates

based on LD were considerably lower than historical N_e, a similar size ratio between cohorts was observed with both approaches (Table 4). The average estimated N_e (±SE) based on the LD method was 40.7±8.3, 18.9±3.7 and 13.1±2.9 in total, above ground, and seed bank cohorts, respectively. The difference between total cohorts and seed bank cohorts was marginally significant (two tailed, P=0.035). However, no other comparisons between cohorts were significantly different (P>0.05). The N_e(T)/N_e(A) ratio was lower than N_e(T)/N_e(S) ratio calculated for both the historical and contemporary approach (Table 4). The average (±SE) of N_e(T)/N_e(A) and N_e(T)/N_e(S) ratio based on maximum likelihood method was 1.45±0.08 and 1.70±0.08, respectively. Based on the LD method the N_e(T)/N_e(A) and N_e(T)/N_e(S) ratio was 2.00±0.37 and 3.17±0.60, respectively. The average N_e(T)/N ratio (±SE) calculated from the maximum likelihood method (1.91±0.59) was considerably higher than the ratio (0.20±0.07) calculated from the LD method (Table 4). The ML based N_e was positively correlated with allelic richness in the seed bank (Spearman's ρ=0.64, P=0.026) and in the above ground cohorts (Spearman's ρ=0.85, P<0.001). *N* was not significantly correlated with N_e in any of the two cohorts (P>0.01).

Discussion

This study has demonstrated that the presence of a seed bank increases the total effective population size (N_e) in natural populations of the self-incompatible, perennial herb *Arabidopsis lyrata*. Seed banks may thus influence population dynamics and microevolution in this species by reducing the loss of genetic variation caused by stochastic processes. However, the genetic effect of the seed bank on total N_e was low in *A. lyrata* compared to the closely related selfing annual *A. thaliana*.

Genetic composition of cohorts

Genetic diversity levels did not differ between above ground and seed bank cohorts, suggesting that most of the genetic variability maintained in the soil seed bank is also present in the above ground population of *A. lyrata*. Our results are thus in accordance with other studies reporting no significant genetic differences between seed bank and above ground populations (e.g., Mahy et al. 1999 and Mandák et al. 2006). This implies that there is a gradual recruitment from the seed

bank to the above ground cohort and vice versa, which homogenizes the genetic composition of the two cohorts. Consequently, allelic richness in above ground plants was also similar to that in the seed bank, although there were some private alleles in both cohorts. Private alleles were mostly rare, with an allele frequency lower than 4% in individual cohorts. Because private alleles in the seed bank were present only at low frequencies, it is possible that they originated from rare above ground genotypes that were not detected by the sampling regime of the adult population employed by Gaudeul et al. (2007). It is also possible that they originated from genotypes no longer represented in the above ground population (Uesugi et al. 2007). Finally, private alleles could be the product of mutations in the seed bank, in which case the seed bank would serve as a source of novel genetic variation (see also Levin 1990). In a review, Honnay et al. (2008) found that high levels of genetic diversity accumulated, and more alleles were present, in the soil seed bank than in the above ground population, but that alleles exclusive for the seed bank were mainly rare alleles. They therefore suggested that selection acts either directly or indirectly as a filter on the alleles present in the seed bank, and, as a consequence, some alleles are not found in seedlings (see also Mandák et al. 2006). This can be explained by a trend of declining homozygosity through a selection process favoring heterozygotes and/or removing inbred individuals during life stage transitions from seed bank to adults, as observed in *Cecropia obtusifolia* (Alvarez-Buylla et al. 1996) and *Plantago lanceolata* (Tonsor et al. 1993).

The average seedling density in soil samples of *A. lyrata*, 7.9 seedlings/dm^3, was low compared to a similar study in *A. thaliana* (94.2 seedlings/dm^3; Lundemo et al. 2009). Several factors may have contributed to this difference. First, the reproductive output of annual species is higher than perennials (Primack 1979; Spira and Pollak 1986), therefore seed density in natural populations of *A. lyrata* are *a priori* expected to be lower than in populations of *A. thaliana*. Second, many *A. lyrata* populations are located close to running water, suggesting that seeds may be washed away before entering the seed bank.

Despite similar partitioning of genetic variation, the proportion of significant pairwise F_{ST} among populations was lower in the seed bank than in the above ground cohort. We also found a low, but significant difference in F_{ST} among combined cohorts compared to only above ground data, suggesting that the seed bank reduces differentiation among populations. This is in accordance

with Vitalis et al. (2004), who proposed that the differentiation among populations would decrease with the presence of seed banks. McCue and Holstford (1998) observed lower differentiation among seed bank cohorts than among above ground cohorts in *Clarkia springvillensis*, and suggested that seed banks may have the effect of slowing differentiation of populations by retaining alleles preserved from different generations. Similar patterns of clustering were indicated by Bayesian methods (implemented in *Structure* and *BAPS*) and F_{ST} for seed bank and above ground cohorts in our study. The mean F_{ST} between cohorts in *A. lyrata* (0.084, range 0.019-0.254) was somehow lower than previously found in *A. thaliana*, (0.12, range 0-0.395), but this difference was not significant (P>0.05). However, this suggests that, compared to *A. thaliana* (Lundemo et al. 2009), differential recruitment from seed banks are less likely to cause rapid fluctuation of allele frequencies in local populations of *A. lyrata*.

Effective population size and seed bank

Maximum likelihood (ML) based on coalescent theory and the linkage disequilibrium (LD) approach revealed an increase in total effective population size in *A. lyrata* populations, i.e. when the genetic diversity of the seed bank and the above ground cohorts were considered together. Although the historical N_e, based on the ML method, was considerably higher than contemporary N_e, based on the LD method, both methods indicated a similar relative size of N_e in the two cohorts. The two approaches to estimate N_e are based on different models and assumptions, which can explain the difference in estimates. The coalescent method uses genealogical relationships among alleles in the populations (Fu and Li 1999), whereas LD is based on allele frequencies within populations. The crucial assumption in the LD method is that populations are completely isolated without migration among populations, whereas for the ML method, which is based on θ, this assumption is relaxed.

According to theoretical expectations, seed banks should reduce the influence of stochastic processes because they increase the effective population size (Templeton and Levin 1979; Turelli 2001; Nunney 2002; Vitalis et al. 2004). However, the importance of seed banks may differ between plants with different life histories. In the present study, both the ML and LD method indicated that the contribution of the seed bank to total N_e was lower than that of the above ground population. The estimate of N_e for the seed bank cohort was lower than that for above

ground plants in about a third of the study populations, which may be due to the higher proportion of private alleles in the above ground cohorts. In contrast, estimates of N_e based on seed bank cohorts were larger than N_e estimates based on above ground plants in more than half of the *A. thaliana* populations examined by Lundemo et al. (2009). This may reflect larger year-to-year variation in population size and in recruitment from the seed bank in populations of annuals compared to perennials (Lundemo et al. 2009), and suggests that the seed bank may contribute less to total N_e in perennial compared to annual species.

The N_e/N ratio in an idealized Fisher-Wright population is equivalent to unity (Kimura and Crow 1963). However, several characteristics of natural populations can cause deviations from this ratio, and low N_e/N ratios are commonly associated with the potential loss of genetic variation (Gomez-Uchida et al. 2006). It has also been suggested that variation in reproductive success in natural populations has a large effect on the N_e/N ratio, so that a high variance in reproductive success will be accompanied by a low N_e/N ratio (Nunney 1993; Hedrick and Goodnight 2005). In this study, we obtained similar N_e/N ratios based on LD (mean=0.20) compared to contemporary estimates obtained for other plant species (0.11, Husband and Barrett 1992; 0.34, Campbell and Husband 2005; for a review, see Frankham 1995). These studies are, however, based on demographic measures and heterozygote excess, but despite the application of different methods for the estimation of contemporary N_e, our results fall within the range of what has been shown previously. However, in our study the N_e/N ratio based on contemporary N_e was of one order of magnitude less than the ratio based on historical N_e. Hence, both approaches should be applied as this can provide important information on population dynamics, in particular for conservation and management purposes.

Conclusions

We have shown that seed banks in Norwegian populations of *A. lyrata* maintain the same amount of genetic variation as the above ground cohort, although in a few populations there was a low level of genetic differentiation between the cohorts. The seed bank contributed to the total effective population size, but this contribution was lower than that of the above ground cohort. The slightly higher proportion of private alleles in the above ground cohort may explain this difference. Still, the results suggest that the seed bank is an indispensable part of the population

dynamics in *A. lyrata* and has the potential to reduce the loss of genetic variation through dampening the effect of stochastic genetic drift. Future studies should examine the importance of seed banks for population dynamics in *A. lyrata*, as well as whether the relationship between the seed bank and total effective population size documented in the present study is typical for other perennial plant species.

Acknowledgements

This work was supported financially by grants from the Norwegian Research council (NFR grant No. 10298100) to HKS, and from the Swedish Research Council (VR 621-2004-3314) to HKS and JÅ. We thank Marianne Doré Hansen for help with genotyping and Per Toräng for comments on the manuscript.

References

Aguilar R, Quesada M, Ashworth L, Herrerias-Diego Y, Lobo J (2008) Genetic consequences of habitat fragmentation in plant populations: susceptible signals in plant traits and methodological approaches. Molecular Ecology, 17, 5177-5188.

Alvarez-Buylla ER, Chaos Á, Piñero D, Garay AA (1996) Demographic genetics of a pioneer tropical tree species: patch dynamics, seed dispersal and seed banks. Evolution, 50, 1155-1166.

Amiaud B, Touzard B (2004) The relationships between soil seed bank, aboveground vegetation and disturbances in old embanked marshlands of Western France. Flora, 199, 25-35.

Baker HG (1989) Some aspects of the natural history of seed banks. In: *Ecology of soil seed banks* (eds. Leck MA, Parker VT, Simpson RL), pp. 9–21. Academic Press Inc., San Diego, CA.

Bartley D, Bagley M, Gall G, Bentley B (1992) Use of linkage disequilibrium data to estimate effective size of hatchery and natural fish populations. Conservation Biology, 6, 365-375.

Beerli P (2004) Migrate: documentation and program, part of LAMARC. Version 2.0. Revised December 23, Distributed over the Internet, 1997-2004.

Beerli P, Felsenstein J (1999) Maximum likelihood estimation of migration rates and effective population numbers of two populations using a coalescent approach. Genetics, 152, 763–773.

Beerli P, Felsenstein J (2001) Maximum likelihood estimation of a migration matrix and effective population sizes in *n* subpopulations by using a coalescent approach. Proceedings of the National Academy of Sciences USA, 98, 4563–4568.

Caballero I, Olano JM, Loidi J, Escudero A (2008) A model for small-scale seed bank and standing vegetation connection along time. Oikos, 117, 1788-1795.

Campbell LG, Husband BC (2005) Impact of clonal growth on effective population size in *Hymenoxys herbacea* (Asteraceae). Heredity, 94, 526–532.

Clauss MJ, Cobban H, Mitchell-Olds T (2002) Cross-species microsatellite markers for elucidating population genetic structure in *Arabidopsis* and *Arabis* (Brassicaeae). Molecular Ecology, 11, 591-601.

Corander J, Marttinen P (2006) Bayesian identification of admixture events using multi-locus molecular markers. Molecular Ecology, 15, 2833-2843.

Corander J, Waldmann P, Marttinen P, Sillanpää MJ (2004) BAPS 2: enhanced possibilities for the analysis of genetic population structure. Bioinformatics, 20, 2363-2369.

Corander J, Waldmann P, Sillanpää MJ (2003) Bayesian analysis of genetic differentiation between populations. Genetics, 163, 367-374.

Corander JP, Marttinen P, Sirén J, Tang J (2008) Enhanced Bayesian modelling in BAPS software for learning genetic structures of populations. BMC Bioinformatics, 9, 539.

Crow JF, Kimura M (1970) *An introduction to population genetic theory*. Harper & Row, NY.

Del Castillo RF (1994) Factors influencing the genetic structure of *Phacelia dubia*, a species with a seed bank and large fluctuations in population size. Heredity, 72, 446-458.

Depeiges A, Farget S, Degroote F, Picard G (2004) A new transgene assay to study microsatellite instability in wild-type and mismatch-repair defective plant progenies. Plant Science, 168, 939-947.

Ellstrand NC, Elam DR (1993) Population genetic consequences of small population size: implications for plant conservation. Annual Review of Ecology and Systematics, 24, 217-242.

Epling C, Lewis H, Ball FM (1960) The Breeding Group and Seed Storage: A Study in Population Dynamics. Evolution 14, 238-255.

Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Molecular Ecology, 14, 2611–2620.

Excoffier L, Laval G, Schneider S (2005) Arlequin ver. 3.0: An integrated software package for population genetics data analysis. Evolutionary Bioinformatics Online, 1, 47-50.

Excoffier L, Smouse P, Quattro J (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: Application to human mitochondrial DNA restriction data. Genetics, 131, 479-491.

Falush D, Stephens M, Pritchard JK (2003) Inference of population structure using multilocus genotype data: Linked loci and correlated allele frequencies. Genetics, 164, 1567-1587.

Falush D, Stephens M, Pritchard JK (2007) Inference of population structure using multilocus genotype data: dominant markers and null alleles. Molecular Ecology Notes, 7, 574–578.

Fisher RA (1930) *The genetical theory of natural selection*. Clarendon Press, Oxford, UK.

Frankham R (1995) Effective population size/adult population size ratios in wildlife: a review. Genetical Research, 66, 95-107.

Frankham R (1996) Relationship of genetic variation to population size in wildlife. Conservation Biology, 10, 1500–1508.

Fu YX, Li WH (1999) Coalescing into the 21st century: An overview and prospects of coalescent theory. Theoretical Population Biology, 56, 1-10.

García MB, Picó FX, Ehrlén J (2008) Life span correlates with population dynamics in perennial herbaceous plants. American Journal of Botany, 95, 258-262.

Garwood NC (1989) Seed banks: General concepts and methodological issues. In: *Ecology of soil seed banks* (eds. Leck MA, Parker VT, Simpson RL), pp. 149–210. Academic Press Inc., San Diego, CA.

Gaudeul M, Stenøien HK, Ågren J (2007) Landscape structure, clonal propagation, and genetic diversity in Scandinavian populations of *Arabidopsis lyrata* (Brassicaceae). American Journal of Botany, 94, 1146–1155.

Gomez-Uchida D, Banks MA (2006) Estimation of effective population size for the long-lived darkblotched rockfish *Sebastes crameri*. Journal of Heredity, 97, 603-606.

Goodson JM, Gurnell AM, Angold PG, Morrissey IP (2001) Riparian seed banks: structure, process and implications for riparian management. Progress in Physical Geography, 25, 301-325.

Goudet J (2002) FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9.3). Available from http://www.unil.ch/izea/softwares/fstat.html. Updated from Goudet (1995).

Guo S, Thompson E (1992) Performing the exact test for Hardy-Weinberg proportion for multiple alleles. Biometrics, 48, 361-372.

Hedrick P, Goodnight C (2005) Large variance in reproductive success and the Ne/N ratio. Evolution, 59, 1596-1599.

Hill WG (1981) Estimation of effective population size from data on linkage disequilibrium. Genetical Research*,* 38, 209–216.

Hopfensperger KN (2007) A review of similarity between seed bank and standing vegetation across ecosystems. Oikos, 116, 1438-1448.

Honnay O, Bossuyt B, Jacquemyn H, Shimono A, Uchiyama K (2008) Can a seed bank maintain the genetic variation in the aboveground population? Oikos, 117, 1-5.

Husband BS, Barrett SCH (1992) Effective population size and genetic drift in tristylous *Eichhornia paniculata* (Pontederiaceae). Evolution, 46, 1875-1890.

Husband BC, Barrett SCH (1998) Spatial and temporal variation in population size of *Eichhornia paniculata* in ephemeral habitats: implications for metapopulation dynamics. Journal of Ecology, 86, 1021-1031.

Kimura M and Crow JF (1963) The measurement of effective population number. Evolution, 17, 279-288.

Leberg P (2005) Genetic approaches for estimating the effective size of populations. Journal of Wildlife Management, 69, 1385-1399.

Levin DA (1990) The seed bank as a source of genetic novelty in plants. The American Naturalist, 135, 563–572.

Lundemo S, Falahati-Anbaran M, Stenøien HK (2009) Seed banks cause elevated generation times and effective population sizes of *Arabidopsis thaliana* in Northern Europe. Molecular Ecology, 18, 2798-2811.

MacArthur RH, Wilson EO (1967) *The Theory of Island Biogeography*. Princeton University Press, Princeton, NJ.

Mahy G, Vekemans X, Jacquemart AL (1999) Patterns of allozymic variation within *Calluna vulgaris* populations at seed bank and adult stages. Heredity, 82, 432-40.

Mandák B, Bímová K, Mahelka V, Plačková I (2006) How much genetic variation is stored in the seed bank? A study of *Atriplex tatarica* (Chenopodiaceae). Molecular Ecology, 15, 2653-2663.

McCue KA, Holstford TP (1998) Seed bank influences on genetic diversity in the rare annual *Clarkia springvillensis* (Onagraceae). American Journal of Botany, 85, 30-36.

Nei M (1987) *Molecular Evolutionary Genetics*. Columbia University Press, NY.

Nunney L (1993) The influence of mating system and overlapping generations on effective population Size. Evolution, 47, 1329-1341.

Nunney L (2002) The effective size of annual plant populations: The interaction of a seed bank with fluctuating population size in maintaining genetic variation. The American Naturalist, 160, 195-204.

Nunney L, Elam DR (1994) Estimating the effective population size of conserved populations. Conservation Biology, 8, 175-184.

Peel D, Ovenden JR, Peel SL (2004) NeEstimator: software for estimating effective population size, Version 1.3. Queensland Government, Department of Primary Industries and Fisheries.

Pudovkin AI, Zaykin DV, Hedgecock D (1996) On the potential for estimating the effective number of breeders from heterozygote-excess in progeny. Genetics, 144, 383-387.

Primack RB (1979) Reproductive effort in annual and perennial species of *Plantago* (Plantaginaceae). The American Naturalist, 114, 51-62.

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. Genetics, 155, 945-959.

Rosenberg NA (2004) DISTRUCT: A program for the graphical display of population structure. Molecular Ecology Notes, 4, 137–138.

Schwartz MK, Tallmon DA, Luikart G (1998). Review of DNA-based census and effective population size estimators. Animal Conservation, 1, 293–299.

Simpson RL, Leck MA, Parker VT (1989) Seed banks; General concepts and methodological issues. In: *Ecology of seed banks* (eds. Leck MA, Parker VT, Simpson RL), pp. 3-8. Academic Press Inc., San Diego, CA.

Spira TP, Pollak OD (1986) Comparative reproductive biology of alpine biennial and perennial gentians (*Gentiana*: Gentianaceae) in California. American Journal of Botany, 73, 39-47.

Stenøien HK, Såstad SM (1999) Genetic structure in three haploid peatmosses (*Sphagnum*). Heredity, 82, 391-400.

Templeton AR, Levin DA (1979) Evolutionary consequences of seed pools. The American Naturalist, 114, 232-249.

Thuillet AC, Bru D, David J, et al. (2002) Direct estimation of mutation rate for ten microsatellite loci in durum wheat, *Triticum turgidum* (L.) Thell. ssp. *durum* desf. Molecular Biology and Evolution, 19, 122-125.

Tonsor SJ, Kalisz S, Fisher J, Holtsford TP (1993) A life-history based study of population genetic structure: seed bank to adults in *Plantago lanceolata*. Evolution, 47, 833-843.

Turelli M, Schemske DW, Bierzychudek P (2001) Conditions for stable two-allele polymorphisms with seed banks and fluctuating selection: maintaining the blues in *Linanthus parryae*. Evolution, 55, 1283-1298.

Udupa SM, Baum M (2001) High mutation rate and mutational bias at (TAA) (n) microsatellite loci in chickpea (*Cicer arietinum* L). Molecular Genetics and Genomics, 265, 1097-1103.

Uesugi R, Nishihiro J, Tsumura Y, Washitani I (2007) Restoration of genetic diversity from soil seed banks in a threatened aquatic plant, *Nymphoides peltata*. Conservation Genetics, 8, 111–121.

Vigouroux YJS, Jaqueth Y, Matsuoka OS, et al. (2002) Rate and pattern of mutation at microsatellite loci in maize. Molecular Biology and Evolution, 19, 1251-60.

Villiers AJD, Rooyen MWV, Theron GK (2003) Similarity between the soil seed bank and the standing vegetation in the Strandveld Succulent Karoo, South Africa. Land Degradation & Development, 14, 527-540.

Vitalis R, Glémin S, Olivieri I (2004) When genes go to sleep: the population genetic consequences of seed dormancy and monocarpic perenniality. The American Naturalist, 163, 295-311.

Wang J (2005) Estimation of effective population sizes from data on genetic markers. Philosophical Transactions of the Royal Society B – Biological Sciences, 360, 1395-1409.

Waples RS (1989). A generalized approach for estimating effective population size from temporal changes in allele frequency. Genetics, 121, 379-391.

Weir BS, Cockerham CC (1984) Estimating F-statistics for the analysis of population structure. Evolution, 38, 1358-1370.

Williams LR (2000) Annual variation in the size of a population of *Cardamine impatiens* L. Watsonia, 23, 209-212.

Wright S (1931) Evolution in mendelian populations. Genetics, 16, 97-159.

Young A, Boyle T, Brown T (1996) The population genetic consequences of habitat fragmentation for plants. Trends in Ecology & Evolution, 11, 413-418.

Tables

Table 1: Summary of multiplex PCR and characteristics of the microsatellites in *Arabidopsis lyrata*. Numbers within parentheses represent the number of private alleles for each cohort in each locus. *AthGAPAb* amplified two microsatellite loci, which differed in size range. The above ground cohort had 14 private alleles, while the seed bank cohort had 6.

	Primer concentration (μM)	Dye	Number of alleles	
			Seed bank	Above ground
Multiplex 1				
AthGAPAb	0.45	NED	2	2
AthGAPAb′	-		3	3
F20D22	0.2	HEX	4	5(1)
ICE5	0.2	6-FAM	1	2(1)
nga151	0.3	6-FAM	2	2
nga162	0.3	6-FAM	1	2(1)
ICE12	0.2	HEX	1	1
Multiplex 2				
SLL2	0.25	HEX	2	2
nga280	0.2	NED	2	2
ATTS0392	0.2	NED	4	6(2)
ICE13	0.2	NED	6	10(4)
AthZFPG	0.5	6-FAM	11(4)	9(2)
ICE14	0.4	6-FAM	5(1)	6(1)
F19K23	0.3	HEX	2(1)	1
AthCDPK9	0.3	HEX	3	4(2)
Total			49(6)	57(14)

Table 2: Average frequency of 62 alleles observed at polymorphic microsatellite loci in the seed bank and above ground cohorts in 12 Norwegian population of *Arabidopsis lyrata.* The mean allele frequencies for seed bank and above ground cohorts were not significantly different (t-test, P=0.99). '0' indicate absence of a specific allele in a given cohort.

Locus	Allele size	Seed bank	Above ground	Locus	Allele size	Seed bank	Above ground
nga280	76	0.017	0.028	ICE14	223	0.041	0
	82	0.983	0.972		225	0.195	0.197
ATTSo392	140	0.131	0.173		228	0	0.007
	143	0.204	0.192		231	0	0.002
	149	0.403	0.399		234	0.624	0.729
	152	0.261	0.226		237	0.121	0.055
	155	0	0.002		240	0.019	0.009
	158	0	0.008	F19K23	185	0.012	0
ICE13	222	0.011	0.037		188	0.988	1
	231	0.001	0.002	AthCDPK9	88	0	0.01
	240	0.286	0.27		90	0.499	0.475
	246	0.438	0.415		94	0.156	0.129
	249	0.167	0.151		96	0.345	0.386
	252	0	0.007	AthGAPAb	123	0.605	0.538
	255	0.097	0.1		132	0.395	0.462
	258	0	0.002	AthGAPAb′	159	0.187	0.302
	261	0	0.011		161	0.589	0.534
	267	0	0.004		162	0.224	0.165
AthZFPG	137	0	0.005	F20D22	173	0.331	0.358
	141	0.002	0.007		176	0.563	0.454
	146	0.107	0.142		178	0.096	0.167
	148	0.053	0.039		180	0.01	0.002
	152	0.064	0.064		182	0	0.019
	154	0.678	0.674	ICE5	167	0	0.002
	156	0.064	0.057		171	1	0.998
	158	0.008	0	nga151	98	0.659	0.739
	162	0.018	0.008		100	0.341	0.261
	164	0.002	0	nga162	79	1	0.998
	166	0.002	0		81	0	0.002
	168	0	0.002	SLL2	300	0.587	0.563
	177	0.002	0		311	0.413	0.437

Table 3: Estimates of within-population diversity in the seed bank and above ground cohorts in natural populations of *Arabidopsis lyrata*. Number of samples genotyped (N), observed heterozygosity (H_O), gene diversity (expected heterozygosity, H_E), inbreeding coefficient (F_{IS}), allelic richness (R_S), proportion of polymorphic loci (PPL) and proportion of pairwise loci in linkage disequilibrium (P_D) in seed bank (S) and above ground (A) cohorts. Estimates were obtained with only one individual per genotype (genets) included in the analyses. No significant differences were found between seed bank and above ground cohorts by comparing mean values of the different parameters based on Wilcoxon signed-rank test (two-tailed; P>0.05).

Population	N		H_O		H_E		F_{IS}		R_S		PPL		P_D	
	S	A	S	A	S	A	S	A	S	A	S	A	S	A
N2	22	18	0.389	0.298	0.337	0.294	-0.18	-0.06	1.94	1.83	0.71	0.79	0.067	0.073
N3	7	16	0.418	0.336	0.385	0.316	-0.10	-0.07	1.99	1.90	0.79	0.71	0.018	0.033
N4	6	22	0.286	0.263	0.258	0.259	-0.12	-0.05	1.69	1.69	0.71	0.71	0.000	0.111
N5	7	19	0.255	0.278	0.293	0.304	0.14	0.09	1.90	1.82	0.57	0.64	0.036	0.069
N6	29	20	0.296	0.312	0.313	0.301	0.06	-0.05	1.82	1.82	0.71	0.64	0.033	0.028
N7	21	17	0.282	0.244	0.246	0.211	-0.15	-0.16	1.71	1.57	0.64	0.64	0.083	0.042
N8	4	20	0.214	0.230	0.204	0.264	-0.06	0.12	1.50	1.68	0.50	0.64	0.024	0.069
N9	24	19	0.199	0.302	0.227	0.321	0.12	0.04	1.72	1.99	0.71	0.79	0.078	0.082
N10	18	19	0.321	0.278	0.322	0.309	0.00	0.10	1.84	1.89	0.79	0.71	0.027	0.044
N13	33	17	0.319	0.369	0.321	0.395	0.00	0.04	1.92	2.20	0.79	0.79	0.100	0.164
N14	15	17	0.257	0.260	0.259	0.253	-0.02	-0.04	1.67	1.65	0.79	0.64	0.027	0.056
N16	5	16	0.343	0.298	0.390	0.324	0.14	0.03	2.12	2.03	0.64	0.79	0.000	0.136
Mean	15.92	18.33	0.298	0.289	0.296	0.296	-0.01	0.00	1.82	1.84	0.70	0.71	0.041	0.076
SE	2.91	0.527	0.019	0.011	0.017	0.013	0.032	0.025	0.049	0.052	0.03	0.02	0.010	0.012

Table 4: Estimates of historical and contemporary effective population sizes (N_e) of the seed bank (S) and above ground (A) cohorts of *Arabidopsis lyrata* populations, obtained with maximum likelihood (ML) and linkage disequilibrium (LD) approaches, respectively. Estimates of total population effective population size (T) were obtained by combining seed bank and above ground cohorts. Population census sizes (*N*) are from (Gaudeul et al. 2007).

Population	Census size (*N*)	ML based N_e						LD based N_e					
		S	A	T	T/*N*	T/A	T/S	S	A	T	T/*N*	T/A	T/S
N2	150	180	226	300	2.00	1.33	1.67	18.7	11.1	28.6	0.19	2.58	1.53
N3	50	238	233	377	7.53	1.62	1.59	-	10.2	39.3	0.79	-	-
N4	500	104	194	216	0.43	1.11	2.08	12.2	18.8	86	0.17	4.57	7.05
N5	350	274	205	308	0.88	1.50	1.12	8.3	26.8	37.4	0.11	1.4	4.51
N6	3000	250	244	361	0.12	1.48	1.44	33.7	47.2	80.9	0.03	1.71	2.4
N7	300	201	169	283	0.94	1.67	1.41	22.9	13.9	9.2	0.03	0.66	0.4
N8	500	108	162	220	0.44	1.36	2.05	3.2	8.3	16.7	0.03	2.01	5.22
N9	250	176	335	333	1.33	0.99	1.90	5.2	10.2	11.4	0.05	1.12	2.19
N10	350	238	255	467	1.33	1.83	1.97	-	19.3	74.6	0.21	-	-
N13	350	286	369	467	1.34	1.27	1.63	8.4	37.6	25.9	0.07	0.69	3.08
N14	90	164	171	318	3.53	1.85	1.94	5.7	3.7	12	0.13	3.24	2.11
N16	110	204	234	331	3.01	1.42	1.63	-	20	66.9	0.61	-	-
Mean	500	202	233	332	1.91	1.45	1.70	13.14	18.93	40.74	0.20	2.00	3.17
SE	231.35	17	18	23	0.59	0.08	0.08	2.91	3.69	8.32	0.07	0.43	0.69

Figures

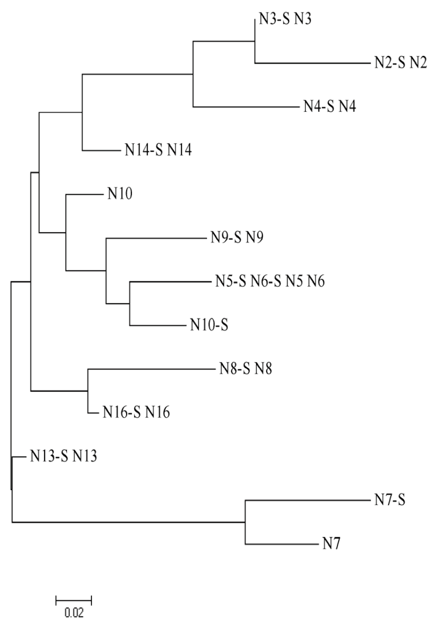


Figure 1: Neighbor-joining (NJ) clustering of seed bank and above ground cohorts of *Arabidopsis lyrata* populations. The NJ tree is based on a Nei divergence matrix generated by *BAPS*. The suffix following the population names (-S) indicates seed bank cohorts.

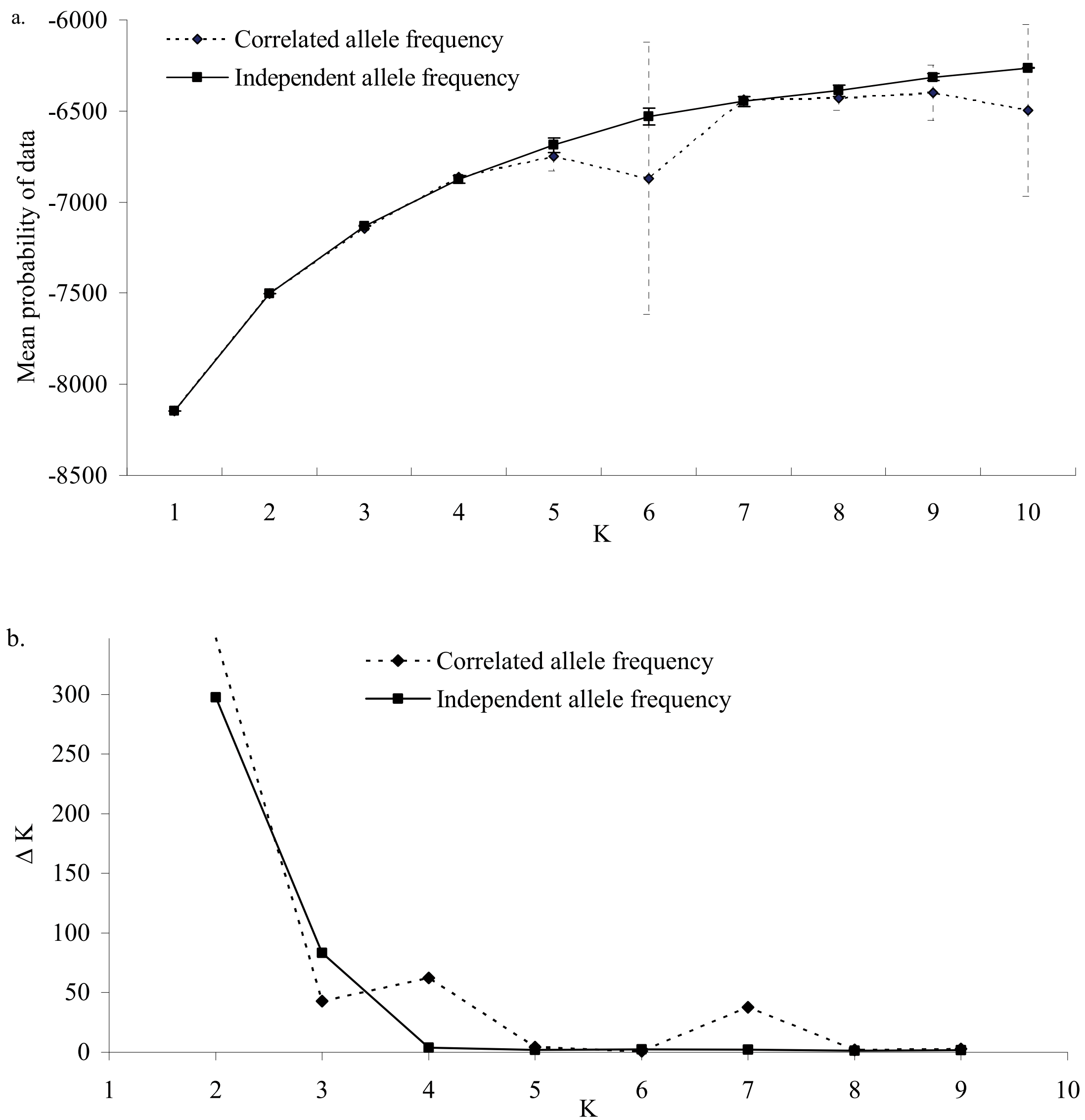


Figure 2: The true number of clusters inferred from *Arabidopsis lyrata* populations using *Structure*. The results presented here are based on an admixture model, and both independent and correlated allele frequency models: a) Mean of Ln probability of data in two different models; and b) ΔK calculated based on an *ad hoc* method indicating the true number of cluster (K=5). Dashed line and solid line represent correlated and independent allele frequency models, respectively.

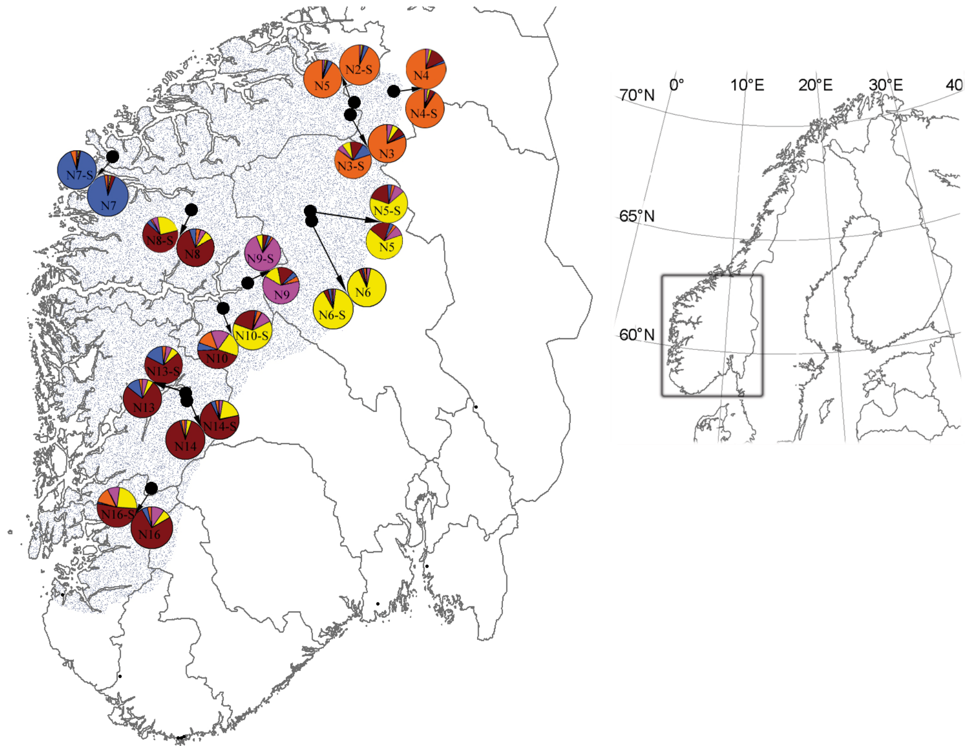


Figure 3: Distribution of genetic structure within seed bank and above ground cohorts of *Arabidopsis lyrata* populations as inferred by *Structure*. Each pie represents proportional membership of individuals within each inferred population. The suffix following the population names (-S) indicates seed bank cohorts.

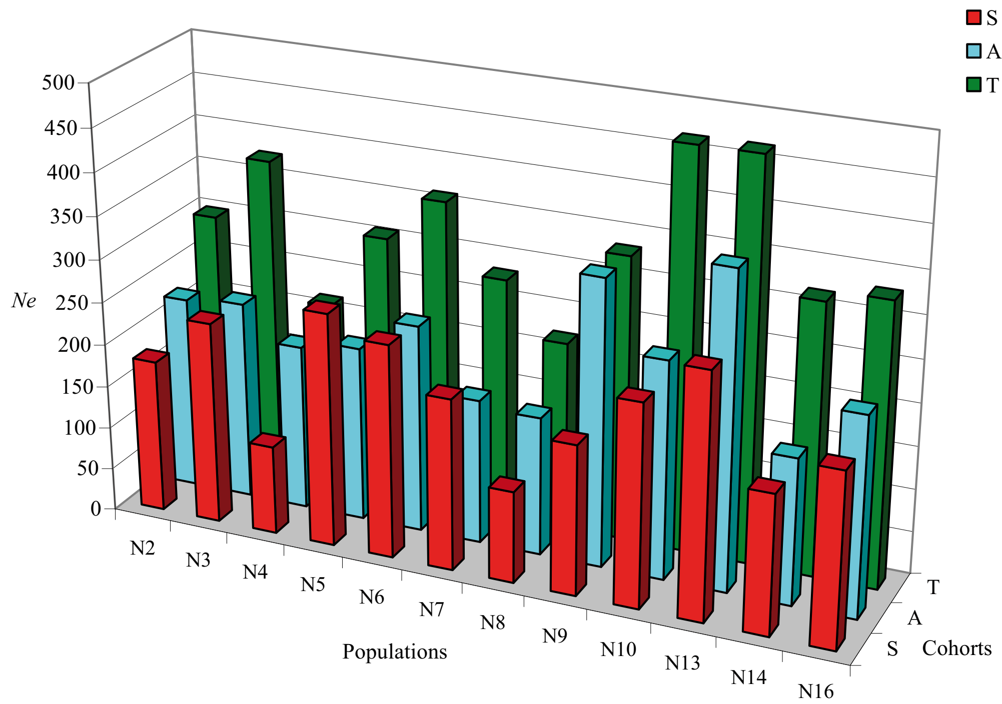


Figure 4: Maximum-likelihood based estimation of effective population size (*Ne*) in seed bank (S), above ground (A) and total population (combined seed bank and above ground, T) cohorts of *Arabidopsis lyrata* populations.

Supplementary material

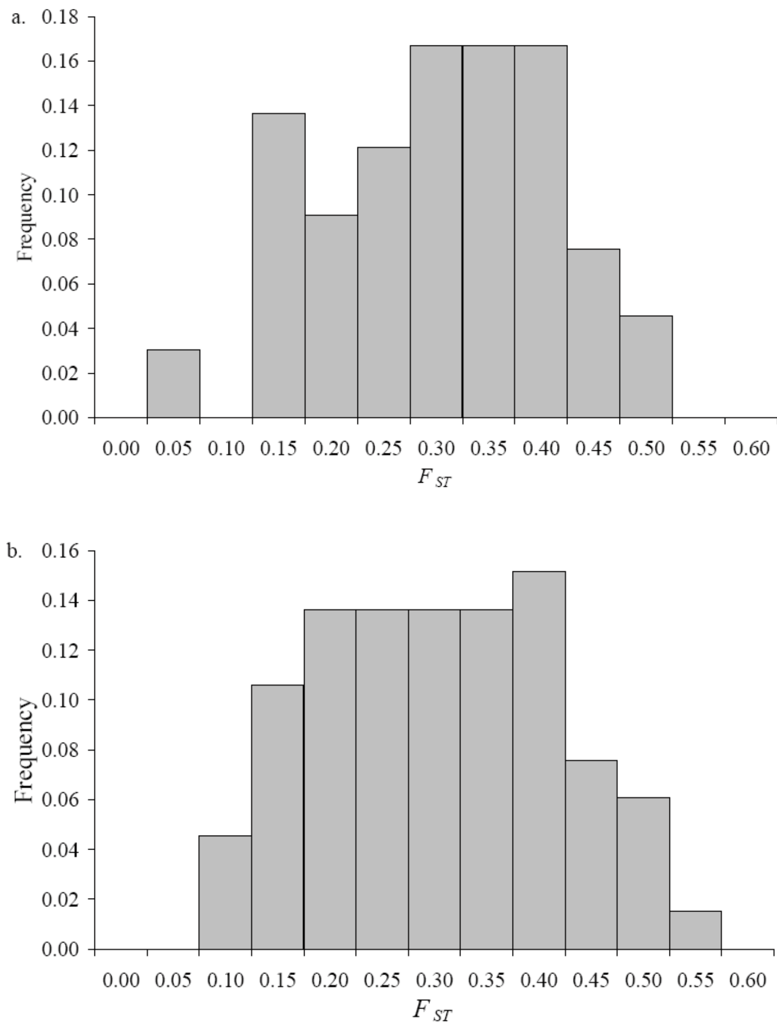


Figure S1: Frequency of F_{ST} distribution among *Arabidopsis lyrata* populations for: a) seed bank and b) above ground cohorts.

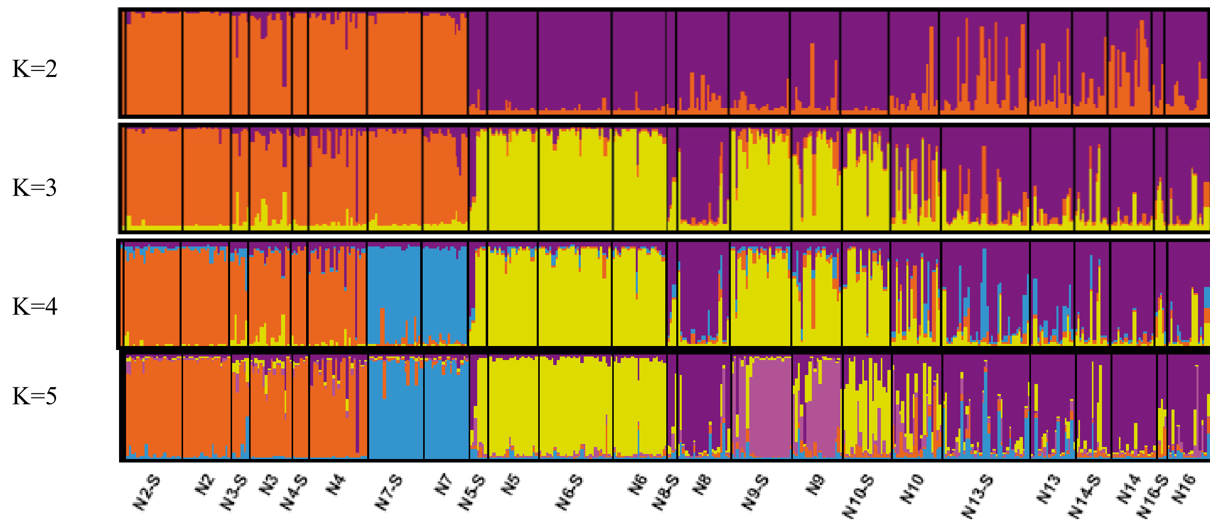


Figure S2: Population structure of seed bank (denoted by the suffix –S) and above ground cohorts in 12 populations of *Arabidopsis lyrata* estimated by the program *Structure* and graphically displayed using Distruct. Individuals are represented by thin vertical lines partitioned into segments corresponding to their membership fractions in K clusters as indicated by the colors. Black lines separate individuals of different cohorts.

Paper III

Investigating the effects of topography and clonality on genetic structuring within a large Norwegian population of *Arabidopsis lyrata*

Sverre Lundemo[1]*, Hans K. Stenøien[2], Outi Savolainen[3]

[1]Department of Biology, Norwegian University of Science and Technology, N-7491 Trondheim

[2]Systematics and Evolution Group, Section of Natural History, Museum of Natural History and Archaeology, Norwegian University of Science and Technology, N-7491 Trondheim

[3]Dept. of Biology and Biocenter Oulu, University of Oulu, P.O. Box 3000, FIN-90014 Oulu, Finland

Abstract

The gene flow through pollen and/or seeds governs the extent of spatial genetic structure in plant populations. Another factor that can contribute to this pattern is clonal growth. The perennial species *Arabidopsis lyrata* ssp. *petraea* (Brassicaceae) is a self-incompatible, clonal species found in disjunctive populations in Central and Northern Europe. We employed 14 microsatellite markers to study the level of kinship and clonality in a high altitude mountain valley at Spiterstulen, Norway. The population has a continuous distribution along the banks of the river Visa for about 1.5 km. A total of 17 (10 m×10 m) squares were laid out in a north-south transect following the river on both sides. We show that clonal growth is far more common than previously shown in this species, although the overall size of the genets is small (mean diameter=6.4 cm). Across the whole population there is no indication of isolation by distance, and spatial genetic structure is only visible on fine spatial scales. In addition, we find no effect of the river on the spatial distribution of genotypes. Unexpectedly, our data thus show that populations of small perennials like *A. lyrata* can behave like panmictic units across relatively large areas at local sites, as opposed to earlier findings in Central Europe.

Introduction

Limited gene flow within a population may give rise to a correlation between genetic and geographic distance. Documenting this structure is important in order to understand the detailed population dynamics and microevolution. In species with leptokurtic pollen and seed dispersal curves, most of the gametes are deposited close to their site of origin (Schaal, 1980; Hardy and Vekemans, 1999; Austerlitz et al., 2004; Vekemans and Hardy, 2004; Otero-Arnaiz et al., 2005; Clauss and Mitchell-Olds, 2006; Vaughan et al., 2007). Vegetative propagation through surface or subsurface structures can also contribute to spatial structure of populations, the exact pattern depending on the growth strategy (see e.g. Jonsson et al., 1996; van Rossum et al., 2004; Honnay and Jacquemyn, 2008). Such clonal plant populations consist of genets, which are formed by groups of genetically identical units (ramets), which can be more or less connected through e.g. stolons and rhizomes (Oborny and Cain, 1997). Thus, all ramets originating from the same zygote together constitute a genet, even if they become detached from each other at a later stage (Eriksson, 1993). Clonality may give rise to two distinct growth forms, referred to as the so-called guerrilla and phalanx strategy, respectively (Doust, 1981). The guerrilla strategy is in its extreme characterized by an individual sending out its vegetative propagation organs in several directions and far from each other (e.g. Parks and Werth, 1993; McLellan et al., 1997). Contrary to this is the phalanx strategy, consisting of slower advancement through shorter and denser groups of vegetative fragments (e.g. Maddox et al., 1989; Reisch et al., 2007). Although many plant species have been found to exhibit one or the other growth form, numerous species have been shown to fall into the continuum between these extremes (Slade and Hutchings, 1987; Jonsson, 1996; McLellan et al., 1997).

Clonal propagation is expected to give rise to high allelic diversity, higher levels of linkage disequilibrium and higher differentiation between populations compared to sexual reproduction, as well as heterozygote excess relative to Hardy-Weinberg expectations (Delmotte et al., 2002; Halkett et al., 2005). These effects should be most prominent in strictly clonal species, as heterozygosity can become fixed within populations and the effect of genetic drift can thus be reduced (Eriksson, 1993; Balloux et al., 2003). For species with both asexual and sexual reproduction, efficient clonal propagation may result in a reduction in sexual reproduction, especially for self-incompatible species or for species experiencing strong inbreeding depression (Ricardo et al., 2006; Honnay and Jacquemyn, 2008). The lack of sexual reproduction also removes the opportunity for creation of new genotypes through

fusion and recombination, and slightly deleterious mutations can accumulate (Muller, 1964). Another disadvantage of clonal reproduction is low dispersal ability and a higher susceptibility to fragmentation (see e.g. Leimu et al., 2006). In contrast, clonality can be beneficial in small populations during colonization of new environments and in peripheral populations when sexual reproduction can be suboptimal due to a lack of suitable mates (Silvertown, 2008). As a consequence, a mixture of both clonal spread and sexual reproduction may be beneficial in a heterogeneous environment, where one or the other mode of reproduction can be emphasized depending on local or microsite characteristics (Gaudeul et al., 2007; Gonzales et al., 2008).

The pattern of genetic structure on a continental (Muller et al., 2008; Ansell et al., 2010) and regional level (Clauss and Mitchell-Olds, 2006; Gaudeul et al., 2007) has already been documented in the perennial *Arabidopsis lyrata* (L.) O'Kane and Al-Shehbaz (Brassicaceae), a close relative of the model plant *A. thaliana*. Two subspecies are currently recognized (Al-Shehbaz and O'Kane, 2002), with *A.l. lyrata* occurring in North America and *A.l. petraea* in Europe. In contrast to its relative, *A. lyrata* is outcrossing (van Treuren et al., 1997; Schierup, 1998) and self-incompatible (SI) (Charlesworth et al., 2000; Clauss and Mitchell-Olds, 2006). However, self-compatible populations have been documented in North America (Mable and Adam, 2007). Like several other *Arabidopsis* species, it is commonly found in highly disturbed habitats such as river beds and edges, screes and roadsides. *A. lyrata* is increasingly used in plant evolutionary and ecological genetics, mainly because of the close phylogenetic relationship to *A. thaliana*, enabling comparative studies of differences in life history traits (Savolainen et al., 2000; Kuittinen et al., 2004; Abel et al., 2009).

Physical obstacles such as surface topography and water bodies can act as barriers to gene flow on large spatial and temporal scales and result in genetic differentiation within a species (Futuyma, 2005). On smaller scales, environmental features can lead to differentiation between populations due to e.g. topography (Walker et al., 2009), moisture (Xie et al., 2001), and availability of pollinators (Kulbaba and Worley, 2008). The presence of physical obstacles may explain differences in genetic structure between mountainous and widely dispersed Norwegian localities compared to a set of geographically closer lowland populations in Sweden (Gaudeul et al., 2007). Furthermore, in Central Europe, populations of *A. lyrata* have been found to be significantly differentiated over distances <16 km, and fine-scale structure has been documented within populations (Clauss and Mitchell-Olds, 2006).

However, the topographic features differ between the different areas, and the results of one area cannot necessarily be generalized. In particular, in Norway, large continuous populations may have a different genetic structure than the forest populations of Central Europe, e.g. due to different pollinator behaviour or substrate quality.

In addition to extensive outcrossing, *A. lyrata* ssp. *petraea* has been shown to exhibit varying degrees of clonal propagation and/or facultative inbreeding on a regional scale (Schierup, 1998; Mable et al., 2005; Clauss and Koch, 2006; Gaudeul et al., 2007). Gaudeul et al. (2007) showed that the level of clonality varied among Scandinavian populations, whereas no clonality was documented in the study by Clauss and Mitchell-Olds (2006). Clonality has also been shown in another perennial relative occurring in Europe, *A. halleri* (van Rossum et al., 2004; Llaurens et al., 2008). Genetic neighbourhood sizes (Wright, 1943) have been shown to be inversely correlated to plant density in populations (Schmitt, 1983). Thus, in a clonal species like *A. lyrata* we may expect a negative relationship between neighbourhood size and the total number of genets. Due to the clonality, this relationship should be stronger when comparing neighbourhood size to number of genets than to census size (number of ramets). In addition, the level of clonality may be density dependent. In sparsely populated patches, variation in clonality could be due to e.g. local variation in nutrient availability, leading to an increased focus on producing large ramets to obtain resources more efficiently (see Alpert and Stuefert, 1997 and references therein). As *A. lyrata* throughout Europe is shown to be strictly outcrossing (Clauss and Mitchell-Olds, 2006), high levels of clonality may result in insects transferring incompatible pollen in small and scattered populations. This can then lead to lower seed set and a reduction in fitness within populations. One could still expect that plant individuals limited by pollen should allocate more energy to vegetative growth, which could lead to increased clonal propagation, resulting in a positive feedback mechanism (Wolf et al., 2000; Honnay and Jacquemyn, 2008). Thus, to investigate how self-incompatibility influences fitness, the contribution from both seed and ramet production has to be considered.

Several studies have documented SGS at a range of spatial scales in *A. lyrata*, from continental down to local scales (Clauss and Mitchell-Olds, 2006; Gaudeul et al., 2007; Schierup et al., 2006; 2008; Ansell et al., 2010). Clauss and Mitchell-Olds (2006) investigated fine scale structure in a German forest population, where plants are found on separate rocks. The detailed genetic structure has not been studied in other kinds of landscapes, such as the open alpine habitat found in the Norwegian mountains. Furthermore, the degree of clonality

may vary between and within populations, as well as how this contributes to any observed spatial genetic pattern. In the present study we address the following questions: 1) what is the level and spatial distribution of clonality within an extensive population of *A. lyrata*, 2) how is clonality related to population genetic parameters within this population, and 3) is the population random mating, or do limited seed or pollen dispersal result in spatial genetic structure? In the presence of SGS, we also want to evaluate to what extent topographic features cause isolation by distance in the population as a whole.

Materials and methods

Site and sampling design

Leaf material was sampled in August 2006 within a large *A. lyrata* population at Spiterstulen, Norway (N61°37' E008°24; 1,104 m a.s.l.) covering approximately 0.4 km^2 (Figure 1). The population occurs mainly along both sides of the river Visa on fluvial and glacial depositions with the growth substrate dominated by sand and/or gravel. On the fluvial depositions, the most common species group is bryophytes, although several species of graminoids occur together with *A. lyrata*. However, plant density is low, and non-vegetated ground constitutes on overall a sizable part of the habitat. The glacial river has high water levels due to melting ice during the main flowering months in July-August. The river is in itself not very wide, but as it is glacier fed, the current is rapid, creating vortices of cold air above the water. This can potentially prohibit or reduce insect movement across it. As most of the *A. lyrata* patches are close to the river, the plants are prone to flooding every year. This may lead to seeds and plants being washed away, but the seeds may be able to establish at downstream sites. Few potential pollinators were seen, but some Dipteran and Lepidopteran species were observed feeding on *A. lyrata* flowers during sampling.

Squares (10 m×10 m) containing at least 50 plants each were marked throughout the population, along both sides of the river, and in a west-east transect across it. In total, 17 squares with a sufficient density of plants were established. In each square, 50 individual rosettes were sampled, and their positions in two-dimensional space (x, y) measured to the nearest centimetre. If possible, only sexually reproducing rosettes were sampled. Sampling within the squares was designed to cover the spatial distribution of plants, i.e. both rosettes close to each other and far from each other were sampled, and more samples were taken from dense patches than from sparse patches. This was done to ensure that clonal growth on different scales could be detected. The total number of *A. lyrata* rosettes, i.e. the census size,

per square varied from 52 to 369. A total of 850 individuals were sampled throughout the population, and plant material was then dried at 35°C for 24 h and afterwards stored at room temperature. A summary of census sizes, altitudes and coordinates for each square is shown in Table 1.

Genotyping

Plant DNA was extracted using the E.Z.N.A.™ Plant Kit (Omega Bio-Tek. Inc., Norcross, GA) and the NucleoSpin® 96 Plant kit (Macherey-Nagel; Düren, Germany), following the manufacturer's protocols. The PCR reaction was carried out in a 10 µl volume consisting of 1µl DNA template (5 ng/µl), 5 µl PCR master mix (QIAGEN Multiplex PCR Master Mix; Qiagen, Hilden, Germany), 3 µl H_2O and 1 µl primer mix. Plant individuals were genotyped for 14 microsatellite loci in two separate multiplex reactions (Table 2). Amplification was performed on an Eppendorf Mastercycler Gradient (Eppendorf, Hamburg, Germany), using the following procedure: 95°C for 15 minutes, 34 cycles of 94°C for 30 s, 50°C for 1 min 30s and 72°C for 1 min, with a final extension for 30 minutes at 60°C. Capillary electrophoresis was performed on a 3730 DNA Analyzer (Applied Biosystems, Foster City, CA). GeneMapper 4.0 (Applied Biosystems, Foster City, CA) was applied to analyze the fragments and score the alleles.

Population genetic structure

In the analyses the number of sampled rosettes, N, is given as number of ramets, while the number of independent multilocus genotypes are defined as genets, G. Unless otherwise specified, analyses and results are based on genet data. For each square, we calculated the following measures of genetic diversity: observed heterozygosity (H_O), expected heterozygosity (H_E), the inbreeding coefficient (F_{IS}) and proportion of polymorphic loci (PPL). H_O, H_E and PPL were estimated in GenAlex 6.2 (Peakall and Smouse, 2006), while F_{IS} was estimated with Arlequin 3.11 (Excoffier et al., 2005). We performed correlation analyses between census size, proportion of genets and H_E (Spearman rank correlation); whereas differences in H_E, H_O, F_{IS} between genets and ramets were tested for using paired Wilcoxon tests. All association analyses, as well as comparisons between genets and ramets, were computed using SPSS version 14.0 (Windows version 14.0, SPSS Inc., Chicago, IL).

Map coordinates from each square were imported in MapSource (Garmin, Olathe, KS) in order to calculate a geographical distance matrix for the squares. This was combined with a

genetic matrix based on Nei genetic distance (Saitou and Nei, 1987) to test for isolation by distance across the whole population using a Mantel test (Mantel, 1967) with 999 permutations as implemented in GenAlex (Peakall and Smouse, 2006).

We tested for Hardy-Weinberg equilibrium across the whole population and within each square using GenePop (Rousset, 2008). AMOVA (analysis of molecular variance) was performed in Arlequin (Excoffier et al., 2005) using geographical position (east or west side of the river; river bank or moraine ridge) as a grouping variable, yielding four groups. To get an initial idea about the degree of substructure in the whole population, Wright's F_{ST} (Weir and Cockerham, 1984) was calculated in a pairwise manner between squares using Arlequin (Excoffier et al., 2005). The same software was also used to perform a global differentiation test (Weir and Hill, 2002) to investigate significant differences between squares. The proportion of linkage disequilibrium, P_D, based on number of locus pairs in LD in relation to all locus pairs, was calculated as described in Stenøien and Såstad (1999).

Clonality

When investigating clonality, there is a possibility that two individuals which are genetically similar may have arisen separately through sexual reproduction and are identical by state, IBS. This can tested for by calculating the probability for observing a particular genet in an individual through the formula

$$P_{cgen} = (\prod p_i)2^h \quad (1)$$

where p_i is the frequency of each allele at a specific locus of the genotype in the sample population, and h is the number of heterozygous loci (Parks and Werth, 1993). When encountering genets containing more than 2 ramets, the extension

$$P_r = (P_{cgen})^{n-1} \quad (2)$$

has to be added to the calculation (Sydes and Peakall, 1998), where n is the number of times ramets belonging to the same genotype are sampled. This gives the probability of sampling (n-1) copies of the specific genotype. The approach is conditioned on the population being in Hardy-Weinberg equilibrium. We can assume that the occurrence of identical multilocus

genotypes in our sample is not due to random resampling if $P<0.05$ in equation (2). We calculated P_{cgen} and P_r for all clonal genets (n=131) encountered in the total population.

Spatial genetic structure within each square was investigated using the software SPAGeDi 1.2 (Hardy and Vekemans, 2002). The program estimates SGS using pairwise comparisons of individuals in relation to predefined geographical distances. We applied the kinship coefficient described by Loiselle et al. (1995) for comparisons of plants within different distance classes. As we expected geographically close individuals to be more related than more distant ones, distance classes were set to 5, 10, 15, 20, 25, 50, 100, 250, 500 and 1000 cm. In addition, the software calculated a final distance class (maximum 1414 cm) in squares where ramets could be found more than 1000 cm apart. For genets in which several ramets were sampled, a median position based on all ramets within the genet was calculated. Kinship was regressed against distance class and 95% confidence intervals were computed using 10,000 permutations.

Neighbourhood size, *Nb*, is defined as the effective number of individuals in a random breeding neighbourhood (Wright, 1946). This has lead to the interpretation of *Nb* as a panmictic unit, although Epperson (2007) pointed out that this is not the case. Hardy and Vekemans (1999) formulated *Nb* as a function of the variation of dispersal distance,

$$Nb = 4\pi D\sigma^2 \qquad (3)$$

where *D* is the effective density of individuals, and σ^2 is the axial variance of dispersal distance. Where direct dispersal estimates are not available, indirect estimates can be calculated as

$$\check{N}b = -\frac{(1-F)}{blog} \qquad (4)$$

where *F* is the average kinship coefficient and *blog* is the regression slope based on the log of spatial distance (Hardy et al., 2006; Hardy and Vekemans, 2007). *F* and *blog* were estimated for each square with SPAGeDi 1.2 (Hardy and Vekemans, 2002). We tested for correlations between *Nb* and census size, as well as number of genets, respectively (Spearman rank correlation). We also estimated number of genets (G), proportion of genets (G/N), and mean

number of ramets per genet. When screening the data set for potential clones, all individuals containing missing data were excluded. This will cause our measures of clonality to be minimum estimates. Clone size was calculated as the maximum distance between ramets within each genet.

Results

Out of the 850 sampled individuals, 827 could be genotyped. All 14 microsatellite markers were polymorphic in the population as a whole, as well as in all squares, although F19K23 was monomorphic (i.e. frequency of most common allele >0.95) in 15 of the 17 squares. The total number of alleles (±SD) per marker varied from 2 (*nga151*) to 16 (*nga112*), with an average of 7.3±3.9 alleles (Table 2).

Population genetic structure

A summary of population genetic parameters for each square and across all squares can be found in Table 3. No combination of population genetic parameters (N, G/N, H_E) was significant after Bonferroni correction (Spearman rank correlation; $P<0.05$). Across the entire population, expected heterozygosity (±SD), H_E, was found to be 0.480±0.031 and 0.484±0.027 in ramets and genets, respectively. For observed heterozygosity, H_O, the population average was 0.462±0.062 and 0.464±0.045 for ramets and genets, respectively. H_E and H_O were not significantly different between ramets and genets (pairwise Wilcoxon tests; $P=0.29$ and $P=0.98$, respectively). The inbreeding coefficient for genets, F_{IS}, varied from -0.17 to 0.19 in the squares, with values positive and significantly different from zero in 8 of 17 squares (1,023 permutations). In ramets, the same procedure resulted in F_{IS} ranging from -0.35 to 0.18, with 9 of 17 squares having values positive and significantly different from zero. The proportion of polymorphic loci, PPL, was on average±SD 0.94±0.03, ranging from 0.93 to 1.00 among squares. We found that 2 out of 14 loci were not in HWE ($P<0.05$) in the total population. Repeating the same test for each individual square, 10 squares had a few loci (1-3) not in HWE. The global test showed only square Q not to be in HWE. The proportion of linkage disequilibrium±SD, P_D, in genets was 0.26±0.11.

Pairwise F_{ST} values (n=136) had a median value of 0.039 in the squares, ranging from 0.004 to 0.123. A global test of square differentiation showed, however, that none of the comparisons were significantly different from zero ($P>0.05$). Furthermore, there was no pattern of isolation by distance across the population ($r=0.069$, $P=0.28$) (Fig. 3). Separating

squares into groups based on location (side of river and river bank/moraine ridge – 4 groups) in an AMOVA analysis explained the highest proportion of variation among groups, although the component of variation was still very low (1.7%; $P<0.001$). Almost all variation (95.3%) was due to variation among individuals within squares. This supports the global differentiation analysis, showing very low differentiation between squares within the population.

Clonality

The total sample consisted of 546 genets, of which 415 were present in a single ramet each. After excluding all genets with missing data, the 423 remaining genets contained on average (±SD) 1.7±1.8 ramets (range 1-20; Fig. 2a). Mean genet size (±SD) was 6.4±22.5 cm (range 0-267.2 cm; Fig. 2b). In most of the squares (n=12), only a subsample of all rosettes was sampled. For those squares (C, J, N, P and Q; Figure 1, Table 1) where most of the rosettes (>80%) within a square were sampled, genet size was on average (±SD) 10.4±33.4 cm (range 0-267.2 cm) and the number of ramets in each clone was 2.1±2.8 (range 1-20). Most genets were small, and the growth form of ramets within genets condensed (Fig S1). Still, for some genets, the ramets were spread out and/or intermixed with other genets (Fig. S1). The proportion of genets, G/N, varied from 0.22 in square P to 0.85 in square G, with a grand mean (±SD) of 0.66±0.19 (Table 3). For genets containing more than one ramet, the probability of encountering a second, identical genotype, P_{cgen}, varied from 7.93×10^{-12} to 0.007, whereas the probability of encountering more than two identical genotypes, P_r, varied from 6.9×10^{-61} to 0.0003, suggesting that identical genotypes are ramets of the same clone in all cases considered. The mean neighbourhood size (*Nb*) ±SD across all squares was 22.6±9.7, ranging from 10.4 to 38.5. Number of genets per square, G, had almost the same range (11-41) as *Nb*, although its mean±SD was higher (32.1±9.0). If only considering squares which were exhaustively sampled, *Nb*±SD was 20.0±9.1. The number of genets, G, was negatively correlated with *Nb* ($P=0.027$), but not after Bonferroni correction. All squares showed indications of spatial genetic structure at a fine scale according to SPAGeDi analyses (Fig. 4). Genetically similar plants were found at distances up to 15-25 cm, but in a few squares there was significant SGS up to 25-50 cm, and even 100-250 cm in square Q.

Discussion

We found extensive clonality in *A. lyrata* at Spiterstulen, resulting in a fine-scale spatial genetic pattern. Despite this clonality, the population as a whole is random mating without any specific substructure.

Clonality

Stochastic events due to biotic or abiotic factors are expected to affect species capable of clonal growth less than non-clonal species (Honnay and Bossuyt, 2005), thus loss of genetic diversity through genetic drift can be reduced in clonally reproducing species (Balloux et al., 2003). In this way, clonality can be viewed as a form of insurance, in the same way as a seed bank, under conditions that are not optimal for sexual reproduction. Frequent disturbances should not favour clonality (Silvertown, 2008), although *A. lyrata* in Norway occurs in habitats prone to seasonal flooding. Through clonal reproduction, plants can increase the chances of survival in harsh environments, a clear advantage at high altitudes and alpine areas (Bliss, 1971). Clonal propagation is likely to influence spatial genetic structure, and can do so over considerable distances, depending on the size and abundance of clones (see e.g. Parks and Werth, 1993; Vaughan et al., 2007). Gaudeul et al. (2007) showed that clonality in *A. lyrata* was evident but highly variable in Scandinavian populations, whereas no indication of clonality was found in a study from Central Europe (Clauss and Mitchell-Olds, 2006). This difference in clonality between Spiterstulen and Central Europe can thus be a reflection of reduced levels of sexual reproduction in the species' distribution margin in Europe (Eckert, 2002; Rasmussen and Kollmann, 2008).

Clonal structure in A. lyrata

It should be stressed that even though our study showed considerable clonality in *A. lyrata* (mean G/N=0.66), this is most likely an underestimate due to several reasons. First, we may have underestimated clone size as we mostly selected rosettes which were sexually reproducing, and did not sample all rosettes within each square. Resource allocation within a genet may enable flowering in some fragments, while others stay vegetative (Alpert and Stuefer, 1997). Also, since we excluded all rosettes lacking full data when identifying clonal genets, we may have missed some parts of clones. The clonality level found in our study is still comparable to what was found in the relative *A. halleri* (van Rossum et al., 2004; G/N=0.35-0.94), although a later study by Llaurens et al. (2008) documented an average G/N of 0.98. In the *A. halleri* populations, however, plant density was considerably higher (33-89

ramets/m², van Rossum et al., 2004; 14 ramets/m², Llaurens et al., 2008) than in our study (0.5-3.7 ramets/m²). The high occurrence of identical genotypes at Spiterstulen could be due to either clonality or low ability to identify different genets, but as our markers had very high power to distinguish between the genets, we can be confident in treating identical genotypes as ramets of the same genet. We find that most genets are rather small (6.4 cm and 10.4 cm on average for all squares and for squares with exhaustive sampling, respectively). Few ramets per genet on average contributes to this, but we also see a pattern where ramets of the same clone either congregate close to each other, i.e. the phalanx strategy, or more rarely, they send out long runners, following the guerrilla strategy. This is also obvious for squares where close to all individual rosettes were sampled. Thus, clone size and shape varies within and between squares (Fig. S1), showing that *A. lyrata*, as many other species (Jonsson, 1996; McLellan et al., 1997; Alberto et al., 2005; Johansen, 2009) does not adhere to either the guerrilla or the phalanx growth strategy, but lies somewhere in between. The former strategy may be more beneficial in patches where resources are more scarce (Doust, 1981), but as we did not find any significant correlation between plant density and level of clonality, competition between ramets is most likely not the main cause of the spatial distribution pattern of plants seen in the squares. Another, perhaps more likely factor, may be fine scale variation in soil texture and resources, where chance establishment in poorer patches could cause an individual plant to put more effort into spreading its ramets far about. Finally, competition from other species could potentially influence the distribution of *A. lyrata* in this population, but as the cover of other plant species was very low in all squares investigated, this was not taken into account in this study.

Genetic diversity varied between squares, but was not correlated with the proportion of genets. As such, clonality does not lead to a loss of alleles in the Spiterstulen population, conforming to the theoretical predictions that genetic diversity can be preserved in clonal species as the effect of genetic drift is reduced (Balloux et al., 2003). Clonality can be considered a way of exploiting existing opportunities to the best possible degree. In favourable patches, a successful genet may perhaps benefit more from expanding its distribution through the production of new ramets rather than creating new, possibly less-adapted genets through sexual reproduction. A potential risk involved in having a high dependence on clonality for a self-incompatible species becomes evident as the clones grow bigger. If pollinator activity is high or pollen transfer efficient, clogging of the stigma with pollen containing a similar S-allele can cause low sexual reproduction in plants (reviewed in

Barrett, 2002). Thus, availability of unrelated, compatible pollen can be reduced, resulting in lower seed set. This could lead to seed production in *A. lyrata* to be more limited by mates than by resources, as was shown in a recent study on the same species (Sandring and Ågren, 2009). Still, our results show that gene flow is not restricted in the population, but as we have not separated the effect of pollen transport from seed transport, we cannot say whether we observe pollen limitation presently.

The concept of neighbourhoods was originally developed by Wright (1943), and can be said to describe an area in which breeding is more or less restricted for the individuals within it. Neighbourhoods can be useful in describing to some degree spatial structure within a population in terms of the relationship between dispersal and drift, but not when the occurrence of long range dispersal is high (Fenster et al., 2003). In our study, neighbourhood size across the whole population was similar to when only considering the squares in which close to all rosettes were sampled. Our estimates of neighbourhoods show a trend for a negative correlation between neighbourhood sizes and level of clonality ($P=0.05$, not significant after Bonferroni correction), thus in squares with many clones exhibiting high numbers of ramets, we expect seed set to be reduced compared to squares with few clonal genets. This can create a differentiation within the population, where marginal patches develop into sites of plants focusing mainly on vegetative propagation, while central patches have plants putting more energy into seed production. The lack of correlation between census size and proportion of genets in the squares indicates that clonality is not density dependent. Thus, a lack of sites for establishment does not necessarily cause plants to switch from sexual reproduction to vegetative propagation through rhizome growth.

In the previous study addressing clonality in Norwegian *A. lyrata* populations (Gaudeul et al., 2007), the proportion of genets was found to vary from 0.55 to 1, with an average±SD of 0.84±0.13. In the Spiterstulen population, however, no clonality was shown due to the absence of identical genets. This contrasts with our study, where we have found clonality to be quite high, resulting in G/N ratios within some squares to be as low as 0.22. The main reason for this deviation compared to the previous study has to do with the sampling scheme. Whereas Gaudeul et al. (2007) in most of the populations sampled plants with at least 50 cm intervals, we sampled plants down to 0.5 cm apart in order to discern in more detail the local genetic structure. For most squares, 50 cm was above the maximum clone size, hence it is no surprise that the sampling scheme of Gaudeul et al. (2007) failed to discover any clonality in

Spiterstulen. In 5 squares (C, J, N, P and Q), G/N was less than 0.5 as a result of either many small or several large genets, all containing two or more ramets. If we consider the three Norwegian populations (N3, N7 and N14) in Gaudeul et al. (2007) where distance between sampled individuals were only 20 cm, the proportion of genets rises to 0.71. This is quite close to our estimate for the Spiterstulen population (0.66), indicating that when sampling *A. lyrata*, a distance of 20 cm between samples is sufficient to detect most fine-scale spatial genetic structure. Another effect of the high proportion of clonal individuals in the Spiterstulen population was the relatively high levels of linkage disequilibrium, similar to what was found in populations exhibiting high levels of clonality in Gaudeul et al. (2007), but considerably higher than for 14 Norwegian *A. lyrata* populations where close to no clonality was evident (P_D=0.08; Falahati-Anbaran et al., in prep.).

Population genetic structure

The lack of any isolation by distance pattern on the population scale illustrates the fact that the spatial genetic structure (SGS) is prevalent only at very fine spatial scales, i.e. within squares, and is caused by clonality. By considering larger distance classes, i.e. larger distances between plants (>15-25 cm), we find very little evidence of spatial structure in neutral loci. This could be compared to results from *A. lyrata* on Iceland, where there are indications of isolation by distance on very local scales (10-20 meters; Schierup et al., 2006), but not on regional scales (200-300 km; Schierup et al., 2008). However, Schierup et al. (2008) looked at SI haplotypes, which are subject to selection in populations across a large region, while in Norway, Gaudeul et al. (2007) found significant isolation by distance on the same scale (hundreds of kilometres) using neutral loci. The reason for this difference is not clear; the Icelandic populations could have been sampled in localities which are more interconnected with each other through an open landscape, or, more likely, as the SI alleles are subject to selection, regional genetic structure based on such markers will be different than for microsatellites. It could also be that the Icelandic population of *A. lyrata* has dispersed and reached its current distribution at a much later time compared to Norway, not giving enough time to create an IBD pattern (Schierup et al., 2008).

Contrary to what we expected, genetic structure on the population level was not found. Only two microsatellite loci deviated from Hardy-Weinberg equilibrium across the whole population, and a test for isolation by distance did not reveal any significant pattern. Thus it seems that despite the topographic barriers, there is a substantial gene flow throughout the

large population. This is in contrast to what has been found in other studies considering SGS on such local scales, where indications have been found of restricted gene flow and subdivision of the population (Schaal, 1980; Loiselle et al., 1995; Clauss and Mitchell-Olds, 2006; Schierup et al., 2006; Vaughan et al., 2007). It is unknown whether this is due to seed or pollen transport. Pollen transport should be considered more efficient in long distance transportation, but as this *A. lyrata* population is mainly situated along streams, water transport of seeds can contribute as well. We anticipated that the river might limit insect movement and result in differentiation between squares on each side of the river. No such differentiation was found, but direct measures on pollen transport and the effect of the river as a vector of gene flow will be necessary. Clauss and Mitchell-Olds (2006) found that much of the genetic variation within the Central European Plech population was a due to substructure, likely a consequence of the specialized habitat of the plants (rock outcrops). In Norwegian populations, *A. lyrata* typically inhabits river banks and have more continuous populations. This may be the main reason for the lack of any obvious structure in the Spiterstulen population.

Conclusions

We have shown that vegetative spread through rhizomes is a common way of propagation in *A. lyrata* at Spiterstulen, shaping the spatial population structure at very fine scales in this Norwegian high altitude population. The lack of a clear structure across the population as a whole shows, however, that *A. lyrata* can act as a more or less random mating unit over considerable distances on a local scale. Contrary to what should be expected, levels of clonality are not related to plant density, and other factors, such as soil texture and nutrient availability, may play a more crucial role for this pattern. Seed transport can be facilitated by water flow, especially during flooding events, but the relationship between pollen transport, seed transport, and population structure is still unclear. Finally, as neutral microsatellite markers in this study show that the overall spatial genetic pattern in this population is random, it may be easier to detect possible patterns of differentiation due to local microsite variation against this background.

Acknowledgements

We would like to thank Marte Sendstad for field assistance, Meeri Otsukka and Mariann Nilsen for helping out with DNA extraction and PCR, and a special thanks to Johanna Leppälä for all advice and help applying microsatellite markers. The comments of two

anonymous reviewers greatly improved the content of the manuscript. This work was financially supported by the Swedish and Norwegian Research councils (to HKS) and the Biosciences and Environment Research Council of Finland and the University of Oulu (to OS).

References

Abel C, Clauss M, Schaub A, Gershenzon J, Tholl D. 2009. Floral and insect-induced volatile formation in *Arabidopsis lyrata* ssp. *petraea*, a perennial, outcrossing relative of *A. thaliana*. *Planta* 230, 1-11.

Alberto F, Gouveia L, Arnaud-Haond S, Pérez-Lloréns JL, Duarte CM, Serrão EA. 2005. Within-population spatial genetic structure, neighbourhood size and clonal subrange in the seagrass *Cymodocea nodosa*. *Molecular Ecology* 14, 2669-2681.

Alpert P, Stuefer JF. 1997. Division of labour in clonal plants. In: de Kroon H, van Groenendael J, eds. *The ecology and evolution of clonal plants*. Leiden: Backhuys Publishers, 137-154.

Al-Shehbaz IA, O'Kane SL. 2002. Taxonomy and phylogeny of *Arabidopsis* (Brassicaceae). In: *The Arabidopsis Book*. American Society of Plant Biologists, Rockville, MD. doi: 10.1199/tab.0001, http://www.aspb.org/publications/arabidopsis/.

Ansell SW, Stenøien HK, Grundmann M, et al. 2010. Population structure and historical biogeography of European *Arabidopsis lyrata*. Heredity. doi:10.1038/hdy.2010.10.

Austerlitz F, Dick CW, Dutech C, et al. 2004. Using genetic markers to estimate the pollen dispersal curve. *Molecular Ecology* 13, 937-954.

Balloux F, Lehmann L, de Meeûs T. 2003. The population genetics of clonal and partially clonal diploids. *Genetics* 164, 1635-1644.

Barrett SCH. 2002. Sexual interference of the floral kind. *Heredity* 88, 154-159.

Bell CJ, Ecker JR. 1994. Assignment of 30 microsatellite loci to the linkage map of *Arabidopsis*. *Genomics* 19, 137-144.

Bliss LC. 1971. Arctic and alpine plant life cycles. *Annual Review of Ecology and Systematics* 2, 405-438.

Charlesworth D, Awadalla P, Mable BK, Schierup MH. 2000. Population-level studies of multiallelic self-incompatibility loci, with particular reference to Brassicaceae. *Annals of Botany* 85 (Supplement A), 227-239.

Clauss MJ, Cobban H, Mitchell-Olds T. 2002. Cross-species microsatellite markers for elucidating population genetic structure in *Arabidopsis* and *Arabis* (Brassicaceae). *Molecular Ecology* 11, 591-601.

Clauss MJ, Koch MA. 2006. Poorly known relatives of *Arabidopsis thaliana*. *Trends in Plant Science* 11, 449-459.

Clauss MJ, Mitchell-Olds T. 2006. Population genetic structure of *Arabidopsis lyrata* in Europe. *Molecular Ecology* 15, 2753-2766.

Delmotte F, Leterme N, Gauthier JP, Rispe C, Simon JC. 2002. Genetic architecture of sexual and asexual populations of the aphid *Rhopalosiphum padi* based on allozyme and microsatellite markers. Molecular Ecology 11, 711-723.

Doust LL. 1981. Population dynamics and local specialization in a clonal perennial (*Ranunculus repens*) I. The dynamics of ramets in contrasting habitats. *Journal of Ecology* 69, 743-755.

Eckert CG. 2002. The loss of sex in clonal plants. Evolutionary Ecology 15, 501-520.

Epperson B. 2007. Plant dispersal, neighbourhood size and isolation by distance. *Molecular Ecology* 16, 3854-3865.

Eriksson O. 1993. Dynamics of genets in clonal plants. Trends in Ecology & Evolution 8, 313-316.

Excoffier L, Laval G, Schneider S. 2005. Arlequin ver. 3.0: An integrated software package for population genetics data analysis. *Evolutionary Bioinformatics Online* 1, 47-50.

Fenster CB, Vekemans X, Hardy OJ. 2003. Quantifying gene flow from spatial genetic structure data in a metapopulation of *Chamaechrista fasciculata* (Leguminosae). Evolution 7, 995-1007.

Futuyma DJ. 2005. *Evolution*. Sunderland: Sinauer.

Gaudeul M, Stenøien HK, Ågren J. 2007. Landscape structure, clonal propagation, and genetic diversity in Scandinavian populations of *Arabidopsis lyrata* (Brassicaceae). *American Journal of Botany* 94, 1146-1155.

Gonzales E, Hamrick JL, Smouse PE. 2008. Comparison of clonal diversity in mountain and Piedmont populations of *Trillium cuneatum* (Melanthiaceae-Trilliaceae), a forest understorey species. American Journal of Botany 95, 1254-1261.

Goudet J. 2001. FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9.3). Available from http://www.unil.ch/izea/softwares/fstat.html. Updated from Goudet (1995).

Halkett F, Simon J-C, Balloux F. 2005. Tackling the population genetics of clonal and partially clonal organisms. Trends in Ecology and Evolution 20, 194-201.

Hardy OJ, Maggia L, Bandou B, et al. 2006. Fine-scale genetic structure and gene dispersal inferences in 10 Neotropical tree species. *Molecular Ecology* 15, 559-571.

Hardy OJ, Vekemans X. 1999. Isolation by distance in a continuous population: reconciliation between spatial autocorrelation and population genetics models. *Heredity* 83, 145-154.

Hardy OJ, Vekemans X. 2002. SPAGeDi: a versatile computer program to analyse spatial genetic structure at the individual or population levels. *Molecular Ecology Notes* 2, 618-620.

Hardy OJ, Vekemans X. 2007. SPAGeDi 1.2 – a program for spatial pattern analysis of genetic diversity. User's manual. Available from http://www.ulb.ac.be/sciences/ecoevol/spagedi.html.

Honnay O, Bossuyt B. 2005. Prolonged clonal growth: Escape route or route to extinction? Oikos 108, 427-432.

Honnay O, Jacquemyn H. 2008. A meta-analysis of the relation between mating system, growth form and genotypic diversity in clonal plant species. *Evolutionary Ecology* 22, 299-312.

Johansen L. 2009. Clonal growth strategies in simultaneously persistent and expanding *Trifolium repens* patches. *Plant Ecology* 201, 435-444.

Jonsson BO, Jónsdóttir IS, Cronberg N. 1996. Clonal diversity and allozyme variation in populations of the arctic sedge *Carex bigelowii* (Cyperaceae). *Journal of Ecology* 84, 449-459.

Kuittinen H, de Haan AA, Vogl C, et al. 2004. Comparing the linkage maps of the close relatives *Arabidopsis lyrata* and *A. thaliana*. *Genetics* 168, 1575-1584.

Kulbaba MW, Worley AC. 2008. Floral design in *Polemonium brandegei* (Polemoniaceae): Genetic and phenotypic variation under hawkmoth and hummingbird pollination. *International Journal of Plant Sciences* 169, 509-522.

Leimu R, Mutikainen P, Koricheva J, Fischer M. 2006. How general are positive relationships between plant population size, fitness and genetic variation? Journal of Ecology 94, 942-952.

Llaurens V, Castric V, Austerlitz F, Vekemans X. 2008. High paternal diversity in the self-incompatible herb *Arabidopsis halleri* despite clonally reproduction and spatially restricted pollen dispersal. Molecular Ecology 17, 1577-1588.

Loiselle BA, Sork VL, Nason J, Graham C. 1995. Spatial genetic structure of a tropical understorey shrub, *Psychotria officinalis* (Rubiaceae). *American Journal of Botany* 82, 1420-1425.

Loudet O, Chaillou S, Camilleri C, Bouchez D, Daniel-Vedele F. 2002. Bay-0 x Shahdara recombinant inbred line population: a powerful tool for the genetic dissection of complex traits in *Arabidopsis*. *Theoretical and Applied Genetics* 104, 1173-1184.

Mable BK, Robertson AV, Dart S, Di Berardo C, Whitham L. 2005. Breakdown of self-incompatibility in the perennial *Arabidopsis lyrata* (Brassicaceae) and its genetic consequences. *Evolution* 59, 1437-1448.

Mable BK, Adam A. 2007. Patterns of genetic diversity in outcrossing and selfing populations of *Arabidopsis lyrata*. Molecular Ecology 16, 3565-3580.

Mantel N. 1967. The detection of disease clustering and a generalized regression approach. *Cancer research* 27, 209-220.

McLellan AJ, Prati D, Kaltz O, Schmid B. 1997. Structure and analysis of phenotypic and genetic variation in clonal plants. In: de Kroon H, van Groenendael J, eds. *The ecology and evolution of clonal plants* eds. Leiden: Backhuys Publishers, 185-210.

Muller HJ. 1964. The relation of recombination to mutational advance. *Mutational Research* 1, 2-9.

Muller MH, Leppälä J, Savolainen O. 2008. Genome-wide effects of postglacial colonization in *Arabidopsis lyrata*. Heredity 100, 47-58.

Oborny B, Cain ML. 1997. Models of spatial spread and foraging in clonal plants. In: de Kroon H, van Groenendael J, eds. *The ecology and evolution of clonal plants*. Leiden: Backhuys Publishers, 155-183.

Otero-Arnaiz A, Casas A, Hamrick JL. 2005. Direct and indirect estimates of gene flow among wild and managed populations of *Polaskia chichipe*, an endemic columnar cactus in Central Mexico. *Molecular Ecology* 14, 4313-4322.

Parks JC, Werth RC. 1993. A study of spatial features of clones in a population of bracken fern, *Pteridium aquilinum* (Dennstaedtiaceae). *American Journal of Botany* 80, 537-544.

Peakall R, Smouse PE. 2006. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes* 6, 288-295.

Rasmussen KK, Kollmann J. 2008. Low genetic diversity in small peripheral populations of a rare European tree (*Sorbus torminalis*) dominated by clonal reproduction. Conservation Genetics 9, 1533-1539.

Reisch C, Schurm S, Poschlod P. 2007. Spatial genetic structure and clonal diversity in an alpine population of *Salix herbacea* (Salicaceae). *Annals of Botany* 99, 647-651.

Ricardo CT, Corrado CA, Mandujano MC, Molina-Freaner F. 2006. Reproductive consequences of clonal growth in *Stenocereus eruca*, a rare clonal cactus of the Sonoran desert. *Evolutionary Ecology* 20, 131-142.

Rousset F. 2008. GENEPOP ' 007: a complete re-implementation of the GENEPOP software for Windows and Linux. Molecular Ecology Resources 8, 103-106.

Savolainen O, Langley CH, Lazarro BP, Fréville H. 2000. Contrasting patterns of nucleotide polymorphism at the alcohol dehydrogenase locus in the outcrossing *Arabidopsis lyrata* and the selfing *Arabidopsis thaliana*. *Molecular Biology and Evolution* 17, 645-655.

Schaal BA. 1980. Measurement of gene flow in *Lupinus texensis*. *Nature* 284, 450-451.

Schmitt J. 1983. Density-dependent pollinator foraging, flowering phenology, and temporal pollen dispersal patterns in *Linanthus bicolor*. *Evolution* 37, 1247-1257.

Saitou N, Nei M. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution* 4, 406-425.

Sandring S, Ågren J. 2009. Pollinator-mediated selection on floral display and flowering time in the perennial herb *Arabidopsis lyrata*. *Evolution* 63, 1292-1300.

Schierup MH. 1998. The effect of enzyme heterozygosity on growth in a strictly outcrossing species, the self-incompatible *Arabis petraea* (Brassicaceae). *Hereditas* 128, 21-31.

Schierup MH, Bechsgaard JS, Nielsen LH, Christiansen FB. 2006. Selection at work in self-incompatible *Arabidopsis lyrata*: Mating patterns in a natural population. *Genetics* 172, 477-484.

Schierup MH, Bechsgaard JS, Christiansen FB. 2008. Selection at work in self-incompatible *Arabidopsis lyrata*. II. Spatial distribution of S haplotypes in Iceland. *Genetics* 180, 1051-1059.

Silvertown J. 2008. The evolutionary maintenance of sexual reproduction: Evidence from the ecological distribution of asexual reproduction in clonal plants. International Journal of plant Sciences 169, 157-168.

Slade AJ, Hutchings MJ. 1987. The effects of nutrient availability on foraging in the clonal herb *Glechoma hederaceae*. *Journal of Ecology* 75, 95-112.

Stenøien HK, Såstad SM. 1999. Genetic structure in three haploid peat mosses (*Sphagnum*). *Heredity* 82, 391-400.

Sydes MA, Peakall R. 1998. Extensive clonality in the endangered shrub *Haloragodendron lucasii* (Haloragaceae) revealed by allozymes and RAPDs. *Molecular Ecology* 7, 87-93.

van Rossum F, Bonnin I, Fénart S, Pauwels M, Petit D, Saumitou-Laprade P. 2004. Spatial genetic structure within a metallicolous population of *Arabidopsis halleri*, a clonal, self-incompatible and heavy metal tolerant species. *Molecular Ecology* 13, 2959-2967.

van Treuren R, Kuittinen H, Kärkkäinen K, Baena-Gonzalez E, Savolainen O. 1997. Evolution of microsatellites in *Arabis petraea* and *Arabis lyrata*, outcrossing relatives of *Arabidopsis thaliana*. *Molecular Biology and Evolution* 14, 220-229.

Vaughan SP, Cottrell JE, Moodley DJ, Connolly T, Russell K. 2007. Distribution and fine-scale spatial-genetic structure in British wild cherry (*Prunus avium* L.). *Heredity* 98, 274-283.

Vekemans X, Hardy OJ. 2004. New insights from fine-scale spatial genetic structure analyses in plant populations. *Molecular Ecology* 13, 921-935.

Weir BS, Cockerham CC. 1984. Estimating *F*-statistics for the analysis of population structure. *Evolution* 38, 1358–1370.

Weir BS, Hill WG. 2002. Estimating F-statistics. *Annual Review of Genetics* 36, 721-750.

Walker NF, Hulme PE, Hoelzel AR. 2009. Population genetics of an invasive riparian species, *Impatiens glandulifera*. *Plant Ecology* 203, 243-252.

Wolf AT, Harrison SP, Hamrick JL. 2000. Influence of habitat patchiness on genetic diversity and spatial structure of a serpentine endemic plant. *Conservation Biology* 14, 454-463.

Wright S. 1946. Isolation by distance under diverse systems of mating. *Genetics* 31, 39-59.

Xie Z-W, Lu Y-Q, Ge S, Hong D-Y, Li F-Z. 2001. Clonality in wild rice (*Oryza rufipogon*, Poaceae) and its implications for conservation management. *American Journal of Botany* 88, 1058-1064.

Table legends

Table 1:

Description of the 17 squares of *A. lyrata* investigated in this study. Census size is number of rosettes (ramets) per square (10 m×10 m), and altitude, meters above sea level, = m a.s.l.

Square	Census size	Altitude	Coordinates
A	307	1,104	N61°37.364’ E008°24.078’
B	108	1,104	N61°37.265’ E008°24.063’
C	369	1,107	N61°37.194’ E008°24.001’
D	70	1,107	N61°37.197’ E008°23.954’
E	188	1,110	N61°37.021’ E008°23.859’
F	225	1,111	N61°37.005’ E008°23.870’
G	102	1,112	N61°36.976’ E008°23.849’
H	55	1,142	N61°36.762’ E008°23.468’
I	161	1,137	N61°36.767’ E008°23.537’
J	52	1,127	N61°36.786’ E008°23.697’
K	58	1,105	N61°37.355’ E008°24.123’
L	100	1,105	N61°37.282’ E008°24.089’
M	109	1,116	N61°36.958’ E008°23.846’
N	314	1,117	N61°36.940’ E008°23.845’
O	68	1,131	N61°36.789’ E008°23.746’
P	55	1,173	N61°36.766’ E008°24.094’
Q	59	1,160	N61°36.798’ E008°24.008’
Mean±SD	141.2±103.4		

Table 2:

Summary of marker information for the two multiplexes used in the study. For each marker, marker name, dye colour (blue=FAM, green=YY, yellow=NED), primer sequences, allele range, number of alleles found in this study, and reference for the marker is shown.

Multiplex 1

Marker name	Colour	Primer sequences	Allele range	# alleles	Reference
AthCDPK9	Blue	TCAATCATTGTCCAAAACTTGG	83-93	5	Clauss et al. (2002)
		GAAACTGACTTGGAGAAGGCA			
ELF3	Green	CGGAAGGACTGATATACAAGC	294-326	9	Kuittinen et al. (2004)
		TGTTGGGTGTTCTGAAGAT			
F20D22	Green	CCCAAGTGACGTCTGGTTTC	170-178	6	Clauss et al. (2002)
		AACAAAATGAGTTTCTCTGCATG			
ICE13	Blue	GATCCTTCACCGGGTCTTG	242-248	5	Clauss et al. (2002)
		GTGGTGGAGACTCTTCGAGC			
ICE3	Yellow	GACTAATCATCACCGACTCAGCCAC	76-111	14	Clauss et al. (2002)
		ATTCTTCTTCACTTTTCTTGATCCCG			
MSAT2.22	Blue	CGATCCAATCGGTCTCTCT	209-226	10	Loudet et al. (2002)
		TGGTAACATCCCGAACTTC			

Multiplex 2

Marker name	Colour	Primer sequences	Allele range	# alleles	Reference
AthZFPG	Yellow	TTGCGTTTCCACATTTGTTT	150-166	9	Clauss et al. (2002)
		TGGGTCAATTCACATGTAGAGA			
F19K23-483	Blue	GGTCTAATTGCCGTTGTTGC	187-190	4	Clauss et al. (2002)
		GAATTCTGTAACATCCCATTTCC			
ICE14	Green	TCGAGGTGCTTTCTGAGGTT	231-237	4	Clauss et al. (2002)
		TACCTCACCCTTTTGACCCA			
MHJ24	Blue	CCGTCCTTGATCCTTGAGATTCTGAG	125-130	6	Clauss et al. (2002)
		CAATTCCGAAAATCATATTCATGCACC			
nga112	Yellow	TAATCACGTGTATGCAGCTGC	176-204	16	Bell and Ecker (1994)
		CTCTCCACCTCCTCCAGTACC			
nga151	Green	GTTTTGGGAAGTTTTGCTGG	93-95	2	Bell and Ecker (1994)
		CAGTCTAAAAGCGAGAGTATGATG			
ATTSO392	Blue	TTTGGAGTTAGACACGGATCTG	136-148	8	Clauss et al. (2002)
		GTTGATCGCAGCTTGATAAGC			
T15M6	Blue	CATCCATGAATCTTGACTTC	196-201	4	Leppälä, unpublished
		GAACAATGCAGAAACTGTG			

Table 3:

Summary of genetic parameters for each square and across the whole population, split into ramets (all rosettes included, n=827) and genets (only independent multilocus genotypes included, n=546). N=number of individual genotypes, G=genets (number of multilocus genotypes) detected, G/N=genets, PPL=proportion of polymorphic loci, H_E=expected heterozygosity, H_O= observed heterozygosity, F_{IS}=inbreeding coefficient. Bold numbers indicate values significantly different from 0 (P<0.05).

					Genets			Ramets		
Square	N	G	G/N	PPL	He	Ho	FIS	He	Ho	FIS
A	45	38	0.84	92.9	0.479	0.435	**0.093**	0.486	0.442	**0.080**
B	49	41	0.84	92.9	0.512	0.518	-0.028	0.521	0.521	**0.127**
C	50	23	0.46	92.9	0.489	0.466	0.018	0.470	0.485	-0.025
D	50	38	0.76	92.9	0.512	0.450	**0.112**	0.515	0.452	**-0.055**
E	50	41	0.82	100.0	0.491	0.437	**0.096**	0.491	0.436	**0.106**
F	50	39	0.78	92.9	0.512	0.482	0.057	0.510	0.483	0.092
G	47	40	0.85	92.9	0.507	0.520	-0.032	0.515	0.524	0.042
H	46	36	0.78	92.9	0.453	0.409	**0.102**	0.444	0.406	-0.036
I	49	34	0.69	92.9	0.496	0.441	**0.097**	0.481	0.428	**0.079**
J	46	20	0.43	92.9	0.460	0.445	0.057	0.442	0.386	**0.092**
K	49	38	0.78	92.9	0.522	0.473	**0.098**	0.523	0.466	**0.104**
L	47	35	0.74	92.9	0.477	0.417	**0.122**	0.480	0.409	**0.135**
M	49	31	0.63	92.9	0.480	0.395	**0.190**	0.475	0.390	**0.180**
N	50	21	0.42	92.9	0.443	0.474	-0.071	0.453	0.484	-0.082
O	50	37	0.74	100.0	0.471	0.451	0.034	0.470	0.405	**0.124**
P	50	11	0.22	100.0	0.494	0.578	-0.137	0.471	0.631	-0.351
Q	50	23	0.46	92.9	0.424	0.489	-0.172	0.415	0.503	-0.234
Mean	48.6	32.1	0.66	94.1	0.484	0.464	0.037	0.480	0.462	
SD	1.7	9.0	0.19	2.8	0.027	0.045	0.097	0.031	0.062	

Figure legends

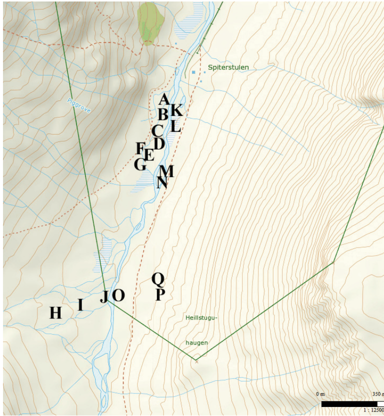


Figure 1: The *Arabidopsis lyrata* locality at Spiterstulen, Norway (N61°37’ E008°24; 1,104 m a.s.l.). Squares (10 m×10 m; n=17) with 50 individuals sampled in each are denoted by letters A-Q. The river Visa flows in a northward direction, as depicted by the arrow.
Source: Norge Digitalt/gislink.no

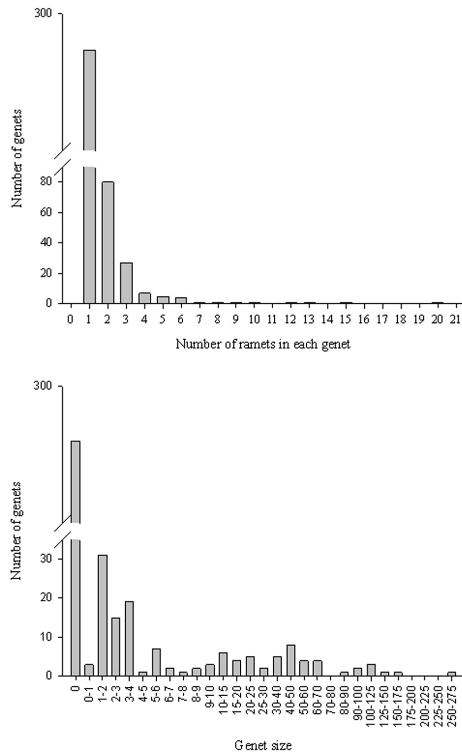


Figure 2: Summary of genet size in the *Arabidopsis lyrata* population at Spiterstulen, Norway. Individuals with missing data are excluded. a): Size distribution of genets based on the number of ramets within each genet; b): Genet size (in cm) shown as the greatest distance measured between two ramets in a genet. A genet size of 0 means the genet was found only in one ramet.

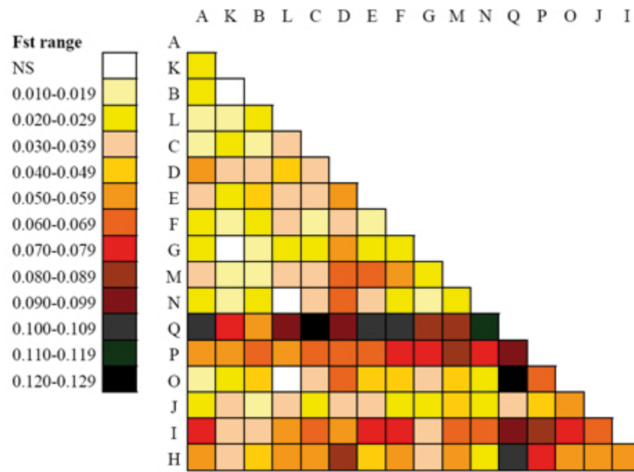


Figure 3: Distribution of pairwise F_{ST}-values (n=136) from 17 squares of *Arabidopsis lyrata* at Spiterstulen, Norway. NS = comparisons not significantly different from 0. The distance between squares A and H was 1.2 km.

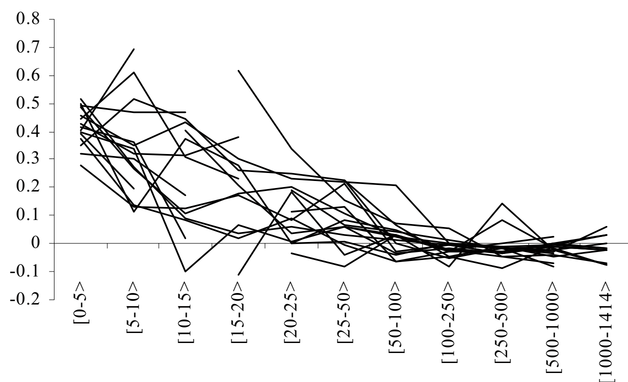
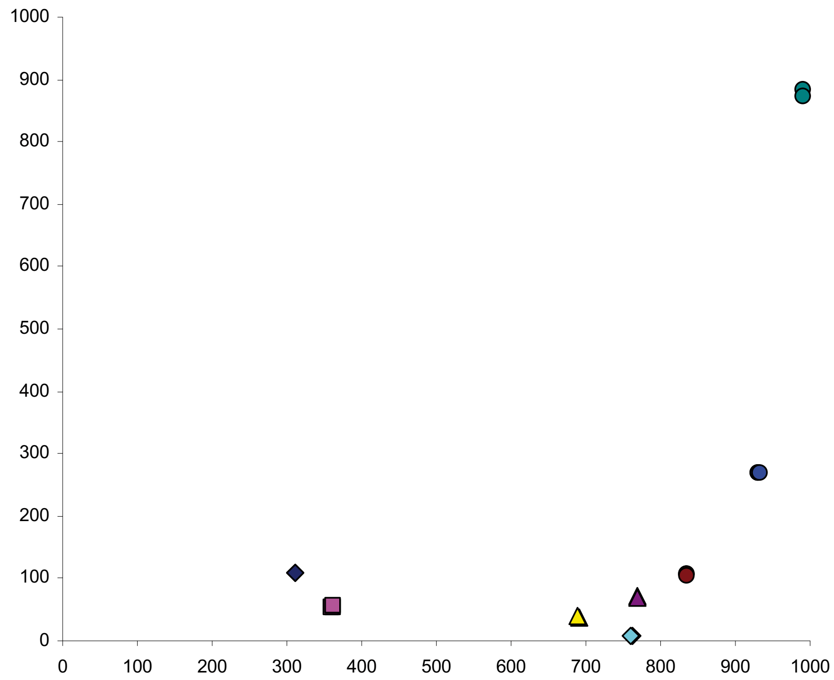


Figure 4: Kinship coefficient plotted against distance interval in all 17 squares from the Spiterstulen population. Kinship coefficient from Loiselle et al. (1995), distance intervals in centimetres.

Supplementary information



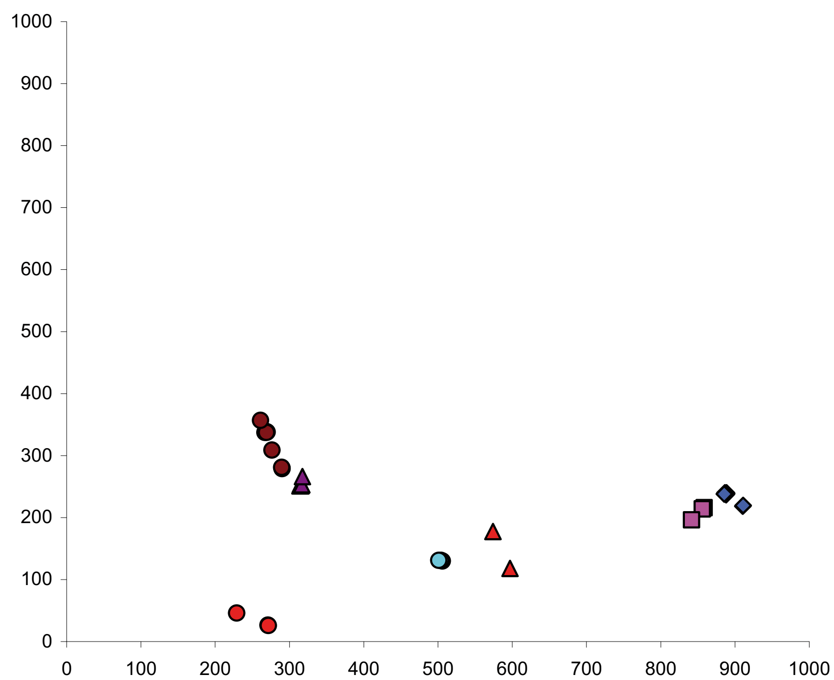


Figure S1: Distribution of *A. lyrata* clones in two squares that were subjected to exhaustive sampling, i.e. >80% of all rosettes within the square were sampled. Square K (top) had 8 genets and square Q (bottom) had 7 genets containing two or more ramets, respectively. In square K, all clones showed condensed growth (phalanx form), whereas in square Q some clones exhibited a more dispersed growth pattern (guerrilla form). Scale is in centimetres.

Paper IV

Flower visitation has small effects on outcrossing levels in a natural population of *Arabidopsis thaliana*

Sverre Lundemo[1]

[1]Department of Biology, Norwegian University of Science and Technology, N-7491 Trondheim.

Abstract

Arabidopsis thaliana (Brassicaceae) is an annual plant growing on disturbed soil, exhibiting small flowers, and a low production of nectar and pollen. It has been shown that almost all reproductive events in this species are due to selfing, and the few chance outcrossing events occurring may therefore be quite important for population genetic structure and dynamics. This has rarely been studied in the field, with the exception of a study documenting very low visitation rates in one German population, where solitary bees and Dipterans were the most common visitors. However, it is unknown to what extent these results can be generalized to other parts of the species' distribution range. A patch of *A. thaliana* plants within a natural population at Lauset, Central Norway (N63˚24.466, E10˚04.878) was studied during the flowering season in May 2007. Visitation rates were much higher than expected (close to 7% of all open flowers were visited), and the solitary bee *Lasioglossum fratinellum* was the main visitor, being responsible for almost 70% of all visits. In addition, Syrphids and other Dipterans were frequent visitors. Despite the high visitation rate found in this study, SNP analyses of the population in the years 2006, 2007 and 2008 showed variable rates of outcrossing (0-6%), with no outcrossing at all observed in 2007. *A. thaliana* flowers in natural populations are thus more commonly visited than what is shown in the literature, but pollen transfer is likely low or inefficient, reducing the possible contribution of floral visitors to outcrossing. The variation in genetic diversity in *A. thaliana* observed at this locality highlights the importance of sufficient sampling, both by covering the whole population and by sampling for more than one season, in order to capture the genetic diversity present.

Introduction

Arabidopsis thaliana (L.) Heynhold (Brassicaceae) is a well known species within the field of molecular and cell biology, with numerous studies being conducted every year. However, as an annual and predominantly selfing species, with selfing rates being as high as 96-99% (Abbott & Gomes 1989; Stenøien et al. 2005; Lundemo et al. 2009), little attention has been paid to plant-pollinator interactions involving *A. thaliana*. The flowers of the species are small, only about 2-4 mm in diameter (Tutin et al. 1993). Clapham et al. (1962) mention it being visited by various insects, but that self-pollination is still the rule in this species. Hence, *A. thaliana* does not seem like an appropriate model for pollinator studies. Previous studies on aspects of ecology and population dynamics in the species mention only that it is most likely visited by insects like hover-flies, Syrphidae (Snape and Lawrence 1971; Lawrence 1976), but never by bees (Lawrence 1976). To my knowledge, only one study has so far described the pollinator assemblage in *A. thaliana*, using a semi-natural population in Germany (Hoffmann et al. 2003). In this study, insects from the orders Hymenoptera, Thysanoptera and Diptera accounted for the main bulk of flower visitors, but on average, the proportion of flowers receiving a visit was as low as 0.8%.

As *A. thaliana* flowers are very small and the plant itself is close to exclusively self-pollinating, one may wonder why insects visit the plant at all. However, while its flowers appear small and white to us, this is not necessarily the case for insects. Most insect species have a visual spectrum extending down to smaller wavelengths than humans can comprehend, hence most flowers appearing white to us may instead be perceived as dark coloured due to their absorption of UV light (Kevan et al. 1996). Emission of volatiles from floral and vegetative organs is another mode of attracting insects, and *A. thaliana* has been found to emit a blend of various chemicals (Tholl et al. 2005). Finally, as a potential way of rewarding visiting insects in addition to pollen, *A. thaliana* possesses two pairs of nectaries, located at the base of the flowers (Kram et al. 2009), although the amount of nectar produced is very small compared to other species in the Brassicaceae (Davis et al. 1998).

Visitation frequency by potential pollinators is dependent upon both abiotic factors like temperature (McCall and Primack 1992), and biotic factors like size of inflorescence (Conner and Rush 1996) and density of nearby flowering individuals, including other species (Kunin 1993; Dauber et al. 2010). The pollinator assemblage, the group of species of potential pollinators regularly visiting a plant species at a given locality, can be expected to be lower at

northern latitudes. This is so because in general, insect diversity is thought to be negatively correlated with latitude (see e.g. Hillebrand 2004). It may thus be expected that fewer species and/or fewer genera act as flower visitors to *A. thaliana* in Central Norway than was found in the German population by Hoffmann et al. (2003).

Knowledge on plant-pollinator interactions is deficient in *A. thaliana*, both in terms of what kind of insects visiting it, and what consequences this may have for the species' population dynamics. The purpose of this study was thus to 1) measure flower visitation rates in a natural population of *A. thaliana* in Central Norway and investigate how this varied through the flowering season, 2) identify the potential pollinator assemblage at this northern locality, and 3) examine what effect flower visitation has on genetic structure in this population.

Materials and methods

Field work was carried out in a natural population of *A. thaliana* at Lauset, Norway (N63˚24.466, E10˚04.878; 28 m a.s.l.) during the flowering season in 2007. The population is growing on exposed soil upon a rock outcrop next to a road, covering an area of approximately 6.5×1 meter, and had a census size of about 400 individuals during the registration period. *A. thaliana* density was quite variable throughout the population and observations were confined to a 95×40 cm patch, containing about 250 plants. Most individuals in this dense patch were small, having approximately 5-10 flowers each and being 5-15 cm tall.

Registrations were conducted from 9th to 30th of May 2007, when very few *A. thaliana* individuals were still flowering. The number of open *A. thaliana* flowers, as well as the temperature within the patch, was registered after each 30 minute observation period. Observations were conducted from when the flowers opened in the morning to when they closed in the afternoon, and only on days without longer periods of rain. During each observation period, all visits by insects to *A. thaliana* flowers were recorded. If an insect visited a flower, left the observation patch, and then returned to the same flower, it was counted as two visits. To avoid depleting the local pollinator pool through exhaustive sampling, insects were identified visually to group (solitary bees, other Hymenopterans, Syrphids, other Dipterans, and beetles) in the field, but a number of individuals from each group were collected for subsequent identification to genus or species level in order to get a more detailed picture of the pollinator assemblage. Of other plant species occurring close to

the observation patch, only *Taraxacum officinale* (Weber) and *Fragaria vesca* (L.) were observed flowering during the observation period, both of which may potentially act as competitors to *A. thaliana* for pollinator attraction.

Data on registration were ln-transformed to improve normality, and associations between daily insect visitation rates, daily mean temperature and maximum temperature, and number of available flowers were tested for using multiple correlation analysis in SPSS version 14.0 (Windows version 14.0, SPSS Inc., Chicago, IL) using a stepwise Bonferroni correction (Holm 1979).

Siliques from *A. thaliana* were sampled at the locality in 2006, 2007 and 2008. The sampling procedure was slightly different between the years. In 2006, sampling was done throughout the whole population, while in 2007, only siliques from the observation patch were sampled. In 2008, the procedure from the previous year was repeated, but another sampling, covering the whole population, was done as well. In addition, the soil seed bank was sampled in 2008 before that year's seed rain. Seeds from siliques, as well as from the soil samples, were grown in a green house until the plants were large enough for sampling. DNA was extracted from leaves of as many maternal families as possible from seedlings germinated from siliques, as well as from all individuals germinating from the soil seed bank, and subsequently screened using 94 single nucleotide polymorphism (SNP) markers. The marker set used was the same as in Lundemo et al. (2009), except for seven markers that did not function and were replaced by five new markers (Table S1). SNP analysis was conducted at the Centre of Integrative Genetics, CIGENE, Ås. For a more detailed description of the methods used, see Lundemo et al. (2009).

Observed heterozygosity (H_O) and expected heterozygosity (H_E) were calculated using GenAlex 6.2 (Peakall and Smouse 2006), whereas the inbreeding coefficient (F_{IS}) was estimated in Arlequin 3.11 (Excoffier et al. 2005). Arlequin was also used to perform a global differentiation test (Weir and Hill 2002) to test for genetic differentiation between cohorts. The selfing rate, *S*, was calculated using the inbreeding equilibrium equation in Allard et al. (1968). Proportion of polymorphic loci (*PPL*) was calculated as the proportion of polymorphic loci, i.e. frequency of most common allele <0.95, divided by the total number of genotyped loci.

Results

Insect visitation was registered for a total of 17 observation days, and totally 119 observation periods were conducted. The number of insects captured, as well as total number of visiting insects, varied greatly throughout the period (Fig. 1). In total 255 different individuals of insects and arachnids were observed to visit the flowers, and 69 of these were captured for subsequent identification. Most of the flower visits were by solitary bees (69.8%), but Syrphids (9.4%) and other Dipterans (12.9%) were also important visitors (Table 1). The group termed solitary bees consisted of one species only, *Lasioglossum fratellum* (Perez), which at this locality was by far the most common flower visitor. The Syrphids, on the other hand, were a far more diverse group, consisting of totally 10 different species (Table 1). Other Dipterans were mainly individuals from the closely related families Sciaridae and Mycetophilidae. Flowers were in general open between 0900 and 1600 hours, although most flower visits occurred during the middle of the day, and 70% of all visits were recorded between 1000 and 1300 hours (results not shown). To calculate the total number of visits to the patch, the mean rate of flowers visited per individual based on only non-captured visitors (mean=1.75) was extrapolated to total number of visitors registered, giving an estimate of 447 visits for the flowering period. When summarizing the maximum number of open flowers each day, this gave an approximate number of open flowers available for insects in the patch throughout the flowering season. The total number of open flowers was thus 6365, giving an estimate of 0.07 visits per flower. This can be interpreted as 7% of all open flowers receiving a visit by a potential pollinator.

Insect visitation rates were positively correlated with daily average temperature ($P=0.047$), but not after a stepwise Bonferroni correction. In addition, insect visitation rates was positively correlated with daily maximum temperature ($P=0.001$), but not with maximum number of open flowers per day ($P>0.5$) (Spearman rank correlation).

In total 90 *A. thaliana* seedlings were screened for SNPs, distributed as following; 2006 (8 individuals from throughout the population), 2007 (18 individuals from the observation patch), 2008 (31 individuals from the observation patch, and 9 individuals from throughout the population), and 2008 seed bank (24 individuals from throughout the population). A summary of population genetic parameters can be found in Table 2. The individuals sampled in 2007 constituted one genotype only, and were thus completely inbreeding (S=1). Sampling the same patch in 2008, S was found to be 0.98. The population sample in 2008 was completely

inbreeding (*S*=1), but genetic diversity was substantially higher than in 2007 (H_E=0.073 and 0, respectively). The seed bank was also completely inbred (*S*=1), though several genotypes were present. The 2008 cohort sampled throughout the whole population, was significantly different from the cohorts sampled within the observation patch in both 2007 and 2008, and the 2008 seed bank, but not from the 2006 sampling, which also covered the whole population and had a similar sample size (global differentiation test; $P<0.05$). No other comparisons were significant (Table 3; $P>0.05$).

Discussion

A. thaliana had higher flower visitation rates in the study population than expected, despite it being located in the northern part (63˚ N) of the species' distribution range in Europe. The most common flowers visitors were solitary bees, but the visits apparently resulted in no outcrossing events.

Plant functions involved in insect attraction can be divided into means of attraction and means of rewarding. *A. thaliana* emits a blend of volatiles dominated by sesquiterpenes, and it has been shown that various accessions have more or less the same volatile composition (Tholl et al. 2005). This is in contrast to the perennial relative *A. lyrata*, which has a blend dominated by benzenoid compounds, as well as rates of volatile emission up to several magnitudes higher than in *A. thaliana* (Abel et al. 2009). In *A. lyrata*, small bees and flies have been found to be the dominant flower visitors in Central Europe (Clauss and Koch 2006), whereas Syrphids and other flies were most common in Sweden (Sandring and Ågren 2009). This shows that the two *Arabidopsis* relatives attract insects from the same insect groups, while at the same time, emission of floral volatiles are very different concerning both composition and amount. Nectar production in *A. thaliana* is also very low compared to other species in the Brassicaceae (Davis et al. 1998). The same is also evident for pollen production in the species (on average 450 pollen grains per stamen; Pylatuik et al. 1998), e.g. when compared to the annual and largely outcrossing species *Raphanus sativus* (on average 26000 pollen grains per stamen; Stanton and Preston 1988).

Compared to the study by Hoffmann et al. (2003), the Norwegian population had a visitation rate of almost one order of magnitude higher (0.8% and 7.0%, respectively). In addition, solitary bees were far more frequent in the Norwegian population than in the German

population (69.8% and 33.6%, respectively). The difference in visitation rates is a bit surprising, especially considering the fact that the German population contained more flowers, and was located within a botanical garden, both of which should contribute to a higher attraction level for insect visitors (Kunin 1993; Dauber et al. 2010). On the other hand, such conditions may cause a higher competition for pollinators among the *Arabidopsis* flowers, as well as competition for attraction with other plant species flowering at the same time (cf. Rathcke 1983). As the Norwegian locality was relatively small compared to the German population, and the presence of other flowering plant species was low, flowering individuals may experience less competition for flower visitors. *A. thaliana* flowers can thus constitute an important resource for the insects visiting this locality. Further studies would be needed to investigate whether numbers of open flowers of own species compared to open flowers of other species is more important for the visitation rate. As expected, the pollinator assemblage was dominated by a few species, in this study the bee *L. fratellum*, and the diversity of Hymenopterans was markedly lower than in Hoffmann et al. (2003). Still, the diversity of Syrphids was as large or perhaps even larger at the Norwegian locality, while Thysanopterans were much less common. As such, making general predictions about the pollinator assemblage is difficult, and the efficiency of the different groups in contributing to pollen transfer is still not known.

Estimates on time since most recent common ancestor between *A. thaliana* and *A. lyrata* vary from 5 (Koch et al. 2000) to 8.7 (Ossowski et al. 2010) million years. The evolution of self-compatibility in *A. thaliana* followed this divergence event, the transition to the selfing state estimated to have taken place about 1 million years ago (Tang et al. 2007). Through time, this has resulted in *A. thaliana* developing rather small flowers compared to *A. lyrata*, and floral organs containing rewards for pollinators have been reduced as well. For this reason, the species can be perceived as insect pollinated although the number of visits are few, and the chances for successful cross fertilization small. Tan et al. (2005) mention that observed heterozygosity should be mainly due to insect pollination, although dispersal of pollen by wind is also possible. Studies of natural populations of *A. thaliana* have shown outcrossing levels (1%; Stenøien et al. 2005; 4%; Lundemo et al. 2009) that suggest chance outcrossing events are somewhat more common than earlier believed (0.3%; Abbott and Gomes 1989).

As only one genotype was registered, no outcrossing was detected in plants sampled within the observation patch in 2007. This could be due to a low sample size, but as the seedlings

that germinated from the seed bank, which should consist of seeds from 2007 and earlier, also were found to have no outcrossing (*S*=1), it seems plausible that none of the observed visits this season lead to successful pollen transfer and cross-fertilization. However, heterozygous individuals were detected both in 2006 and 2008, indicating that rare outcrossing events may occur almost every year. As such, this study shows that the plants growing outside the main patch within the population can contain different genotypes, making them important for the overall population diversity. When e.g. sampling for seed stocks, sampling of one or a few individuals may lead to the capture of only a part of a population's actual genetic diversity. This stresses the importance of a thorough sampling, that one should not expect populations to be monomorphic (cf. Stenøien et al. 2005; Lundemo et al. 2009).

Conclusions

As *A. thaliana* flowers are small and potential rewards for flower visitors are not present in large amounts, very low visitation rates by potential pollinators were expected. Furthermore, the main flowering period of the species at this latitude (63º N) is May, and at this time of the year the locality can still experience bad weather and low temperatures, causing a reduction in the frequency of flower visits. Still, the number of visiting insects was quite high during the flowering period. However, there was no evidence of outcrossing in the population this season, which may indicate that a substantial number of flower visits is required for the few outcrossing events that are detected in natural populations. Despite these initial findings, the interpretation is limited by the size of the data set used, and field studies on this topic should ideally be conducted over several seasons and if possible, in several populations, to ensure consistency. This would enable a more thorough evaluation of the contribution of pollinators to population dynamics in *A. thaliana*.

Acknowledgements

I would like to thank Hans K. Stenøien for valuable discussion and comments to the manuscript, Nina Sletvold and Kirsti Stengrundet for help during field work, Mohsen Falahati-Anbaran for help with laboratory work, and Frode Ødegaard (Apidae and Curculionidae), Tore R. Nielsen (Syrphidae) and Vera Sandlund for assistance in insect identification.

References

Abbott RJ, Gomes MF (1989) Population genetic structure and outcrossing rate of *Arabidopsis thaliana* (L.) Heynh. Heredity, 62, 411-418.

Abel C, Clauss M, Schaub A, Gershenzon J, Tholl D (2009) Floral and insect-induced volatile formation in *Arabidopsis lyrata* ssp. *petraea*, a perennial, outcrossing relative of *A. thaliana*. Planta, 230, 1-11.

Clapham AR, Tutin TG, Warburg EF (1962) *Flora of the British Isles* (2nd ed). Cambridge University Press, Cambridge, UK.

Clauss MJ, Koch MA. (2006) Poorly known relatives of *Arabidopsis thaliana*. Trends in Plant Science, 11, 449-459.

Conner JK, Rush S (1996) Effects of flower size and number on pollinator visitation to wild radish, *Raphanus raphanistrum*. Oecologia, 105, 509-516.

Dauber J, Biesmeijer JC, Gabriel D, et al. (2010) Effects of patch size and density on flower visitation and seed set of wild plants: a pan-European approach. Journal of Ecology, 98, 188-196.

Davis AR, Pylatuik JD, Paradis JC, Low NH (1998) Nectar-carbohydrate production and composition vary in relation to nectar anatomy and location within individual flowers of several species of Brassicaceae. Planta, 205, 305-318.

Excoffier L, Laval G, Schneider S (2005) Arlequin ver. 3.0: An integrated software package for population genetics data analysis. Evolutionary Bioinformatics Online, 1, 47-50.

Hillebrand H (2004) On the generality of the latitudinal diversity gradient. The American Naturalist, 163, 192-211.

Hoffmann MH, Bremer M, Schneider K, et al. (2003) Flower visitors in a natural population of *Arabidopsis thaliana*. Plant Biology, 5, 491-494.

Holm S (1979) A simple sequentially rejective multiple test procedure. Scandinavian Journal of Statistics, 6, 65-70.

Kevan P, Giurfa M, Chittka L (1996) Why are there so many and so few white flowers? Trends in Plant Science, 1, 280-284.

Koch MA, Haubold B, Mitchell-Olds T (2000) Comparative evolutionary analysis of chalcone synthase and alcohol dehydrogenase loci in *Arabidopsis*, *Arabis*, and related genera. Molecular Biology and Evolution, 17, 1483-1498.

Kram BW, Xu WW, Carter CJ (2009) Uncovering the *Arabidopsis thaliana* nectary transcriptome: investigation of differential gene expression in floral nectariferous tissues. BMC Plant Biology, 9, 92. doi:10.1186/1471-2229-9-92.

Kunin WE (1993) Sex and the single mustard: Population density and pollinator behavior effects on seed-set. Ecology, 74, 2145-2160.

Lawrence MJ (1976) Variations in natural populations of Arabidopsis thaliana (L.) Heynh. In: *The biology and chemistry of the Cruciferae* (eds. Vaughan JG, Macleod AJ, Jones BMG), pp 167-190. Academic Press, London, UK.

Lundemo S, Falahati-Anbaran M, Stenøien HK (2009) Seed banks cause elevated generation times and effective population sizes of *Arabidopsis thaliana* in Northern Europe. Molecular Ecology, 18, 2798-2811.

McCall C, Primack RB (1992) Influence of flower characteristics, weather, time of day, and season on insect visitation rates in three plant communities. American Journal of Botany, 79, 434-442.

Ossowski S, Schneeberger K, Lucas-Lledó JI, et al. (2010) The rate and molecular spectrum of spontaneous mutations in *Arabidopsis thaliana*. Science, 327, 92-94.

Peakall R, Smouse PE (2006) GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. Molecular Ecology Notes, 6, 288-295.

Pylatuik JD, Bonham-Smith PC, Davis AR (1998) The additional stamens of *flo*10-1 mutants of *Arabidopsis thaliana* are compromised in production and viability of pollen. Canadian Journal of Botany, 76, 1733-7142.

Rathcke B (1983) Competition and facilitation among plants for pollination. In: *Pollination biology* (ed. Real L), pp 305-329. Academic Press, Orlando, FL.

Sandring S, Ågren J (2009) Pollinator-mediated selection on floral display and flowering time in the perennial herb *Arabidopsis lyrata*. Evolution, 63, 1292-1300.

Snape JW, Lawrence MJ (1971) The breeding system of *Arabidopsis thaliana*. Heredity, 27, 299-302.

Stanton ML, Preston RE (1988) Ecological consequences and phenotypic correlates of petal size variation in wild radish, *Raphanus sativus* (Brassicaceae). American Journal of Botany, 75, 528-539.

Stenøien HK, Fenster CB, Kuittinen H, Savolainen O (2005) Genetic variability in natural populations of *Arabidopsis thaliana* in Northern Europe. Molecular Ecology, 14, 137-148.

Tan YY, Xu HH, Tao WJ, et al. (2005) Transgenic GFP as a molecular marker for approaches to quantify pollination mechanism and gene flow in *Arabidopsis thaliana*. Plant Biology, 7, 405-410.

Tang C, Toomajian C, Sherman-Broyles S, et al. (2007) The evolution of selfing in *Arabidopsis thaliana*. Science, 317, 1070-1072.

Tholl D, Chen F, Petri J, Gershenzon J, Pichersky E (2005) Two sesquiterpene synthases are responsible for the complex mixture of sesquiterpenes emitted from *Arabidopsis* flowers. The Plant Journal, 42, 757-771.

Tutin TG, Burges NA, Chater AO (eds.) et al. (1993) *Flora Europaea – Vol 1 Psilotaceae to Platanaceae* (2nd ed). Cambridge University Press, Cambridge, UK.

Weir BS, Hill WG (2002) Estimating F-statistics. Annual Review of Genetics, 36, 721-750.

Tables

Table 1: Insects and arachnids captured (n=69) visiting *A. thaliana* flowers during the flowering season in 2007.

Class	Order	Superfamily	Family	Species	Number of inds
Insecta	Hymenoptera		Apidae	*Lasioglossum fratellum* (Perez)	26
			Formicidae	*Lasius niger* (L.)	1
			Ichneumonidae		1
		Chalcidoidea			1
		Cynipoidea			1
		Proctotrupoidea			1
	Diptera		Syrphidae	*Rhingia campestris* (Meigen)	3
				Parasyrphus lineolus (Zett.)	2
				Parasyrphus macularis (Zett.)	1
				Dasysyrphus pauxillus (Will.)	1
				Dasysyrphus pinastri (De Geer)	1
				Neoascia tenur (Harris)	1
				Orthonevra geniculata (Meigen)	1
				Platycheirus albimanus (Fabr.)	1
				Platycheirus ambiguus (Fall.)	1
				Melanostoma mellinum (L.)	1
			Sciaridae		5
			Mycetophilidae		5
			Phoridae		1
			Anthomyiidae		1
			Carnidae		1
	Coleoptera		Scarabaeidae		2
			Curculionidae	*Miarus campanulae* (L.)	4
			Cholevinae		1
	Thysanoptera				2
Arachnida	Araneae		Tetragnathidae		1
	Prostigmata		Tetranychidae		2

Table 2: Summary of population genetic parameters for the different sampling batches of *A. thaliana* individuals from Lauset in 2006, 2007 and 2008. AB-a=above ground individuals from the observation patch only, AB-b=above ground individuals from throughout the whole population, SB=seed bank. H_O=observed heterozygosity, H_E=expected heterozygosity, F_{IS}=inbreeding coefficient, *PPL*=proportion of polymorphic loci, *N*=number of samples for SNP analysis, *S*=selfing rate. For H_O and H_E, average values ± SE are shown.

	H_O	H_E	F_{IS}	*PPL*	*N*	*S*
2006 AB-b	0.001±0.001	0.015±0.006	0.92	0.07	8	0.96
2007 AB-a	0	0	1	0	11	1
2008 AB-a	0.003±0.001	0.081±0.017	0.96	0.23	31	0.98
2008 AB-b	0	0.073±0.017	1	0.17	9	1
2008 SB	0	0.040±0.008	1	0.23	24	1

Table 3: Global differentiation test (P-values) for the different sampling batches of *A. thaliana* individuals sampled at Lauset in 2006, 2007 and 2008. AB-a=above ground individuals from the observation patch only, AB-b=above ground individuals from throughout the whole population, SB=seed bank. Significant values are in bold.

	2006 AB-b	2007 AB-a	2008 AB-a	2008 AB-b
2007 AB-a	0.201			
2008 AB-a	0.633	0.409		
2008 AB-b	0.098	**<0.001**	**0.013**	
2008 SB	0.626	0.669	0.324	**0.008**

Figures

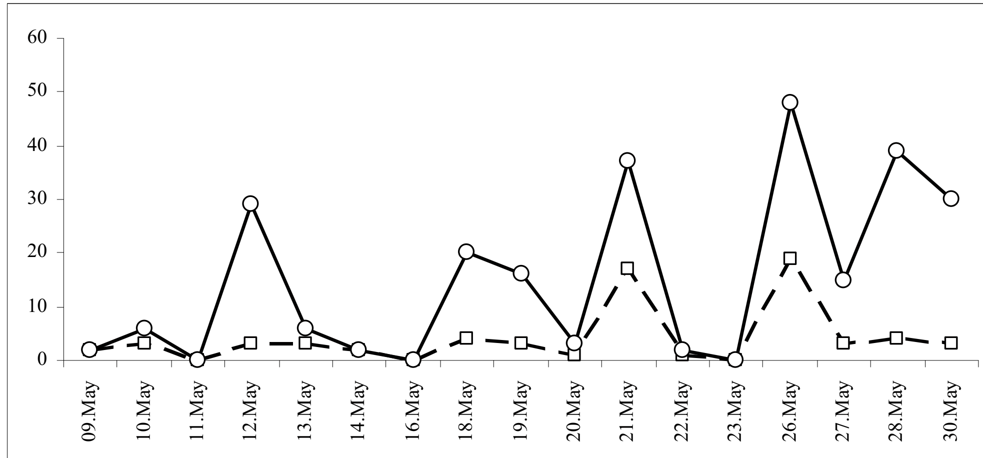


Figure 1: Number of insects and arachnids registered in the observation patch at Lauset in May 2007, split into number of individuals captured (squares; thick, dotted line), and total number of visiting individuals (circles; thick, solid line).

Supplementary material

Table S1: Summary of markers that did not function in this study, as well as new markers that were added, compared to the marker set used in Lundemo et al. (2009). Marker names are from the MASC single nucleotide polymorphism (SNP) data base (http://www2.mpiz-koeln.mpg.de/masc/search_masc_snps.php).

Non-functioning markers	New markers
MASC04170	MASC02812
MASC04199	MASC03154
MASC04209	MASC04005
MASC04819	MASC04209
MASC05258	MASC04317
MASC05360	
MASC05962	

Doctoral theses in Biology

Norwegian University of Science and Technology

Department of Biology

Year	Name	Degree	Title
1974	Tor-Henning Iversen	Dr. philos Botany	The roles of statholiths, auxin transport, and auxin metabolism in root gravitropism
1978	Tore Slagsvold	Dr. philos. Zoology	Breeding events of birds in relation to spring temperature and environmental phenology.
1978	Egil Sakshaug	Dr.philos Botany	"The influence of environmental factors on the chemical composition of cultivated and natural populations of marine phytoplankton"
1980	Arnfinn Langeland	Dr. philos. Zoology	Interaction between fish and zooplankton populations and their effects on the material utilization in a freshwater lake.
1980	Helge Reinertsen	Dr. philos Botany	The effect of lake fertilization on the dynamics and stability of a limnetic ecosystem with special reference to the phytoplankton
1982	Gunn Mari Olsen	Dr. scient Botany	Gravitropism in roots of *Pisum sativum* and *Arabidopsis thaliana*
1982	Dag Dolmen	Dr. philos. Zoology	Life aspects of two sympartic species of newts (*Triturus, Amphibia*) in Norway, with special emphasis on their ecological niche segregation.
1984	Eivin Røskaft	Dr. philos. Zoology	Sociobiological studies of the rook *Corvus frugilegus.*
1984	Anne Margrethe Cameron	Dr. scient Botany	Effects of alcohol inhalation on levels of circulating testosterone, follicle stimulating hormone and luteinzing hormone in male mature rats
1984	Asbjørn Magne Nilsen	Dr. scient Botany	Alveolar macrophages from expectorates – Biological monitoring of workers exosed to occupational air pollution. An evaluation of the AM-test
1985	Jarle Mork	Dr. philos. Zoology	Biochemical genetic studies in fish.
1985	John Solem	Dr. philos. Zoology	Taxonomy, distribution and ecology of caddisflies (*Trichoptera*) in the Dovrefjell mountains.
1985	Randi E. Reinertsen	Dr. philos. Zoology	Energy strategies in the cold: Metabolic and thermoregulatory adaptations in small northern birds.
1986	Bernt-Erik Sæther	Dr. philos. Zoology	Ecological and evolutionary basis for variation in reproductive traits of some vertebrates: A comparative approach.
1986	Torleif Holthe	Dr. philos. Zoology	Evolution, systematics, nomenclature, and zoogeography in the polychaete orders *Oweniimorpha* and *Terebellomorpha*, with special reference to the Arctic and Scandinavian fauna.
1987	Helene Lampe	Dr. scient. Zoology	The function of bird song in mate attraction and territorial defence, and the importance of song repertoires.
1987	Olav Hogstad	Dr. philos. Zoology	Winter survival strategies of the Willow tit *Parus montanus*.

1987 Jarle Inge Holten	Dr. philos Botany	Autecological investigations along a coust-inland transect at Nord-Møre, Central Norway
1987 Rita Kumar	Dr. scient Botany	Somaclonal variation in plants regenerated from cell cultures of *Nicotiana sanderae* and *Chrysanthemum morifolium*
1987 Bjørn Åge Tømmerås	Dr. scient. Zoology	Olfaction in bark beetle communities: Interspecific interactions in regulation of colonization density, predator - prey relationship and host attraction.
1988 Hans Christian Pedersen	Dr. philos. Zoology	Reproductive behaviour in willow ptarmigan with special emphasis on territoriality and parental care.
1988 Tor G. Heggberget	Dr. philos. Zoology	Reproduction in Atlantic Salmon (*Salmo salar*): Aspects of spawning, incubation, early life history and population structure.
1988 Marianne V. Nielsen	Dr. scient. Zoology	The effects of selected environmental factors on carbon allocation/growth of larval and juvenile mussels (*Mytilus edulis*).
1988 Ole Kristian Berg	Dr. scient. Zoology	The formation of landlocked Atlantic salmon (*Salmo salar* L.).
1989 John W. Jensen	Dr. philos. Zoology	Crustacean plankton and fish during the first decade of the manmade Nesjø reservoir, with special emphasis on the effects of gill nets and salmonid growth.
1989 Helga J. Vivås	Dr. scient. Zoology	Theoretical models of activity pattern and optimal foraging: Predictions for the Moose *Alces alces*.
1989 Reidar Andersen	Dr. scient. Zoology	Interactions between a generalist herbivore, the moose *Alces alces*, and its winter food resources: a study of behavioural variation.
1989 Kurt Ingar Draget	Dr. scient Botany	Alginate gel media for plant tissue culture,
1990 Bengt Finstad	Dr. scient. Zoology	Osmotic and ionic regulation in Atlantic salmon, rainbow trout and Arctic charr: Effect of temperature, salinity and season.
1990 Hege Johannesen	Dr. scient. Zoology	Respiration and temperature regulation in birds with special emphasis on the oxygen extraction by the lung.
1990 Åse Krøkje	Dr. scient Botany	The mutagenic load from air pollution at two work-places with PAH-exposure measured with Ames Salmonella/microsome test
1990 Arne Johan Jensen	Dr. philos. Zoology	Effects of water temperature on early life history, juvenile growth and prespawning migrations of Atlantic salmion (*Salmo salar*) and brown trout (*Salmo trutta*): A summary of studies in Norwegian streams.
1990 Tor Jørgen Almaas	Dr. scient. Zoology	Pheromone reception in moths: Response characteristics of olfactory receptor neurons to intra- and interspecific chemical cues.
1990 Magne Husby	Dr. scient. Zoology	Breeding strategies in birds: Experiments with the Magpie *Pica pica*.
1991 Tor Kvam	Dr. scient. Zoology	Population biology of the European lynx (*Lynx lynx*) in Norway.
1991 Jan Henning L'Abêe Lund	Dr. philos. Zoology	Reproductive biology in freshwater fish, brown trout *Salmo trutta* and roach *Rutilus rutilus* in particular.
1991 Asbjørn Moen	Dr. philos Botany	The plant cover of the boreal uplands of Central Norway. I. Vegetation ecology of Sølendet nature reserve; haymaking fens and birch woodlands
1991 Else Marie Løbersli	Dr. scient Botany	Soil acidification and metal uptake in plants

1991 Trond Nordtug	Dr. scient. Zoology	Reflctometric studies of photomechanical adaptation in superposition eyes of arthropods.
1991 Thyra Solem	Dr. scient Botany	Age, origin and development of blanket mires in Central Norway
1991 Odd Terje Sandlund	Dr. philos. Zoology	The dynamics of habitat use in the salmonid genera *Coregonus* and *Salvelinus*: Ontogenic niche shifts and polymorphism.
1991 Nina Jonsson	Dr. philos.	Aspects of migration and spawning in salmonids.
1991 Atle Bones	Dr. scient Botany	Compartmentation and molecular properties of thioglucoside glucohydrolase (myrosinase)
1992 Torgrim Breiehagen	Dr. scient. Zoology	Mating behaviour and evolutionary aspects of the breeding system of two bird species: the Temminck's stint and the Pied flycatcher.
1992 Anne Kjersti Bakken	Dr. scient Botany	The influence of photoperiod on nitrate assimilation and nitrogen status in timothy (*Phleum pratense* L.)
1992 Tycho Anker-Nilssen	Dr. scient. Zoology	Food supply as a determinant of reproduction and population development in Norwegian Puffins *Fratercula arctica*
1992 Bjørn Munro Jenssen	Dr. philos. Zoology	Thermoregulation in aquatic birds in air and water: With special emphasis on the effects of crude oil, chemically treated oil and cleaning on the thermal balance of ducks.
1992 Arne Vollan Aarset	Dr. philos. Zoology	The ecophysiology of under-ice fauna: Osmotic regulation, low temperature tolerance and metabolism in polar crustaceans.
1993 Geir Slupphaug	Dr. scient Botany	Regulation and expression of uracil-DNA glycosylase and O^6-methylguanine-DNA methyltransferase in mammalian cells
1993 Tor Fredrik Næsje	Dr. scient. Zoology	Habitat shifts in coregonids.
1993 Yngvar Asbjørn Olsen	Dr. scient. Zoology	Cortisol dynamics in Atlantic salmon, *Salmo salar* L.: Basal and stressor-induced variations in plasma levels ans some secondary effects.
1993 Bård Pedersen	Dr. scient Botany	Theoretical studies of life history evolution in modular and clonal organisms
1993 Ole Petter Thangstad	Dr. scient Botany	Molecular studies of myrosinase in Brassicaceae
1993 Thrine L. M. Heggberget	Dr. scient. Zoology	Reproductive strategy and feeding ecology of the Eurasian otter *Lutra lutra*.
1993 Kjetil Bevanger	Dr. scient. Zoology	Avian interactions with utility structures, a biological approach.
1993 Kåre Haugan	Dr. scient Bothany	Mutations in the replication control gene trfA of the broad host-range plasmid RK2
1994 Peder Fiske	Dr. scient. Zoology	Sexual selection in the lekking great snipe (*Gallinago media*): Male mating success and female behaviour at the lek.
1994 Kjell Inge Reitan	Dr. scient Botany	Nutritional effects of algae in first-feeding of marine fish larvae
1994 Nils Røv	Dr. scient. Zoology	Breeding distribution, population status and regulation of breeding numbers in the northeast-Atlantic Great Cormorant *Phalacrocorax carbo carbo*.
1994 Annette-Susanne Hoepfner	Dr. scient Botany	Tissue culture techniques in propagation and breeding of Red Raspberry (*Rubus idaeus* L.)

1994 Inga Elise Bruteig	Dr. scient Bothany	Distribution, ecology and biomonitoring studies of epiphytic lichens on conifers
1994 Geir Johnsen	Dr. scient Botany	Light harvesting and utilization in marine phytoplankton: Species-specific and photoadaptive responses
1994 Morten Bakken	Dr. scient. Zoology	Infanticidal behaviour and reproductive performance in relation to competition capacity among farmed silver fox vixens, *Vulpes vulpes*.
1994 Arne Moksnes	Dr. philos. Zoology	Host adaptations towards brood parasitism by the Cockoo.
1994 Solveig Bakken	Dr. scient Bothany	Growth and nitrogen status in the moss *Dicranum majus* Sm. as influenced by nitrogen supply
1994 Torbjørn Forseth	Dr. scient. Zoology	Bioenergetics in ecological and life history studies of fishes.
1995 Olav Vadstein	Dr. philos Botany	The role of heterotrophic planktonic bacteria in the cycling of phosphorus in lakes: Phosphorus requirement, competitive ability and food web interactions.
1995 Hanne Christensen	Dr. scient. Zoology	Determinants of Otter *Lutra lutra* distribution in Norway: Effects of harvest, polychlorinated biphenyls (PCBs), human population density and competition with mink *Mustela vision*.
1995 Svein Håkon Lorentsen	Dr. scient. Zoology	Reproductive effort in the Antarctic Petrel *Thalassoica antarctica*; the effect of parental body size and condition.
1995 Chris Jørgen Jensen	Dr. scient. Zoology	The surface electromyographic (EMG) amplitude as an estimate of upper trapezius muscle activity
1995 Martha Kold Bakkevig	Dr. scient. Zoology	The impact of clothing textiles and construction in a clothing system on thermoregulatory responses, sweat accumulation and heat transport.
1995 Vidar Moen	Dr. scient. Zoology	Distribution patterns and adaptations to light in newly introduced populations of *Mysis relicta* and constraints on Cladoceran and Char populations.
1995 Hans Haavardsholm Blom	Dr. philos Bothany	A revision of the *Schistidium apocarpum* complex in Norway and Sweden.
1996 Jorun Skjærmo	Dr. scient Botany	Microbial ecology of early stages of cultivated marine fish; inpact fish-bacterial interactions on growth and survival of larvae.
1996 Ola Ugedal	Dr. scient. Zoology	Radiocesium turnover in freshwater fishes
1996 Ingibjørg Einarsdottir	Dr. scient. Zoology	Production of Atlantic salmon (*Salmo salar*) and Arctic charr (*Salvelinus alpinus*): A study of some physiological and immunological responses to rearing routines.
1996 Christina M. S. Pereira	Dr. scient. Zoology	Glucose metabolism in salmonids: Dietary effects and hormonal regulation.
1996 Jan Fredrik Børseth	Dr. scient. Zoology	The sodium energy gradients in muscle cells of *Mytilus edulis* and the effects of organic xenobiotics.
1996 Gunnar Henriksen	Dr. scient. Zoology	Status of Grey seal *Halichoerus grypus* and Harbour seal *Phoca vitulina* in the Barents sea region.
1997 Gunvor Øie	Dr. scient Bothany	Eevalution of rotifer *Brachionus plicatilis* quality in early first feeding of turbot *Scophtalmus maximus* L. larvae.
1997 Håkon Holien	Dr. scient Botany	Studies of lichens in spurce forest of Central Norway. Diversity, old growth species and the relationship to site and stand parameters.

1997 Ole Reitan	Dr. scient. Zoology	Responses of birds to habitat disturbance due to damming.
1997 Jon Arne Grøttum	Dr. scient. Zoology	Physiological effects of reduced water quality on fish in aquaculture.
1997 Per Gustav Thingstad	Dr. scient. Zoology	Birds as indicators for studying natural and human-induced variations in the environment, with special emphasis on the suitability of the Pied Flycatcher.
1997 Torgeir Nygård	Dr. scient. Zoology	Temporal and spatial trends of pollutants in birds in Norway: Birds of prey and Willow Grouse used as Biomonitors.
1997 Signe Nybø	Dr. scient. Zoology	Impacts of long-range transported air pollution on birds with particular reference to the dipper *Cinclus cinclus* in southern Norway.
1997 Atle Wibe	Dr. scient. Zoology	Identification of conifer volatiles detected by receptor neurons in the pine weevil (*Hylobius abietis*), analysed by gas chromatography linked to electrophysiology and to mass spectrometry.
1997 Rolv Lundheim	Dr. scient. Zoology	Adaptive and incidental biological ice nucleators.
1997 Arild Magne Landa	Dr. scient. Zoology	Wolverines in Scandinavia: ecology, sheep depredation and conservation.
1997 Kåre Magne Nielsen	Dr. scient Botany	An evolution of possible horizontal gene transfer from plants to sail bacteria by studies of natural transformation in *Acinetobacter calcoacetius*.
1997 Jarle Tufto	Dr. scient. Zoology	Gene flow and genetic drift in geographically structured populations: Ecological, population genetic, and statistical models
1997 Trygve Hesthagen	Dr. philos. Zoology	Population responces of Arctic charr (*Salvelinus alpinus* (L.)) and brown trout (*Salmo trutta* L.) to acidification in Norwegian inland waters
1997 Trygve Sigholt	Dr. philos. Zoology	Control of Parr-smolt transformation and seawater tolerance in farmed Atlantic Salmon (*Salmo salar*) Effects of photoperiod, temperature, gradual seawater acclimation, NaCl and betaine in the diet
1997 Jan Østnes	Dr. scient. Zoology	Cold sensation in adult and neonate birds
1998 Seethaledsumy Visvalingam	Dr. scient Botany	Influence of environmental factors on myrosinases and myrosinase-binding proteins.
1998 Thor Harald Ringsby	Dr. scient. Zoology	Variation in space and time: The biology of a House sparrow metapopulation
1998 Erling Johan Solberg	Dr. scient. Zoology	Variation in population dynamics and life history in a Norwegian moose (*Alces alces*) population: consequences of harvesting in a variable environment
1998 Sigurd Mjøen Saastad	Dr. scient Botany	Species delimitation and phylogenetic relationships between the Sphagnum recurvum complex (Bryophyta): genetic variation and phenotypic plasticity.
1998 Bjarte Mortensen	Dr. scient Botany	Metabolism of volatile organic chemicals (VOCs) in a head liver S9 vial equilibration system in vitro.
1998 Gunnar Austrheim	Dr. scient Botany	Plant biodiversity and land use in subalpine grasslands. – A conservtaion biological approach.
1998 Bente Gunnveig Berg	Dr. scient. Zoology	Encoding of pheromone information in two related moth species

1999 Kristian Overskaug	Dr. scient. Zoology	Behavioural and morphological characteristics in Northern Tawny Owls *Strix aluco*: An intra- and interspecific comparative approach
1999 Hans Kristen Stenøien	Dr. scient Bothany	Genetic studies of evolutionary processes in various populations of nonvascular plants (mosses, liverworts and hornworts)
1999 Trond Arnesen	Dr. scient Botany	Vegetation dynamics following trampling and burning in the outlying haylands at Sølendet, Central Norway.
1999 Ingvar Stenberg	Dr. scient. Zoology	Habitat selection, reproduction and survival in the White-backed Woodpecker *Dendrocopos leucotos*
1999 Stein Olle Johansen	Dr. scient Botany	A study of driftwood dispersal to the Nordic Seas by dendrochronology and wood anatomical analysis.
1999 Trina Falck Galloway	Dr. scient. Zoology	Muscle development and growth in early life stages of the Atlantic cod (*Gadus morhua* L.) and Halibut (*Hippoglossus hippoglossus* L.)
1999 Marianne Giæver	Dr. scient. Zoology	Population genetic studies in three gadoid species: blue whiting (*Micromisistius poutassou*), haddock (*Melanogrammus aeglefinus*) and cod (*Gradus morhua*) in the North-East Atlantic
1999 Hans Martin Hanslin	Dr. scient Botany	The impact of environmental conditions of density dependent performance in the boreal forest bryophytes *Dicranum majus*, *Hylocomium splendens*, *Plagiochila asplenigides*, *Ptilium crista-castrensis* and *Rhytidiadelphus lokeus*.
1999 Ingrid Bysveen Mjølnerød	Dr. scient. Zoology	Aspects of population genetics, behaviour and performance of wild and farmed Atlantic salmon (*Salmo salar*) revealed by molecular genetic techniques
1999 Else Berit Skagen	Dr. scient Botany	The early regeneration process in protoplasts from *Brassica napus* hypocotyls cultivated under various g-forces
1999 Stein-Are Sæther	Dr. philos. Zoology	Mate choice, competition for mates, and conflicts of interest in the Lekking Great Snipe
1999 Katrine Wangen Rustad	Dr. scient. Zoology	Modulation of glutamatergic neurotransmission related to cognitive dysfunctions and Alzheimer's disease
1999 Per Terje Smiseth	Dr. scient. Zoology	Social evolution in monogamous families: mate choice and conflicts over parental care in the Bluethroat (*Luscinia s. svecica*)
1999 Gunnbjørn Bremset	Dr. scient. Zoology	Young Atlantic salmon (*Salmo salar* L.) and Brown trout (*Salmo trutta* L.) inhabiting the deep pool habitat, with special reference to their habitat use, habitat preferences and competitive interactions
1999 Frode Ødegaard	Dr. scient. Zoology	Host spesificity as parameter in estimates of arhrophod species richness
1999 Sonja Andersen	Dr. scient Bothany	Expressional and functional analyses of human, secretory phospholipase A2
2000 Ingrid Salvesen, I	Dr. scient Botany	Microbial ecology in early stages of marine fish: Development and evaluation of methods for microbial management in intensive larviculture
2000 Ingar Jostein Øien	Dr. scient. Zoology	The Cuckoo (*Cuculus canorus*) and its host: adaptions and counteradaptions in a coevolutionary arms race
2000 Pavlos Makridis	Dr. scient Botany	Methods for the microbial econtrol of live food used for the rearing of marine fish larvae
2000 Sigbjørn Stokke	Dr. scient. Zoology	Sexual segregation in the African elephant (*Loxodonta africana*)

2000 Odd A. Gulseth	Dr. philos. Zoology	Seawater tolerance, migratory behaviour and growth of Charr, (*Salvelinus alpinus*), with emphasis on the high Arctic Dieset charr on Spitsbergen, Svalbard
2000 Pål A. Olsvik	Dr. scient. Zoology	Biochemical impacts of Cd, Cu and Zn on brown trout (*Salmo trutta*) in two mining-contaminated rivers in Central Norway
2000 Sigurd Einum	Dr. scient. Zoology	Maternal effects in fish: Implications for the evolution of breeding time and egg size
2001 Jan Ove Evjemo	Dr. scient. Zoology	Production and nutritional adaptation of the brine shrimp *Artemia* sp. as live food organism for larvae of marine cold water fish species
2001 Olga Hilmo	Dr. scient Botany	Lichen response to environmental changes in the managed boreal forset systems
2001 Ingebrigt Uglem	Dr. scient. Zoology	Male dimorphism and reproductive biology in corkwing wrasse (*Symphodus melops* L.)
2001 Bård Gunnar Stokke	Dr. scient. Zoology	Coevolutionary adaptations in avian brood parasites and their hosts
2002 Ronny Aanes	Dr. scient	Spatio-temporal dynamics in Svalbard reindeer (*Rangifer tarandus platyrhynchus*)
2002 Mariann Sandsund	Dr. scient. Zoology	Exercise- and cold-induced asthma. Respiratory and thermoregulatory responses
2002 Dag-Inge Øien	Dr. scient Botany	Dynamics of plant communities and populations in boreal vegetation influenced by scything at Sølendet, Central Norway
2002 Frank Rosell	Dr. scient. Zoology	The function of scent marking in beaver (*Castor fiber*)
2002 Janne Østvang	Dr. scient Botany	The Role and Regulation of Phospholipase A_2 in Monocytes During Atherosclerosis Development
2002 Terje Thun	Dr.philos Biology	Dendrochronological constructions of Norwegian conifer chronologies providing dating of historical material
2002 Birgit Hafjeld Borgen	Dr. scient Biology	Functional analysis of plant idioblasts (Myrosin cells) and their role in defense, development and growth
2002 Bård Øyvind Solberg	Dr. scient Biology	Effects of climatic change on the growth of dominating tree species along major environmental gradients
2002 Per Winge	Dr. scient Biology	The evolution of small GTP binding proteins in cellular organisms. Studies of RAC GTPases in *Arabidopsis thaliana* and
2002 Henrik Jensen	Dr. scient Biology	Causes and consequenses of individual variation in fitness-related traits in house sparrows
2003 Jens Rohloff	Dr. philos Biology	Cultivation of herbs and medicinal plants in Norway – Essential oil production and quality control
2003 Åsa Maria O. Espmark Wibe	Dr. scient Biology	Behavioural effects of environmental pollution in threespine stickleback *Gasterosteus aculeatur* L.
2003 Dagmar Hagen	Dr. scient Biology	Assisted recovery of disturbed arctic and alpine vegetation – an integrated approach
2003 Bjørn Dahle	Dr. scient Biology	Reproductive strategies in Scandinavian brown bears
2003 Cyril Lebogang Taolo	Dr. scient Biology	Population ecology, seasonal movement and habitat use of the African buffalo (*Syncerus caffer*) in Chobe National Park, Botswana
2003 Marit Stranden	Dr.scient Biology	Olfactory receptor neurones specified for the same odorants in three related Heliothine species (*Helicoverpa armigera, Helicoverpa assulta* and *Heliothis virescens*)

Year	Name	Degree	Title
2003	Kristian Hassel	Dr.scient Biology	Life history characteristics and genetic variation in an expanding species, *Pogonatum dentatum*
2003	David Alexander Rae	Dr.scient Biology	Plant- and invertebrate-community responses to species interaction and microclimatic gradients in alpine and Artic environments
2003	Åsa A Borg	Dr.scient Biology	Sex roles and reproductive behaviour in gobies and guppies: a female perspective
2003	Eldar Åsgard Bendiksen	Dr.scient Biology	Environmental effects on lipid nutrition of farmed Atlantic salmon (*Salmo Salar* L.) parr and smolt
2004	Torkild Bakken	Dr.scient Biology	A revision of Nereidinae (Polychaeta, Nereididae)
2004	Ingar Pareliussen	Dr.scient Biology	Natural and Experimental Tree Establishment in a Fragmented Forest, Ambohitantely Forest Reserve, Madagascar
2004	Tore Brembu	Dr.scient Biology	Genetic, molecular and functional studies of RAC GTPases and the WAVE-like regulatory protein complex in *Arabidopsis thaliana*
2004	Liv S. Nilsen	Dr.scient Biology	Coastal heath vegetation on central Norway; recent past, present state and future possibilities
2004	Hanne T. Skiri	Dr.scient Biology	Olfactory coding and olfactory learning of plant odours in heliothine moths. An anatomical, physiological and behavioural study of three related species (*Heliothis virescens, Helicoverpa armigera* and *Helicoverpa assulta*).
2004	Lene Østby	Dr.scient Biology	Cytochrome P4501A (CYP1A) induction and DNA adducts as biomarkers for organic pollution in the natural environment
2004	Emmanuel J. Gerreta	Dr. philos Biology	The Importance of Water Quality and Quantity in the Tropical Ecosystems, Tanzania
2004	Linda Dalen	Dr.scient Biology	Dynamics of Mountain Birch Treelines in the Scandes Mountain Chain, and Effects of Climate Warming
2004	Lisbeth Mehli	Dr.scient Biology	Polygalacturonase-inhibiting protein (PGIP) in cultivated strawberry (*Fragaria* x *ananassa*): characterisation and induction of the gene following fruit infection by *Botrytis cinerea*
2004	Børge Moe	Dr.scient Biology	Energy-Allocation in Avian Nestlings Facing Short-Term Food Shortage
2005	Matilde Skogen Chauton	Dr.scient Biology	Metabolic profiling and species discrimination from High-Resolution Magic Angle Spinning NMR analysis of whole-cell samples
2005	Sten Karlsson	Dr.scient Biology	Dynamics of Genetic Polymorphisms
2005	Terje Bongard	Dr.scient Biology	Life History strategies, mate choice, and parental investment among Norwegians over a 300-year period
2005	Tonette Røstelien	ph.d Biology	Functional characterisation of olfactory receptor neurone types in heliothine moths
2005	Erlend Kristiansen	Dr.scient Biology	Studies on antifreeze proteins
2005	Eugen G. Sørmo	Dr.scient Biology	Organochlorine pollutants in grey seal (*Halichoerus grypus*) pups and their impact on plasma thyrid hormone and vitamin A concentrations.

Year	Name	Degree	Title
2005	Christian Westad	Dr.scient Biology	Motor control of the upper trapezius
2005	Lasse Mork Olsen	ph.d Biology	Interactions between marine osmo- and phagotrophs in different physicochemical environments
2005	Åslaug Viken	PhD Biology	Implications of mate choice for the management of small populations
2005	Ariaya Hymete Sahle Dingle	PhD Biology	Investigation of the biological activities and chemical constituents of selected *Echinops* spp. growing in Ethiopia
2005	Anders Gravbrøt Finstad	ph.d Biology	Salmonid fishes in a changing climate: The winter challenge
2005	Shimane Washington Makabu	ph.d Biology	Interactions between woody plants, elephants and other browsers in the Chobe Riverfront, Botswana
2005	Kjartan Østbye	Dr.scient Biology	The European whitefish *Coregonus lavaretus* (L.) species complex: historical contingency and adaptive radiation
2006	Kari Mette Murvoll	ph.d Biology	Levels and effects of persistent organic pollutans (POPs) in seabirds Retinoids and α-tocopherol – potential biomakers of POPs in birds?
2006	Ivar Herfindal	Dr.scient Biology	Life history consequences of environmental variation along ecological gradients in northern ungulates
2006	Nils Egil Tokle	ph.d Biology	Are the ubiquitous marine copepods limited by food or predation? Experimental and field-based studies with main focus on *Calanus finmarchicus*
2006	Jan Ove Gjershaug	Dr.philos Biology	Taxonomy and conservation status of some booted eagles in south-east Asia
2006	Jon Kristian Skei	Dr.scient Biology	Conservation biology and acidification problems in the breeding habitat of amphibians in Norway
2006	Johanna Järnegren	ph.d Biology	Acesta Oophaga and Acesta Excavata – a study of hidden biodiversity
2006	Bjørn Henrik Hansen	ph.d Biology	Metal-mediated oxidative stress responses in brown trout (*Salmo trutta*) from mining contaminated rivers in Central Norway
2006	Vidar Grøtan	ph.d Biology	Temporal and spatial effects of climate fluctuations on population dynamics of vertebrates
2006	Jafari R Kideghesho	Ph.d Biology	Wildlife conservation and local land use conflicts in western Serengeti, Corridor Tanzania
2006	Anna Maria Billing	ph.d Biology	Reproductive decisions in the sex role reversed pipefish *Syngnathus typhle*: when and how to invest in reproduction
2006	Henrik Pärn	ph.d Biology	Female ornaments and reproductive biology in the bluethroat
2006	Anders J. Fjellheim	ph.d Biology	Selection and administration of probiotic bacteria to marine fish larvae
2006	P. Andreas Svensson	ph.d Biology	Female coloration, egg carotenoids and reproductive success: gobies as a model system
2007	Sindre A. Pedersen	ph.d Biology	Metal binding proteins and antifreeze proteins in the beetle *Tenebrio molitor* - a study on possible competition for the semi-essential amino acid cysteine

2007	Kasper Hancke	ph.d Biology	Photosynthetic responses as a function of light and temperature: Field and laboratory studies on marine microalgae
2007	Tomas Holmern	ph.d Biology	Bushmeat hunting in the western Serengeti: Implications for community-based conservation
2007	Kari Jørgensen	ph.d Biology	Functional tracing of gustatory receptor neurons in the CNS and chemosensory learning in the moth *Heliothis virescens*
2007	Stig Ulland	ph.d Biology	Functional Characterisation of Olfactory Receptor Neurons in the Cabbage Moth, (*Mamestra brassicae* L.) (Lepidoptera, Noctuidae). Gas Chromatography Linked to Single Cell Recordings and Mass Spectrometry
2007	Snorre Henriksen	ph.d Biology	Spatial and temporal variation in herbivore resources at northern latitudes
2007	Roelof Frans May	ph.d Biology	Spatial Ecology of Wolverines in Scandinavia
2007	Vedasto Gabriel Ndibalema	ph.d Biology	Demographic variation, distribution and habitat use between wildebeest sub-populations in the Serengeti National Park, Tanzania
2007	Julius William Nyahongo	ph.d Biology	Depredation of Livestock by wild Carnivores and Illegal Utilization of Natural Resources by Humans in the Western Serengeti, Tanzania
2007	Shombe Ntaraluka Hassan	ph.d Biology	Effects of fire on large herbivores and their forage resources in Serengeti, Tanzania
2007	Per-Arvid Wold	ph.d Biology	Functional development and response to dietary treatment in larval Atlantic cod *(Gadus morhua* L.) Focus on formulated diets and early weaning
2007	Anne Skjetne Mortensen	ph.d Biology	Toxicogenomics of Aryl Hydrocarbon- and Estrogen Receptor Interactions in Fish: Mechanisms and Profiling of Gene Expression Patterns in Chemical Mixture Exposure Scenarios
2008	Brage Bremset Hansen	ph.d Biology	The Svalbard reindeer (*Rangifer tarandus platyrhynchus*) and its food base: plant-herbivore interactions in a high-arctic ecosystem
2008	Jiska van Dijk	ph.d Biology	Wolverine foraging strategies in a multiple-use landscape
2008	Flora John Magige	ph.d Biology	The ecology and behaviour of the Masai Ostrich (Struthio camelus massaicus) in the Serengeti Ecosystem, Tanzania
2008	Bernt Rønning	ph.d Biology	Sources of inter- and intra-individual variation in basal metabolic rate in the zebra finch, (*Taeniopygia guttata*)
2008	Sølvi Wehn	ph.d Biology	Biodiversity dynamics in semi-natural mountain landscapes. - A study of consequences of changed agricultural practices in Eastern Jotunheimen
2008	Trond Moxness Kortner	ph.d Biology	"The Role of Androgens on previtellogenic oocyte growth in Atlantic cod (*Gadus morhua*): Identification and patterns of differentially expressed genes in relation to Stereological Evaluations"

2008	Katarina Mariann Jørgensen	Dr.Scient Biology	The role of platelet activating factor in activation of growth arrested keratinocytes and re-epithelialisation
2008	Tommy Jørstad	ph.d Biology	Statistical Modelling of Gene Expression Data
2008	Anna Kusnierczyk	ph.d Bilogy	*Arabidopsis thaliana* Responses to Aphid Infestation
2008	Jussi Evertsen	ph.d Biology	Herbivore sacoglossans with photosynthetic chloroplasts
2008	John Eilif Hermansen	ph.d Biology	Mediating ecological interests between locals and globals by means of indicators. A study attributed to the asymmetry between stakeholders of tropical forest at Mt. Kilimanjaro, Tanzania
2008	Ragnhild Lyngved	ph.d Biology	Somatic embryogenesis in *Cyclamen persicum.* Biological investigations and educational aspects of cloning
2008	Line Elisabeth Sundt-Hansen	ph.d Biology	Cost of rapid growth in salmonid fishes
2008	Line Johansen	ph.d Biology	Exploring factors underlying fluctuations in white clover populations – clonal growth, population structure and spatial distribution
2009	Astrid Jullumstrø Feuerherm	ph.d Biology	Elucidation of molecular mechanisms for pro-inflammatory phospholipase A2 in chronic disease
2009	Pål Kvello	ph.d Biology	Neurons forming the network involved in gustatory coding and learning in the moth *Heliothis virescens:* Physiological and morphological characterisation, and integration into a standard brain atlas
2009	Trygve Devold Kjellsen	ph.d Biology	Extreme Frost Tolerance in Boreal Conifers
2009	Johan Reinert Vikan	Ph.d Biology	Coevolutionary interactions between common cuckoos *Cuculus canorus* and *Fringilla* finches
2009	Zsolt Volent	ph.d Biology	Remote sensing of marine environment: Applied surveillance with focus on optical properties of phytoplankton, coloured organic matter and suspended matter
2009	Lester Rocha	ph.d Biology	Functional responses of perennial grasses to simulated grazing and resource availability
2009	Dennis Ikanda	ph.d Biology	Dimensions of a Human-lion conflict: Ecology of human predation and persecution of African lions (*Panthera leo*) in Tanzania
2010	Huy Quang Nguyen	pd.d Biology	Egg characteristics and development of larval digestive function of cobia (*Rachycentron canadum*) in response to dietary treatments -Focus on formulated diets
2010	Eli Kvingedal	ph.d Biology	Intraspecific competition in stream salmonids: the impact of environment and phenotype