Conservation Biology of the Rare and Threatened *Drymocallis rupestris*

A Study of Genetic Variation, Seed Germination, and Self-fertility

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Illustration of *Drymocallis rupestris* by Jacob Strum (1796)

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Abstract

Studying the ecology and measuring levels of population genetic diversity are important steps for assessing the conservation status and implementing appropriate conservation strategies for rare or endangered plant species. The Norwegian populations of *Drymocallis rupestris*, a perennial plant in the Rosaceae family, are critically endangered and limited to only a few locations around the Oslo fjord. Samples from these populations were investigated using SNP markers, generated by ddRADseq, to determine levels and structure of genetic variability. To allow for comparisons the Norwegian samples were compared to individuals of cultivated origin and samples from Sweden. Two separate seed germination experiments and a self-fertility test was conducted in order to assess the viability of seeds. Results showed that the genetic variability varied within each population, and there was no correlation between large populations having more diversity than small. Each of the Norwegian populations were genetically distinct from the samples of cultivated origin and generally showed high differentiation between each other. Two localities from the same peninsula in Asker (Akershus) showed low genetic differentiation, the same did the population from Jeløya and the Swedish population. Several of the populations showed signs of inbreeding, whereas others did not, but did in turn have low genetic variation. Results from the self-fertility experiment suggested that the species in self-incompatible. Overall, the low genetic diversity, inbreeding and limited germination of dark grown seeds could indicate low viability for the remaining Norwegian populations of *D. rupestris*, and a close conservation management is recommended.

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1. Introduction

In every region of the world there is a significant decline in biodiversity. Every day species are disappearing due to habitat stress, such as overexploitation, unsustainable use of natural resources and pollution from fossilized fuels, as well as invasive, alien species. Through the earth's history there have been five major extinction events, and we are now in the middle of the sixth. The only difference between the current situation and the previous ones, is that this extinction event is mainly caused by humankind. According to IPBES's report from 2018, the loss of biodiversity is now so substantial that it is in fact threatening the nature's capacity to contribute to human well being (Scholes et al., 2018). The decline in biodiversity affects many areas of biological variation, ranging from the genetic diversity within a species to whole ecosystems. This master thesis will focus on *Drymocallis rupestris*, a regionally rare and endangered plant and its genetic condition within its northernmost distribution.

The Nature Act of 2009 issued by the Norwegian Environment Agency, states that species and their genetic diversity should be conserved within their natural area of distribution in such a way that they will survive in the long term. In order to conserve biodiversity it is vital to maintain a sustainable management of nature reserves and gain more knowledge on the ecology and genetic composition of the endangered species. *D. rupestris* has a very limited distribution and has been considered to be a relict population of one that was previously more widespread in the country (Engan, Båtvik, & Lindberg, 2006). In 2012 a report on the current state of the Norwegian populations of *D. rupestris* was published. It stated that measures must be taken in order to better preserve the remaining populations. These included mapping of the genetic diversity and research of the species' ecology (Thylén, 2012).

The genetic and ecological state of endangered species has been the subject of many studies for several years. The aim being to assess which conservational measures should be taken in order to preserve species in their natural habitat, and understand whether genetic factors associated with small population sizes negatively affect species in an evolutionary context (Crichton, Dalrymple, Woodin, & Hollingsworth, 2016; Edwards, Lindsay, Bailey, & Lance, 2013; Gentili et al., 2015; Gustafson et al., 2016). Knowledge of the ecology and the genetic status of an endangered species may be critical for its management. Unfortunately, there are many shortfalls within gathering data due to the overwhelming complexity of nature. Limited amounts of data and intentional or unintentional biased sampling are some of the many

pitfalls with any research (Simundic, 2013). Hortal et al. (2015) listed and discussed seven key shortfalls on current biodiversity data. Some of these are well known and has been described before, like the Linnean shortfall, referring to the fact that most of Earth's species has still not been described. Other shortfalls are presented for the first time like the Raunkiæran shortfall referring to the lack of knowledge on species traits. By traits, they mean all characteristics that impact a species' fitness in some way. Perhaps, the most important factors relate to reproduction and survival, which includes reproductive systems, seeds, and seed germination traits. It is possible that also genetic makeup can be regarded as a trait even if not explicitly stated (Hortal et al., 2015).

1.1 Population Genetics

It has usually been assumed that a certain amount of genetic diversity is crucial for the persistence of populations. Populations with high levels of variability are seen as healthy, because they will be well equipped when having to respond to threats such as disease, predators, and environmental change (Amos & Harwood, 1998). In the short term, populations with low genetic variation are subject to inbreeding and genetic drift. This reduction in heterozygosity and number of different alleles may lead to lower fitness of individuals and will reduce the species' ability to respond to evolutionary pressures (Reed & Frankham, 2003). In the long term, populations that lose genetic diversity will not be able to evolve since evolution cannot proceed without genetic variation. When conditions change, alleles that have been selectively neutral may become selectively advantageous (Höglund, 2009). In order to respond to rapid changes in the environment, a population must be able to adapt or it will become extinct (Spielman, Brook, & Frankham, 2004).

As a consequence of shifts in climatic conditions, human activities and stochastic events, endangered species often have limited distribution and low numbers of individuals per population. The individuals of small populations carry less selectively neutral genetic diversity than populations with a high number of individuals (Amos & Harwood, 1998). Such populations are usually more affected and are in higher risk of extinction by factors that reduce genetic variation than large populations would be. Especially when subjected to stochastic effects such as environmental changes. Today the most rapid environmental shifts are often caused by human activity.

One of the effects of human population growth is fragmentation of habitats. Fragmentation creates gene flow barriers and will usually lead to a reduction in population size. In extreme cases, fragmentation can cause a genetic bottle neck, where a population will automatically lose much of its genetic variation. The rate at which genetic diversity is lost through genetic drift will increase in a smaller population. Because of this there is a broad concern about the consequences of habitat fragmentation (Amos & Harwood, 1998). A study of fragmentation in meadows showed that habitat fragmentation affects not only rare species in an ecosystem but also reduces the survival probabilities of common species (Hooftman, Billeter, Schmid, & Diemer, 2003).

Another human caused threat to biodiversity, which also may reduce genetic diversity, is the introduction of species that grow aggressively beyond their acceptable distribution and to some extent replace natural vegetation. It has been suggested that plants that have coevolved with herbivores and competitors will grow untamed if moved to a different habitat (Höglund, 2009). There have not yet been any confirmed recordings of plants who have become extinct because of an invasive plant, but it has been proposed that this is because the invasion of alien plants has not been around long enough (Downey & Richardson, 2016). Cultivation of exotic species that escape into the wild has been a problem in Norway for several years. In 2007 and 2012 the Norwegian Biodiversity Information Centre released an ecological risk assessment where they presented a list of alien, blacklisted species that should be monitored and battled if necessary (Gederaas, Loennechen Moen, Skjelseth, & Larsen, 2012).

When population sizes drop and little gene flow occur between them, mating between closely related individuals will be higher than expected by chance. Even if the mating is random, the level of inbreeding will increase over time and reduce heterozygosity (Höglund, 2009). Some species display methods for minimizing the effects of inbreeding, such as self-incompatibility and effective dispersal mechanisms. Edwards et al. (2013) found that the rare cliff endemic *Erigeron lemmonii,* known from just one population, had low genetic diversity, when compared to a more widespread congener, but also a very low inbreeding coefficient. They suggested that the species is highly outcrossing and may be self-incompatible. Inbreeding may not in itself be harmful for species that self-fertilize. Such species are naturally highly homozygous, and low genetic diversity is expected. Crichton et al. (2016) found that in endangered populations of the plant *Melampyrum sylvaticum,* the levels of inbreeding was high and genetic diversity low when compared to other more widespread populations. They

suggested that the natural inbreeding nature of the species protected it from the immediate effects of inbreeding depression, but that the low levels of diversity may become a constraint on evolutionary change in the long term. We see that populations that are naturally inbreeding, may have a buffer against inbreeding depression because deleterious alleles become genetically purged. However, if the amount of recessive deleterious alleles becomes too prominent in the population, or if the decline in heterozygosity results in individuals losing valuable heterozygous traits, inbreeding becomes a concern for the conservation of the population (Höglund, 2009).

In Norway, *D. rupestris,* contains a handful of wild populations, which are patchily distributed. The consequences of this spatial structure for genetic diversity depend on the rate of gene flow between these local populations, which relies on the maximum distance of seed or pollen dispersal. If the distance between the local populations is too great, it would make contact between them impossible. In cases like these, where gene flow is restricted, it is possible for specific alleles to become fixed in the small populations. In an extreme case, all the genetic variation will be retained and possibly even increased between each population, while the variation within each population decreases. However, the low level of migration that is necessary to maintain this distinctness means that individual local populations are likely to be prone to extinction from stochastic processes (Amos & Harwood, 1998). On the other hand, if gene flow occurs between populations, the amount of genetic diversity would be retained within populations and none among. Nybom (2004) found that there were differences in among population genetic diversity and within population diversity between different plant species. Analyses of dominantly inherited markers suggested that long-lived, outcrossing plants retained most of the genetic diversity within population, whereas short-lived, self-fertile species retained most of the genetic variation among populations (Nybom, 2004). Predicting the effects of fragmentation and inbreeding on levels of variation and differentiation is less certain in plant populations, as they are strongly influenced by factors such as self-incompatibility, breeding systems, and methods of dispersal (Cole, 2003).

1.1.1 Using genome-wide SNP discovery for measuring genetic variation in plants

Studies of population genetics often use a mix of analytical methods that focus on the differentiation within and between populations. The choice of analytical method is dependent on the type of genetic marker used. Furthermore, the different aspects of variation that can be assessed, depend on whether the marker is non-neutral and subject to selection, or if it is

selectively neutral (Höglund, 2009). An advantage of using codominant markers is that they distinguish between homo- and heterozygote genotypes. In contrast dominant markers, such as AFLPs, cannot truly measure heterozygosity. A variety of different methods and genetic markers are being used for analyzing genetic diversity in plants. Edwards et al. (2013) and Crichton et al. (2016) used a relatively small number of microsatellites, which are perhaps the most widely used markers. However, some studies argue that in order to gain a more thorough understanding of the genetic diversity within a species, it can be an advantage to use genome-wide data. Several recent studies have used SNPs on a global scale for genetic markers in plants (Kujur et al., 2015; Pan, Wang, Sun, Li, & Gong, 2016; Torres-Martínez & Emery, 2016). SNPs are abundant in a genome, and modern methods of high-throughput sequencing has made creating genome-wide SNP datasets possible. For SNP discovery, double digest restriction site-associated DNA-sequencing (ddRADseq) is a much used method (Peterson, Weber, Kay, Fisher, & Hoekstra, 2012). This is a simple and cost effective method that produces genomic data at population level. By digesting DNA with restriction enzymes and tagging the fragments with barcodes, ddRAD-tags are created. These fragments are then Illumina sequenced and assembled bioinformatically. By comparing loci one can study the genetic variation within and between populations (Andrews, Good, Miller, Luikart, & Hohenlohe, 2016). One of the advantages with ddRADseq is that one can sequence a relatively high number of samples, and at the same time avoid the huge costs needed for whole genome sequencing. Also, by sequencing a subset of the genome rather than the whole genome, the depth coverage per locus is increased. This leads to greater confidence in calling genotypes (Andrews et al., 2016). Another advantage is that ddRADseq does not require any prior genomic information for the taxa being studied. A disadvantage with ddRADseq is that it may introduce PCR artifacts during library preparation. PCR artifacts can skew RAD allele frequencies within loci and thereby cause consistent genotyping errors (Puritz et al., 2014). Multiple programs for handling high-throughput sequence data and SNP genotyping are easily available. However, most studies working with ddRADseq use the Stacks pipeline (Catchen, Hohenlohe, Bassham, Amores, & Cresko, 2013). With SNP data one can calculate a number of within population genetic measures including: percentage of polymorphic loci, number of private alleles, allelic richness, expected heterozygosity, observed heterozygosity and the inbreeding coefficient. In order to measure genetic distance between populations, the most widely used measure is the pairwise F_{ST} estimates. For any given genetic distance

measure it is often useful to visualize the distances between populations graphically. Principal coordinate analyses (PCoA) is a much used method for this.

To assess if isolated populations of a locally rare species actually have low genetic variability, it can be useful to compare these with a population from an area where the species or a congener is more widespread. If an isolated population has low levels of genetic variation, the comparison between the more widespread group can aid in the identification of the factors that have negatively affected levels of genetic diversity. Since the two groups share evolutionary history and have similar morphological and life-history traits, the reasons behind one's low genetic variability may be attributed to the characteristics that differ between the two groups. If limited population size and distribution range are factors that characterize the endangered group and not the other, the low genetic variation can be connected to factors specific for small populations, such as inbreeding, genetic drift or a genetic bottle neck event. Nybom (2004), Edwards et al. (2013) and Cole (2003) all found that the genetic variability was significantly reduced in isolated populations compared to more widespread congeners.

1.2 Study Species

1.2.1 Phylogeny and morphology

Drymocallis rupestris belongs to the Rosaceae family. It was previously part of the *Potentilla* genus, but Eriksson et al. (1998) stated that the genus was not monophyletic and should in fact be split into several genera (Eriksson, Donoghue, & Hibbs, 1998). The species *Potentilla rupestris* was therefore placed in a separate genus *Drymocallis* together with several species from North-America, Europe and Asia. This split was further backed up by Dobes and Paule (2010) in their chloroplast DNA-based phylogeny of *Potentilla*, suggesting that *Drymocallis* was indeed a monophyletic group (Dobes & Paule, 2010).

D. rupestris is a fairly tall standing and sturdy, perennial herb. The roots are mainly adventitious and form a white, tangled web that reach deep into the soil (Wilson, Whittington, & Humphries, 1995). The stem can be 20–60 cm tall, usually green, sometimes red in color. It is hollow, erect and has glandular hairs. The leaves are 10-15 cm long, elliptical, with two to three pairs of distinct leaflets (Mossberg & Stenberg, 2007/2012). The leaflets are 1–7 cm long, green and hairy on both sides. The terminal leaflet is the largest, the other leaflets decrease in size towards the base of the leaf. The stipules are triangular, fringed with hairs but

mostly glabrous. The flowers measures 1–2 cm across, and are in a loose dichotomous cyme with five or six divisions. There are five green sepals that are hairy on the abaxial surface and five white petals that are 0.5–1.5 cm long, and obovate. The style is sub-basal and fusiform and the fruit is an achene. *D. rupestris* has a woody stem and a rootstock. It is a hemicryptophyte with buds forming in the axils of leaves or from the woody stock in old leaf axils. The species has no effective vegetative reproduction, other than growing in mounds (Wilson et al., 1995).

1.2.2 Achene characters, seed germination and pollination

The achenes are semiglobular with a recurved tip and a netted surface. There does not seem to be any particular mechanism for seed dispersal and they are often retained at the plant, enclosed by the bracts, until the following season. The achenes are detached from the plants by mechanical force, like wind, animals or when the stem falls (Wilson et al., 1995). Whittington et al. (1988) studied germination of seeds from one locality in Wales, United Kingdom. Over 90% germination was achieved after eight days' in a 20°C heating cabinet. Germination of seeds did not occur below 12°C, nor in the dark. It was considered that *D. rupestris* mostly germinates in the spring when favorable environmental conditions occur regarding both light and heat (Whittington, Wilson, & Humphries, 1988). Seeds from Norwegian populations of *D. rupestris* have been collected for *ex situ* conservation. Germination percentage of these seeds varied from 67% – 100% when they were treated with alternating temperatures (20°C and 10°C) every 12 hrs., and alternating light conditions (12 hrs. in light, 12 hrs. in dark) (Bjureke, 2015)

The flowers open from the bottom of the inflorescence upwards. There are about 20 stamens positioned in two rings around the style (Thylén, 2012). The pollen grains are oblong with four longitudinal furrows (Wilson et al., 1995). Wilson et al. (1995) stated that *D. rupestris* is entomophilous and visited by bees, ants and flies. The same article also stated that the plant is self-fertile, but it is not known whether any experiments have been conducted on the subject.

1.2.4 Distribution and ecology of Drymocallis rupestris throughout its distribution range

The species' distribution is wide, extending from northwest Africa through west Asia and Europe (Lid & Lid, 1944/2013). On the continent *D. rupestris* is mainly found in sub-alpine areas on open lime-rich shallow-soil habitats, often on dry grasslands or on east- and west facing rocky slopes (Wilson et al., 1995). In Scandinavia the species is found in lowland

systems sometimes associated with the coast. The Norwegian population is the northernmost occurrence of the species, with the nearest population being in the southwest of Sweden (Figure 1).

Figure 1: Map showing the northernmost distribution of *D. rupestris* (Hultén, 1971), previously known under the name *Potentilla rupestris* L.

D. rupestris is generally considered as intolerant of shade and prefer open, to partially shaded areas. The plant does not have high competitive capabilities, and is easily threatened by other fast and high growing herbs if the vegetation becomes too dense and shady. Established mounds of the plant are not so easily outcompeted, but young plants will not prosper in a highly competitive environment (Thylén, 2012).

1.3 Norwegian Populations of *Drymocallis rupestris*

The Norwegian populations of *D. rupestris* are restricted to the south-east and eastern part of the country, with most known populations occurring around the Oslo fjord. The first recorded

find of the species was by Axel Blytt in 1861, who discovered it in Ekeberg (Artsdatabanken). Today the species is considered to be found at six localities, three in Akershus county, Hellvik, Munkesletta and Esviken; two in Oslo county; Tåsen and Ekeberg and one in Østfold county; Jeløya. It was previously recorded an additional four localities in Oslo (Rodeløkka, Ensjøveien, North Ekeberg and Lillefrogner) and there exists herbarium samples and recordings from a few other locations (e.g. Sandvika). It is assumed that the distribution of *D. rupestris* has been more widespread and dense but it is difficult to precisely say how many populations there have been (Thylén, 2012). An overview of all recorded present and past populations can be viewed in Figure 2.

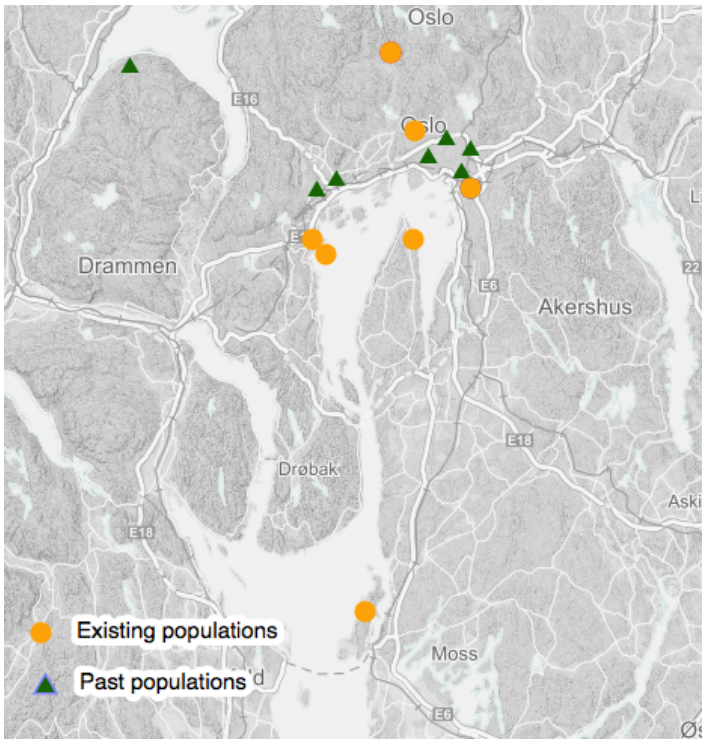

Figure 2: Map of existing and past occurrences of *D. rupestris* in Norway

The populations in Norway are found in open areas with shallow, base-rich soils. The plants usually grow on rocky, sunny hills, that descend towards the sea. These open and lime-rich, coastal habitats are declining in Norway due to increased human activity and overgrowth. Both the nature types, NA-T25; 5,6 (Bratli et al., 2016), and the species are red listed in Norway, with the species status categorized as endangered (EN) (Henriksen & Hilmo, 2015; Lindgaard & Henriksen, 2011). The factors that seem to negatively affect the populations around the Oslo fjord are mainly increased human traffic and development, overgrowth and competition from invasive foreign species (Thylén, 2012).

1.3.1 The populations, their threats and ongoing conservation measures

The population at Hellvik at Nesodden is in a plant conservation area and is one of the larger populations of the species in Norway. In 2012, 125 individuals were recorded (artsobservasjoner.no). The plant grows on top of, in and at the bottom of an east-facing cliff, close to the sea. The immediate area close to the locality is mostly comprised of houses and gardens. The plant is therefore threatened by a certain amount of human activity. The area around the species has been cleared of competing, invasive plants at several occasions as an organized conservation measure. There has also been a successful attempt of reinforcing the population (Bjureke, Bredesen, Gadja, & Røsok, 2016).

The population at Tåsen in Oslo contains three fertile and two sterile individuals (personal observations 2016 & 2017). This population is found in a small meadow situated between housing properties in a quite central place in Oslo where they grow on a hill along the edge of a tree and shrub stand. The development of this population has been mapped well, and there have been recorded a dramatic decrease of plants in the population the last 50 years. The area around the species has been cleared of competitive species through the years, the last two by the Norwegian Botanical Association. This population has also been successfully reinforced (Bjureke et al., 2016). The plant was reintroduced at Ekeberg in Oslo with seeds from Tåsen as this was considered to be the population within closest geographic proximity (Stabbetorp & Wesenberg, 1990).

The population at Jeløya contains one sterile and three fertile individuals (personal observations 2017) that grow on a west-facing rocky hill descending towards the sea. This population was discovered in 2005 (Engan et al., 2006), and is in an area well protected from the public, as it is quite hilly and difficult to get to. In 2010 another individual was discovered about 400 m north of the rest of the population (Bjureke, personal communication).

The population at Munkesletta in Asker contains roughly 50 fertile individuals (personal observations 2016 & 2017). The plants grow on a south-facing, rocky hill that descends towards the sea. The locality is found on a private property, but is not immediately surrounded by houses and gardens. It is therefore somewhat protected from the public but it is still threatened from competition from other plants. There is a separate small occurrence of the species a few hundred meters across the peninsula at Esviken, in what seems to be an abandoned garden. This population contains 5 fertile individuals, but it is unclear whether this

population is part of a larger population comprised of the two, if it has purposely been moved there, or if it stems from a plant nursery.

1.3.2 History as an ornamental garden plant

There has been some discussion among Norwegian botanists as to what degree the occurrence of *D. rupestris* is in fact wild or whether it is a product of escaped garden plants (Bjureke, personal communication). Most of the recorded populations (except Jeløya) are within close proximity to houses and gardens and the species has been sold as a perennial from Steen & Wormsen perennial nursery for some years. A while back, a comprehensive work on registration and collection of old garden perennials in Southeastern Norway was conducted. *D. rupestris* was only found at two locations, Løkenes farm and Esviken (Langeland, personal communication). It has also been recorded at Blankvann (Thylén, 2012), however, this is without doubt of garden origin (Bjureke, personal communication). It is unclear whether the plants in gardens are of cultivated origin or if they are wild plants or seeds brought back for planting. We do not have substantial information regarding how common it is as a garden plant and there may be many more than those officially recorded.

1.4 Objective and Hypotheses

Drymocallis rupestris is an endangered species, and it is therefore of high importance to gather data of traits that influence the species' survival at its present localities. Moreover, knowledge that can form the basis for possible reinforcement of the species is also needed. In order to achieve this, I wish to study the genetic variation within and between Norwegian populations by means of ddRADseq and compare the variation with one population from Sweden. The latter population represents parts of the distribution range where the species is more common. As the species has been used as a garden plant, the Norwegian populations will also be compared with material from a nursery to hopefully rule out the possibility that the Norwegian populations are of garden origin. I will also test the germination ability of seeds and do a test of self-fertility to get an indication of the populations' viability.

The following hypotheses for the Norwegian populations of *D. rupestris* will be tested.

1. The individuals of cultivated origin are genetically distinct from the Norwegian and Swedish populations, i.e. the Norwegian populations are most likely of wild origin.
2. Small populations harbor less genetic diversity than larger populations.
3. Small populations are more inbred than large populations.
4. Genetic variation in the *ex situ* collection is equivalent to the wild population from where it was sampled.
5. The Norwegian populations are less diverse than the Swedish population.
6. The Norwegian populations are genetically distinct from each other and from the Swedish populations.
7. Seed germination is higher in light than in darkness.
8. Percentage of seed germination is similar in all Norwegian populations.
9. Self-pollinated flowers have successful fruit set (the flowers are self-compatible)

If it is concluded that the individuals of Norwegian populations are garden escapes (Hypothesis 1), it would not be of interest to discuss the other hypotheses.

2. Materials and Methods

2.1 Sampling

Six localities were visited visited during the flowering season from June to August in 2016 and 2017. Populations from Norway were chosen based on locality records from Artskart (artskart.artsdatabanken.no). We did not sample from Ekeberg because this population originated from Tåsen, neither from Blankvann due to its garden origin. To allow for comparisons with a wider more continuous distribution of *D. rupestris*, one population in Sweden was sampled. In addition to the known wild populations of *D. rupestris*, samples were also collected from the *ex situ* collection at the Botanical Garden in Oslo, the so-called "red list flowerbed", as well as two samples of *D. rupestris* bought from Steen & Wormsen perennial nursery. A map showing the wild Norwegian localities are shown in Figure 3. A table with descriptions of each locality is shown in Table 1, a more detailed description can be viewed in Appendix nr. 1.

In order to collect samples from the Norwegian populations of *D. rupestris*, the proper permits from the Norwegian government (Appendix nr. 2) was needed. For Sweden no permit was necessary We had permission to collect samples from roughly 10 individuals from each wild population. Leaf tissue, from four to eleven individuals, were sampled from each population, and immediately transferred to plastic containers with silica gel to ensure rapid drying and minimal degradation of the DNA. Seeds were gathered late summer 2017 by squeezing off deflowered buds and storing them in homemade seed bags (made from plain writing paper). Seeds from all Norwegian populations of *D. rupestris* were collected from, except Jeløya (due to difficulties reaching the locality).

Pop. abbrev.	Country	Locality	Collector	Samples collected
HE	Norway	Hellvik	LH, BS	11
EV	Norway	Esviken	LH, BS	5
MS	Norway	Munkesletta	LH, BS	10
JO	Norway	Jeløya	LH, BS	4
TA	Norway	Tåsen	LH, BS	4
RO	Norway	Botanisk hage, Tøyen	LH, BS	17
VN	Sweden	Vingnäs	KB, LH, BS	10
DR	S. & W. Perennial Nursery	Pamir Mts. & unknown	VH, NA	6

Table 1: Sampled populations of *D. rupestris.* Collectors: KB = Kristina Bjureke, LH = Lise Huseby, BS = Brita Stedje, VH = Vojtec Holubec, NA = Not available.

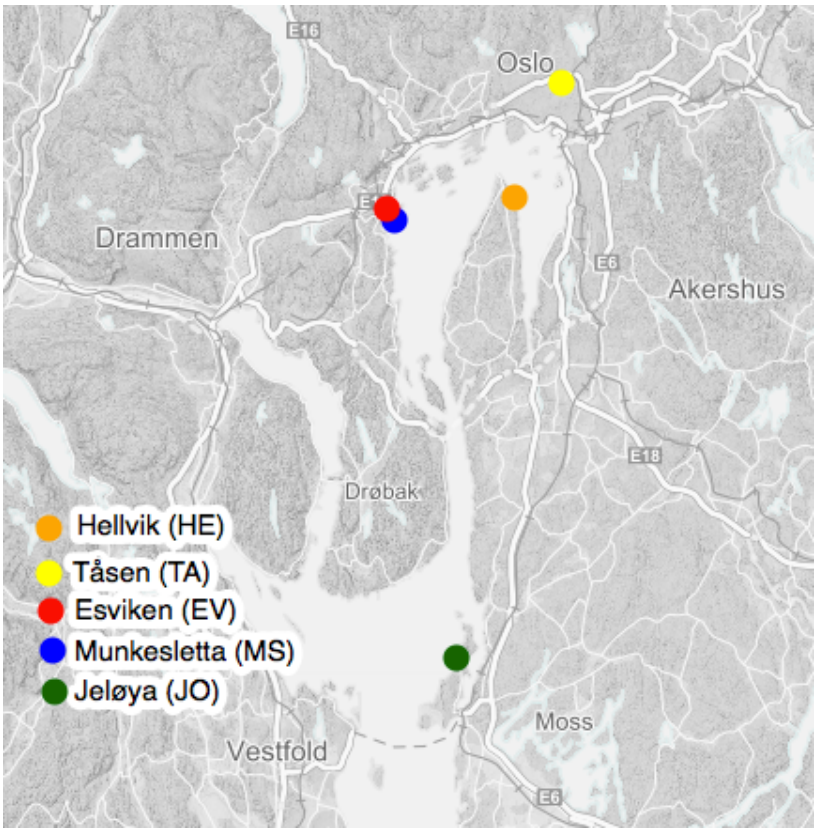

Figure 3: Map showing the sampling area of *D. rupestris* in Norway.

2.2 Molecular Work

All molecular work was carried out at the DNA-lab at the Natural History Museum, University of Oslo.

2.2.1 DNA extraction

Up to 15 mg of dried leaf tissue was taken from 67 samples. The samples were placed into 2 ml microcentrifuge tubes with two sterilized 3 mm Tungsten-Carbide Beads (Qiagen), crushed using a Mixer Mill MM301 (Retsch GmbH & Co.) and grinded for 2×1 min at 20Hz to ensure pulverization. DNA was extracted using the E.Z.N.A. SP Plant DNA Kit (Omega Bio-Tek) following the manufacturer's protocol, including the suggested additional elution step: samples were eluted twice (with 50 µl elution buffer in each) in a Safe-Lock TubesTM (Eppendorf) to increase the DNA yield. Prior to elution, the samples were incubated at 65°C for 5 min to increase DNA yield and concentration. DNA extracts were then stored at -30°C.

2.2.2 Assessing genomic DNA integrity and quantity

Integrity of the extracted DNA was visualized with electrophoresis on 1% agarose gels, mixing SeaKem LE Agarose (Lonza group) with 0.5×Tris-borate-EDTA-buffer. GelRedTM nucleic acid dye (Biotum) was added to the agarose to stain DNA. Each well was loaded with 2 µl genomic DNA mixed with 3 µl homemade loading buffer (50 mM EDTA, 30% glycerol, 0.25% bromphenol blue and 0.2% xylene cyanol). One well per row was loaded with 3 µl FastrulerTM Low Range DNA Ladder (Fermentas Thermo Fisher Scientific) for sequence length reference.

An Invitrogene Qubit 2.0 fluormeter (Thermo Scientific) was used to determine the DNA concentrations of the extracted samples. 2 µl of genomic DNA was measured using either the HS (high sensitivity) or BR (broad range) dsDNA kit (including buffers, dye, and standards) based on the amount of DNA in the sample.

2.2.3 Library preparation

A restriction enzymatic digestion test was carried out using eight out of 67 samples before proceeding with the library prep. The restriction enzymes tested were the frequent cutter Mse and the rare cutter Nsi (New England Biolabs), as well as a combination of the two. The restriction enzymes shared the genomic DNA as expected and library prep continued as follows.

The ddRADseq library preparation was carried out using the protocol described in Peterson et. al (2012) with a few modifications, as described in this section. The 67 samples where split into two groups of 8 and 59. The samples were then normalized to 300 ng of DNA per sample manually or by using the BioMek 4000 (Beckman-Coulter) and run through a double digestion with a reaction volume of 40 µl. Using the restriction enzymes mentioned above, the double digest was run at 37° for 2 hrs. Forward and reverse adapters (Appendix nr. 3) were ligated to the overhangs of the fragmented DNA in a 30 µl reaction volume. One adapter was sample specific and contained a unique oligo of four to eight nucleotides. The sample sequences where then size selected for 350 bp – 550 bp with a BluePippin (Sage Science) and amplified with PCR on a Doppio Thermal Cycler (VWR) using Q5 High-Fidelity DNA polymerase. The PCR program was as follows:

98°C – 30 s (98°C – 10 s, 60°C – 15 s, 72°C – 15 s)x12- 72°C – 2 min.

After each enzymatic step the DNA was purified using 1.2 X Ampure XP beads except after the PCR where the library was purified twice using 0.8 X beads to eliminate short adapter leftovers and primer dimers from the PCR. The final libraries were quality checked with a Fragment Analyzer (Advanced Analytical). A qPCR on a CFX96 machine (Bio-Rad) was conducted using the Illumina p5 + p7 amplification primers in order to check the libraries' molarity before sequencing.

2.2.4 Sequencing

DNA-sequencing was outsourced to the Norwegian Sequencing Center. The 67 barcoded ddRAD libraries were multiplexed on a single flow cell and paired-end sequencing was conducted on an Illumina HiSeq 3000 machine using 150 cycles per end, resulting in paired-end 150 bp reads.

2.2.5 Data assembly

The raw Illumina sequence reads of the 67 samples were demultiplexed using the iPyRAD pipeline (Eaton, 2015) by matching the raw reads to a reference file containing the sample ID and the corresponding barcode. Further processing of the sequence data was conducted using the STACKS software v. 2.0 (Catchen et al., 2013). Given that there was considerable variation in read coverage between samples, any individual with less than 300,000 reads was removed from the data set at this point to produce the most complete data matrix from STACKS as possible. The demultiplexed reads were assembled *de novo* using the

denovo_map.pl script in STACKS. This script initiates a pipeline of software containing *ustacks*, *cstacks*, *sstacks* and *populations*. *ustacks* builds loci and calls SNPs *de novo* in each sample, *cstacks* creates a catalog of all loci across the populations, the loci are then clustered across samples according to sequence similarity. *sstacks* matches each sample against the catalog created in *cstacks*. *populations* was run with the –p parameter set to 8, which means that it will exclude any locus which is not present in all the populations. We also set –r to 0.5, so in order for a locus to be processed for a population it must be present in at least 50% of the individuals of that population. The other parameters were set to default. *populations* then randomly selects one SNP for each locus and generates population-level summary statistics and produces several different output formats (e.g. vcf and structure). VCFtools (Danecek et al., 2011) was used to filter the dataset so that any locus with more than 20 percent missing data was removed. This left us with a data set of 63 individuals and 6146 polymorphic SNP loci.

2.2.6 Genetic analyses of within population variation

To indicate levels of genetic diversity and assess levels of inbreeding within each population, percentage of polymorphic loci, total number of alleles per population, number of private alleles, expected (H_E) and observed (H_O) heterozygosity, were calculated for each population using the Excel add-in software GenAlEx (Peakall & Smouse, 2012). Allelic richness was calculated with the R package PopGenReport (Adamack, Gruber, & Dray, 2014; Gruber & Adamack, 2015), correcting for uneven sample size by using a subset of four individuals. The inbreeding coefficient, F_{IS}, was calculated by the following formula:

$$F_{IS} = \frac{(H_E - H_O)}{H_E}$$

2.2.7 Genetic analyses of among population variation

Initial analyses of the genetic structuring of populations were conducted using the Bayesian Markov Chain Monte Carlo (MCMC) program STRUCTURE (Falush, Stephens, & Pritchard, 2007; Hubisz, Falush, Stephens, & Pritchard, 2009; Pritchard, Stephens, & Donnelly, 2000), which infers population structuring between individuals from the different populations based on information from the data matrix. The software ignores the information about the actual population of each individual. Instead it uses the genetic information within the SNP dataset to assign each individual to a genetic group. The number of groups, denoted *K*, can be varied within an interval, in our case ranging from 1 to 9. Based on the dataset, the software uses an

MCMC simulation to find the most likely group for each individual. The analysis was carried out assuming the presence of admixture and correlated allele frequencies among populations. The program was run with a data set comprised of all the sampled individuals from each population, and also a data set where the samples of cultivated origin (DR), the redlist flowerbed (RO) and Tåsen (TA) were excluded. Six replicates were run for each value of *K*. The results of all of the STRUCTURE runs were analyzed with Structure Harvester (Earl & vonHoldt, 2011) to find the optimal value of *K* (e.g. the *K* value with the highest likelihood). This program implements the Evanno method (Evanno, Regnaut, & Goudet, 2005) for finding the number of groups that best fit the data set. I then ran CLUMPAK (Kopelman, Mayzel, Jakobsson, Rosenberg, & Mayrose, 2015) on the STRUCTURE results, which identifies clustering modes and population structures across *K* and provides a visualization of the genetic structure of the optimal *K*.

A pairwise F_{ST} test and a Principal coordinates analysis (PCoA) was carried out using GenAlEx. The F_{ST} distance values give a relative measure of the genetic difference between the populations. I used an analysis of molecular variance (AMOVA) with 999 permutations, for estimating the F_{ST} values. These can range from 0 to 1. In the case of $F_{ST} = 0$, the allele frequencies are exactly the same in each population, indicating very strong genetic similarities. If $F_{ST} = 1$, there are no similarities between the comparing populations, which when comparing populations of the same species should, in theory, be impossible. When considering if a F_{ST} value between two populations is significant, there is no official consensus. However, Hartl and Clark (1997) suggested F_{ST} values can be divided into the following classes:

< 0.05 = little genetic difference

0.05 – 0.15 = moderate genetic difference

0.15 – 0.25 = great genetic difference

> 0.25 = very great genetic difference

In the PCoA analysis, the different populations are represented as points in a multidimensional space, such that the Euclidian distances are the same as the corresponding genetic distances. In order to plot the points, they are projected down to a plane, chosen so that the larger distances are preserved as much as possible. A necessary condition for this to work is that the genetic distance measure is a "proper" distance measure. Unfortunately, the F_{ST} distance measure does not always satisfy this condition. Nei's standard genetic distance was therefore used (Nei, 1972):

$$D_{ST} = -\ln \frac{J_{XY}}{\sqrt{J_X J_Y}}$$

where J_X, J_Y, and J_{XY} were replaced by unbiased estimators.

2.3 Seed Germination

The seed germination experiment was carried out in the seed bank of the Natural History Museum, University of Oslo, and studied for all populations except Jeløya (JO). All seeds were sown in petri dishes filled with agar (see Appendix nr. 4 for recipe) and placed in an incubator. One experiment was conducted to test the effect of light on germination. For this experiment 5 seeds from each of the 11 individuals from the Hellvik (HE) population were sown and put in an incubator at a constant temperature of 20°C, and either exposed to light or completely excluded from light using two layered bags of aluminum foil.

In a separate experiment, which was conducted to compare seed germination between populations, seeds from all populations (except Jeløya) were germinated in an incubator that alternated between 20°C and 10°C every 12 hrs. The light was turned on and off in the same interval as the change in temperature. Germination was checked every 7 days. The reason for the different temperature regimes in the two experiments was merely for convenience, as standard routines of the seed bank had changed. As the results of the two experiments are only compared to a very limited degree this is not regarded as a problem.

2.4 Self-fertility Test

A test of self-pollination was carried out by carefully isolating a small number of flowers with thin bags of fabric at bud stage. To avoid physical stress of the flowers the fabric bags were fixed to a stick. No open flowers were left inside the bag, but there were flowers at other parts of the plant that were exposed to insects. After the flower buds had opened and the stigmas were mature, pollen was transferred to own styles for each flower. Infruitescenses were collected from flowers that had been exposed to the outside as well as from the actively self-pollinated flowers inside the bag. They were later examined for successful fruit set.

3. Results

3.1 Molecular Analysis

3.1.1 Genetic diversity within populations

The total number of loci for the whole data set was 6146, with 11.5% missing data. The allelic patterns for each population are shown in Table 2. We see that the cultivated material (DR) has the highest number of private alleles, allelic richness and polymorphic loci. The populations Tåsen (TA), red list flowerbed (RO) and Hellvik (HE) show the next to highest values. Observed heterozygosity was lower than would be expected under Hardy-Weinberg equilibrium for the populations DR, HE, RO, and TA, whereas the other populations showed opposite effects. The inbreeding coefficient, F_{IS}, was negative for Esviken (EV), Jeløya (JO), and Munkesletta (MS), indicating excess heterozygosity. DR, HE, RO and TA had positive F_{IS} values, indicating reduced heterozygosity.

Pop	A_N	A_P	$Mean\, A_R$	$Polymorphic\, loci$	H_O	H_E	F_{IS}
DR	8838	948	1.358	43.80%	0.024	0.194	0.877
EV	6359	146	1.018	3.47%	0.011	0.009	-0.233
HE	7325	351	1.116	19.18%	0.037	0.063	0.412
JO	6309	97	1.016	2.65%	0.011	0.008	-0.322
MS	6396	172	1.013	4.07%	0.009	0.007	-0.309
RO	7305	519	1.057	18.86%	0.020	0.030	0.316
TA	8349	372	1.267	35.84%	0.050	0.138	0.638
VN	6430	189	1.015	4.62%	0.011	0.008	-0.340

Table 2: Allelic patterns based on 6146 loci in sampled populations of *D. rupestris*. A_N = number of alleles, A_P = number of private alleles, A_R = allelic richness, H_O = observed heterozygosity, H_E = expected heterozygosity, F_{IS} = inbreeding coefficient. Refer to Table 1 for population abbreviations.

3.1.2 Genetic variation among populations

The results from STRUCTURE and Structure Harvester can be viewed in Table 3. In column three and four of this table I have listed respectively the mean and the standard deviation of the natural logarithm of the estimated posterior probabilities of *K*.

Column five, labeled ln'(*K*), represents the change in mean values when *K* is increased. Thus, e.g., in row two, 178075.8 is the difference between the mean of ln P(2) and the mean of ln P(1). The other rows are computed similarly. The greatest positive change occurs when *K* is increased from 1 to 2.

Column six, labeled |ln'' (*K*)|, represents the absolute value of the change in column five, when *K* is increased. In this case the number in row two, 118427.8, is the absolute value of the difference between ln' (3) and ln' (2), etc.

Finally, column seven, labeled Delta *K*, is calculated by taking the |ln'' (*K*)| divided by Stdev ln P(*K*). Structure Harvest uses Delta *K* for selecting the optimal *K*. That is, the optimal *K* is the one with the highest Delta *K*. Note, that since Delta *K* is obtained by dividing |ln'' (*K*)| by Stdev ln P(K), changes are scaled with respect to these standard deviations. Thus, if the standard deviation is small for a chosen *K*, meaning that uncertainty in the mean value is small, Delta *K* becomes large even if the change is not that great. We observe that |ln'' (8)|is very large, but the corresponding standard deviation is also high. Thus, the resulting value of Delta *K* is relatively small.

Evanno table						
K	$Reps$	$Mean\ \ln P(K)$	$Stdev\ \ln P(K)$	$\ln'(K)$	$\lvert\ln''(K)\rvert$	$Delta\ K$
1	6	-385807.2833	25.6199	NA	NA	NA
2	6	-207731.4833	24.0403	178075.800000	118427.800000	4926.212722
3	6	-148083.4833	34.7272	59648.000000	30370.383333	874.540488
4	6	-118805.8667	4988.9728	29277.616667	29061.083333	5.825063
5	6	-118589.3333	19600.0833	216.533333	433211.466667	22.102532
6	6	-551584.2667	494786.8242	-432994.933333	545777.066667	1.103055
7	6	-438802.1333	537681.7417	112782.133333	1745934.583333	3.247152
8	6	-2071954.5833	2087769.9133	-1633152.450000	2728067.600000	1.306690
9	6	-6433174.6333	12601557.5998	-4361220.050000	NA	NA

Table 3: Output of the Evanno method results showing the likelihoods for each number of groups (*K*).

The result from the STRUCTURE analysis for the data set containing all populations showed best support for a population clustering of two groups. This follows since Delta *K* has the highest value for $K = 2$. We note that for $K = 2$, we also have the highest positive value of ln' (*K*). This indicates that the greatest improvement in probability is obtained when the number of populations is increased from 1 to 2. For higher values of *K* the number of parameters in the probability model increases. This also introduces more uncertainty into the model, which is seen by the increasing standard deviations. The Evanno method will tend to give priority to a model with few parameters.

The clustering into genetic groups was analyzed further by using the program CLUMPAK. The program calculates membership probabilities with respect to the genetic groups for each individual in the data set. These probabilities are illustrated by vertical bars with colors representing the relative likelihoods of each genetic group. If an individual has a vertical bar with only one color, the portion of the genome sampled from this individual is assumed to belong to this particular genetic group. If the vertical bar contains more than one color, then the portion of the genome sampled from this individual is a mixture of two genetic groups. All these vertical bars are presented in a plot (Figure 4). Here the individuals are grouped with respect to their geographical population, which are separated by vertical lines.

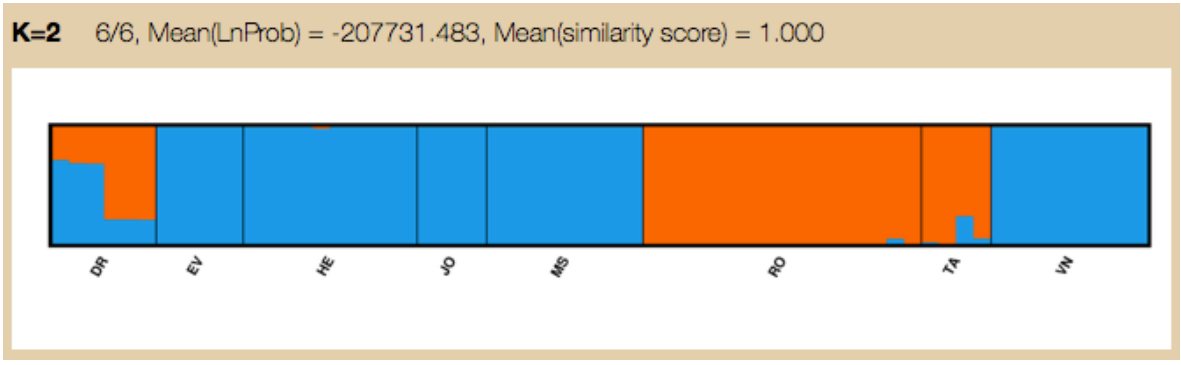

Figure 4: CLUMPAK results, showing the two groups by different color indicating two different genetic groups. Refer to Table 1 for population abbreviations.

All populations were classified into one or the other based on two genetic groups. One of the groups, represented by the color orange in Figure 4, includes the individuals of cultivated origin (DR), the Tåsen population (TA) and the redlist flowerbed (RO). The other genetic group, indicated by the color blue, clusters the rest of the Norwegian populations (HE, EV, MS, JO) together with the Swedish population (VN) as well as DR. We see that the individuals in DR are comprised of two different genetic groups, which is reflected by the color split.

To investigate the potential for further population structure between the Norwegian populations Esviken (EV), Hellvik (HE), Jeløya (JO), Munkesletta (MS) and the Swedish population (VN), they were analyzed in a separate test. The STRUCTURE and Structure Harvester results showed best support for three genetic groups ($K = 3$). See Table 4.

Evanno table						
K	$Reps$	$Mean\ \ln P(K)$	$Stdev\ \ln P(K)$	$\ln'(K)$	$\lvert\ln''(K)\rvert$	$Delta\ K$
1	6	-87617.566667	355.568596	NA	NA	NA
2	6	-56172.316667	2700.759461	31445.250000	9118.483333	3.376266
3	6	-33845.550000	16.918599	22326.766667	14522.716667	858.387652
4	6	-26041.500000	13.164650	7804.050000	10377.683333	788.299242
5	6	-28615.133333	32.032837	-2573.633333	1741.416667	54.363485
6	6	-29447.350000	2193.855580	-832.216667	2474.833333	1.128075
7	6	-32754.400000	7043.321944	-3307.050000	5794.866667	0.822746
8	6	-30266.583333	2509.679892	2487.816667	7310.716667	2.913008
9	6	-35089.483333	4198.262691	-4822.900000	NA	NA

Table 4: Output of the Evanno method results, showing the likelihood of each K-value.

The CLUMPAK results for these five populations are shown in Figure 5. The populations could be placed in three different genetic groups. The populations MS and EV were placed together, indicated by the color blue. The population JO was placed in the same genetic group as the Swedish population (VN), indicated by the color orange. The population HE was placed in a third genetic group, with some admixture of the other genetic groups.

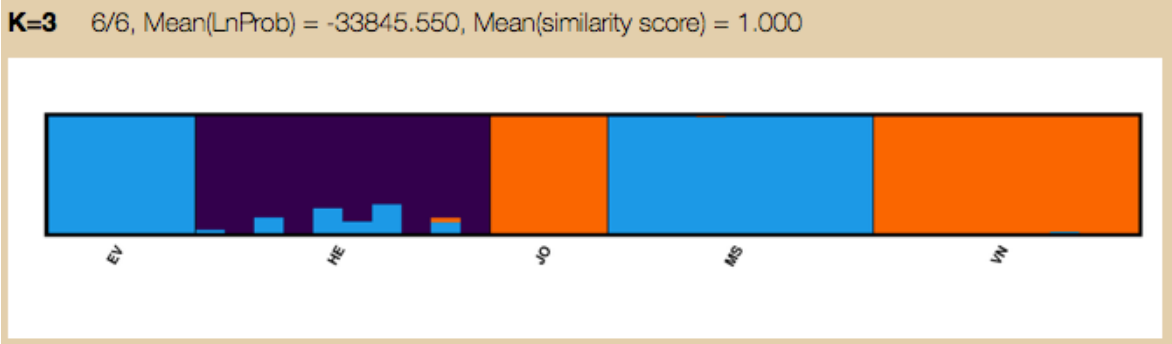

Figure 5: CLUMPAK results, showing three different genetic groups indicated by different colors. Some admixture in the Hellvik population. Refer to Table 1 for population abbreviations.

The pairwise F_{ST} test showed low values when comparing Tåsen (TA) and the red list flowerbed (RO), Esviken (EV) and Munkesletta (MS), and Jeløya (JO) and the Swedish population (VN) (Table 5). The highest values were found between MS and TA (F_{ST} = 0.563) and MS and RO (F_{ST} = 0.536), indicating very high genetic difference. The lowest values were found between the populations RO and TA (F_{ST} = 0.012), and MS and EV (F_{ST} = 0.032), indicating little genetic difference.

Pairwise Population F_{ST} Values

	DR	**EV**	**HE**	**JO**	**MS**	**RO**	**TA**	**VN**
DR	0.000							
EV	0.291	0.000						
HE	0.333	0.124	0.000					
JO	0.281	0.106	0.207	0.000				
MS	0.460	0.032	0.221	0.329	0.000			
RO	0.352	0.437	0.464	0.390	0.536	0.000		
TA	0.183	0.365	0.414	0.300	0.563	0.012	0.000	
VN	0.415	0.222	0.266	0.039	0.328	0.466	0.454	0.000

Table 5: Pairwise F_{ST} among the 8 populations of *D. rupestris* included in this study.

The PCoA was based on the values given by the pairwise population comparison test of Nei's unbiased standardized genetic distance (Table 6). The results of the analysis can be viewed in Figure 6. Almost all of the of the variation between the populations is explained by the first two axes, axis 1 = 78.12% and axis 2 = 18.31%. Since the two first axes explain such a big part of the variation in the data matrix, a 2-dimensional plot based on these two axes gives

almost a complete picture of the genetic distances between the different populations. If the two axes did not explain as much of the variation, important information of distances would be lost when limiting the presentation to a 2-dimensional plot. DR was clearly separated from the wild populations, indicating genetic distance between this group and the rest. The populations TA and RO were distant from the remaining populations along the first axis. Population MS and EV were placed at the same spot, demonstrating that these are very genetically similar. The same applied to JO and VN.

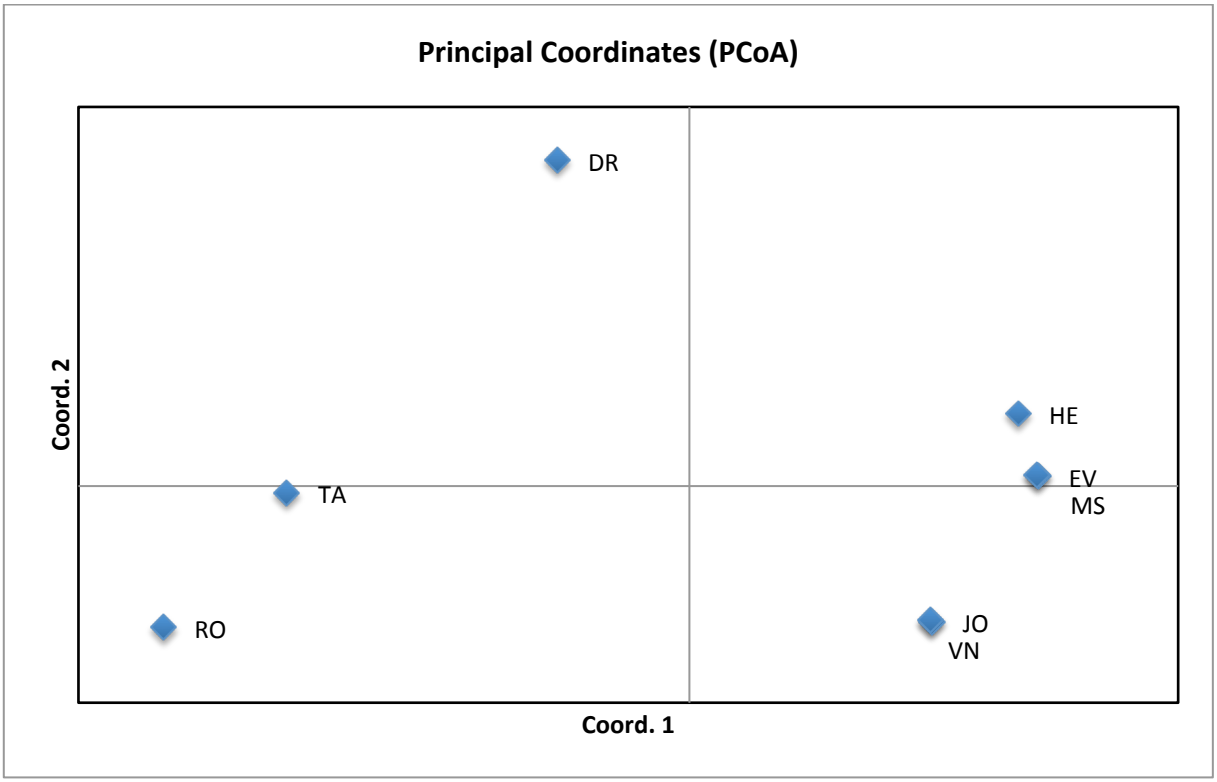

Figure 6: Showing PCoA result for all populations. Esviken (EV) and Munkesletta (MS) ordinated at the same spot, same with Jeløya (JO) and the Swedish population (VN). More information on the population abbreviations in Table 1.

Pairwise population matrix of Nei's unbiased genetic distance								
DR	EV	HE	JO	MS	RO	TA	VN	
0.000								DR
0.256	0.000							EV
0.234	0.065	0.000						HE
0.275	0.083	0.106	0.000					JO
0.255	0.000	0.065	0.083	0.000				MS
0.278	0.374	0.376	0.317	0.374	0.000			RO
0.183	0.328	0.319	0.273	0.327	0.014	0.000		TA
0.274	0.084	0.106	0.001	0.084	0.316	0.272	0.000	VN

Table 6: Values for Nei's unbiased genetic distance. Basis for PCoA (Figure 6). Population abbreviations in Table 1.

3.2 Seed Germination

3.2.1 The effect of light for germination

The germination was both quicker and the germination percentage was higher for seeds germinated in the light than for those germinated in darkness (Table 7, Figure 7). A photo comparing the two treatments is shown in Figure 8. Those kept in the light had germinated as much as 67% after 21 days, whereas the same figure for darkness was 7%. When the experiment was terminated after 42 days, 73% of the seeds kept in the light had germinated versus 35% for those kept in darkness.

Hellvik	**Light**	**Dark**
14 days	38%	0
21 days	67%	7%
28 days	71%	22%
35 days	73%	31%
42 days	73%	35%

Table 7: Germination percentages of light- and dark- grown seeds from Hellvik. Recording of germination started 14 days after sowing.

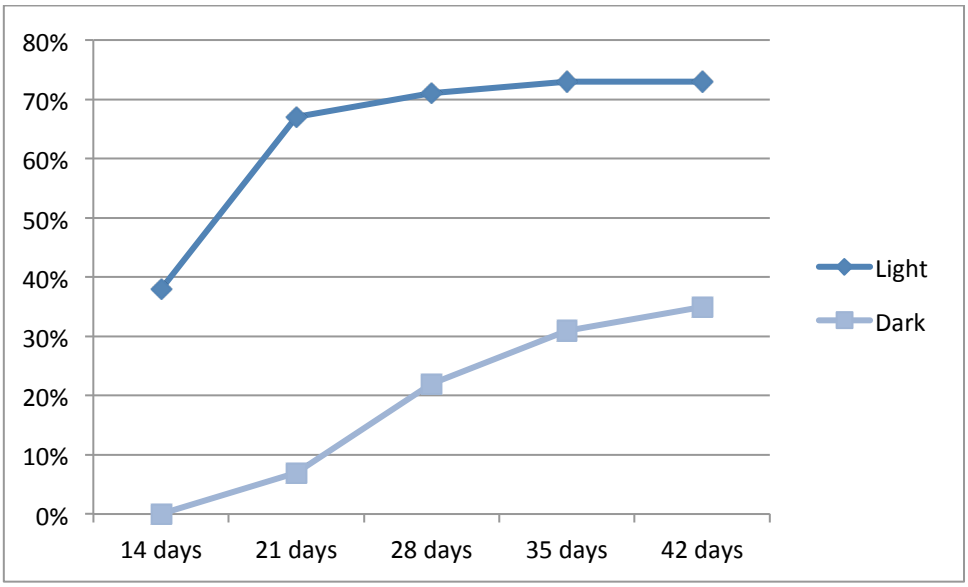

Figure 7: Variation in germination percentage with number of days from sowing of light- and dark-grown seeds of *D. rupestris*.

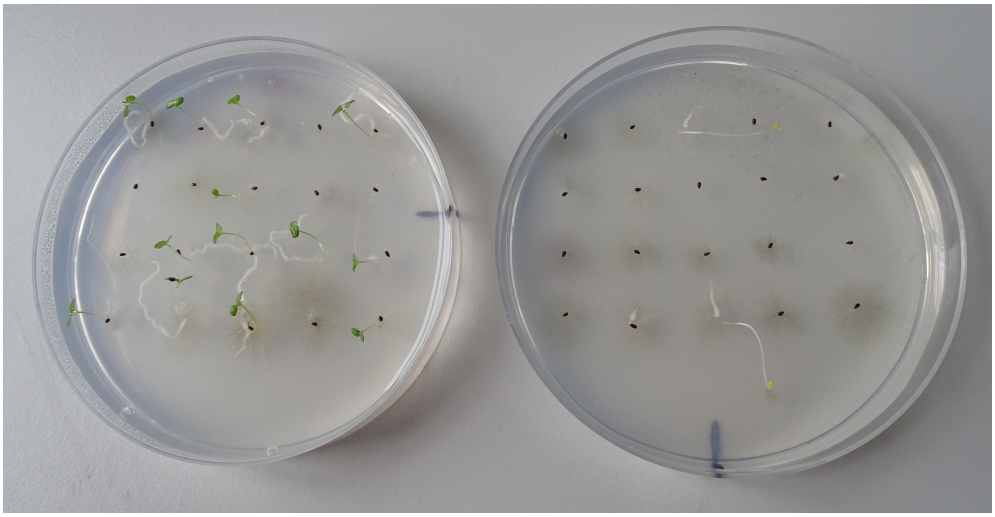

Figure 8: Photo showing differences in germination between light grown seeds in the petri dish to the left, and dark grown in dish to the right. One row represents five seeds from one individual, and is from the same individuals in both treatments.

3.2.2 Variation in seed germination between populations

The germination was quite similar in the populations Tåsen (TA), Esviken (EV) and Munkesletta (MS), with a maximum between 56% and 65% (Table 8, Figure 9). For the Hellvik (HE) population the germination percentage was clearly higher than the others, with 96% as maximum, and also higher than for the same population under constant temperature, 73% (see Table 7).

	TA	EV	MS	HE
14 days	60%	56%	56%	89%
21 days	65%	56%	60%	91%
28 days	65%	56%	62%	96%
35 days	65%	56%	62%	96%
42 days	65%	56%	62%	96%

Table 8: Germination percentages for four populations of *D. rupestris*. Population abbreviations in Table 1.

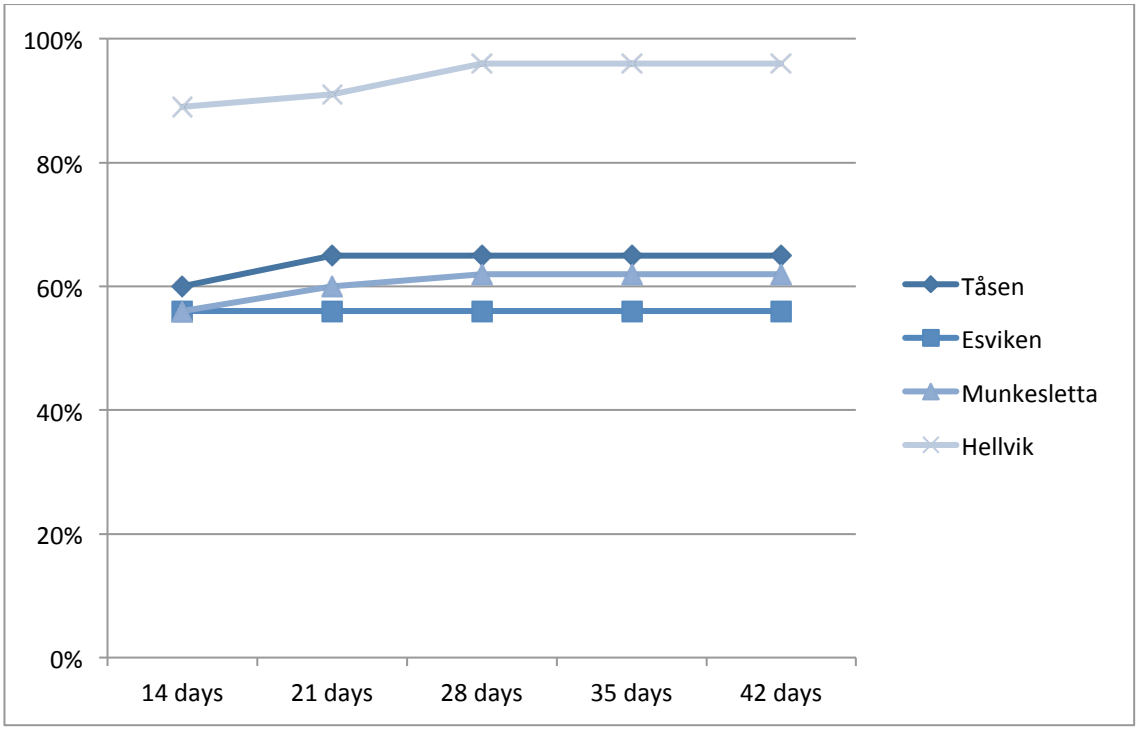

Figure 9: Variation in germination percentage with number of days from sowing of seeds from some populations of *D. rupestris*.

The first recording of seed germination was done at 14 days. Most of the seeds that actually germinated did so already within that time. All populations experienced a slight increase in germination percentages between 14 and 28 days. After 28 days, there was no further increase in germination percentages.

3.3 Examination of fruit set

The actively self-pollinated flowers, which had been isolated with fabric bags (Figure 10), did not successfully produce seeds, only one or two poorly developed seeds were observed. In contrast, the flowers that had been exposed to the outside produced a large amount of seeds.

Figure 10: Self-fertility tested by isolating flowers with thin bags of fabric.

4. Discussion

This study was initiated to gain a greater understanding of the biology of the perennial plant species *Drymocallis rupestris* that is classified as endangered in Norway, in order to aid successful conservation management. In this study I used 6146 SNP markers to assess the genetic structure and diversity in five wild populations in Norway in addition to one *ex situ* collection from the Botanical Garden in Oslo, and compared these to one population from Sweden and samples of cultivated origin. I also tested if light was a limiting factor for seed germination, recorded percentage of germination for each of the wild populations of *D. rupestris* in Norway, and did a simple test on self-fertilization. The sampling done in this study is obviously not as extensive as one would wish under ideal circumstances. With such a rare species as *D. rupestris* one just have to do the best with what is available and I believe that my results nevertheless will be valuable for future conservation measures.

4.1 Genetic Relationship

4.1.1 Distinctness of the Norwegian populations from those of cultivated origin (Hypothesis 1)

The results from the genetic structure test clustered the samples of cultivated origin (DR) together with the Tåsen population (TA) and the redlist flowerbed (RO), and within a different genetic group than the rest of the Norwegian and Swedish populations. However, upon investigating genetic difference, the F_{ST} values between DR and the wild populations were consistently high (most >0.25), indicating a significant genetic distance. This was also supported by the PCoA, which placed DR far away from the other sampled populations. The nursery from where the plants were bought is the only I have found that sells this species in Norway. Their material is based on seeds imported from a German company and has been in their selection for several years. The above mentioned evidence together with the fact that the species has hardly been used as a garden plant in Southeast Norway (Langeland, personal communication), make me conclude that the likelihood that the Norwegian populations are a result of escaped garden plants is very low. I will therefore not discuss the DR material further and rather proceed with discussing my other hypotheses.

4.1.2 Diversity and inbreeding in small versus large populations (Hypothesis 2 & 3)

The levels of genetic diversity, measured by percentage of polymorphic loci, number of private alleles, allelic richness and levels of heterozygosity, varied between the populations.

The clearest difference in variation within the Norwegian populations was given by the percentage of polymorphic loci, which ranged from 2.65% – 35.84%, in respectively two small populations. Crichton et al. (2016) reported similar levels of variation in genetic diversity in populations of a self-fertile plant in the UK. No convincing correlation of genetic diversity and population size was found. The populations Esviken (EV), Hellvik (HE) and Jeløya (JO) seem to match the assumption that larger population harbor more genetic variation and vice versa, while the populations Munkesletta (MS), Tåsen (TA) and the Swedish population (VN) seem to deviate from this pattern. The small population TA (4 individuals in population) showed the highest genetic diversity (Table 2), whereas the larger population MS showed one of the lowest levels of diversity of the whole sample area. The large population VN also showed low levels of diversity. In their study of endemic and vulnerable *Symphyotrichum georgianum,* Gustafson et al. (2016) also found that some large populations had lower diversity than small populations, which is similar to my findings. They suggested that the large populations with low genetic diversity might be reproducing asexually and/or be affected by inbreeding. Asexual reproduction is not reported for *D. rupestris* and can probably be ruled out as cause for low genetic variation.

The average heterozygosity values for *D. rupestris* were markedly lower (H_E = 0.057, H_O = 0.022) than those reported for short-lived perennials (H_E = 0.55, H_O = 0.53), species with narrow geographic range (H_E = 0.56, H_O = 0.52), and self-fertile individuals (H_E = 0.41, H_O = 0.05) in Nybom (2004). However, the levels of heterozygosity corresponds to those found in the highly sefling UK populations of *Melampyrum sylvaticum* in Crichton et al. (2016) (H_O = 0.017, H_E = 0.202). This may indicate that *D. rupestris* also self-fertilize in contrast to the test of self-fertility that was conducted. I found that expected heterozygosity (H_E) was higher than observed (H_O) in three of the Norwegian populations, respectively the large population at Hellvik (HE), the small population at Tåsen (TA) and the redlist flowerbed (RO). This yielded a positive inbreeding coefficient (F_{IS}) (Table 2), which is similar to the results for short-lived perennials in Nybom (2004) and to Crichton et al. (2016), which also showed a positive inbreeding coefficient. The other populations showed no signs of inbreeding, regardless of population size. The geographic isolation and small population sizes could certainly explain why HE, TA and RO may be affected by inbreeding, however, the other populations, who are just as isolated and small, showed no signs of inbreeding and also very low diversity. The populations showing low levels of diversity but no inbreeding could be more affected by genetic drift than inbreeding. It could also be that the species prefer to crossbreed but that it is

also self-fertile if forced to. The small populations showing no signs of inbreeding could be crossbreeding, thereby maintaining a certain level of heterozygosity. However, the low allelic variation and heterozygosity could indicate that they are on the verge of becoming inbred. The populations already showing high levels of inbreeding could be selfing, due to small population sizes or other environmental barriers, although, this is pure speculation. The discrepancy of the populations showing high inbreeding coefficients having the highest levels of diversity (e.g. percentage of polymorphic loci) could also potentially be explained by the presence of technical artifacts, like null-alleles. Null alleles in ddRADseq projects can be caused by undetected alleles that are created by differential amplification success during library preparation (Puritz et al., 2014), and may result in a synthetic reduction of observed heterozygosity and cause large inbreeding coefficients.

The low levels of heterozygosity found in the Norwegian populations of *D. rupestris* may have a deleterious effect on the population's fitness, as described by Reed and Frankham (2003) in a metastudy of 34 datasets on fitness and genetic variations in both plants and animals. However, the signs of high allelic diversity in the inbreeding populations is in a way a potential for an increase in heterozygosity. The high levels of inbreeding could be caused by the fact that within the populations there is little crossbreeding between individuals. As mentioned, in Hellvik (HE) there are a number of plants growing on a plateau while the rest of the population is situated at the base of the cliff. When sampling, I collected material from individuals from both places. The high levels of polymorphism could possibly be explained by the fact that individuals from these two places are genetically different, and that they rarely breed, giving a high number of homozygotes. However, if they were to crossbreed, e.g. by cross-pollinating by hand as a conservation measure, the levels of heterozygosity would perhaps increase giving a lower inbreeding coefficient.

4.1.5 Genetic variation in the ex situ collection (Hypothesis 4)

The initial genetic structure result showed that the Tåsen population (TA) and the samples collected from the redlist flowerbed (RO) share genetic structure. The PCoA ordinated the two populations close together, but not at the exact same spot. The F_{ST} value from the pairwise test suggest that these two populations are genetically similar, and more related than compared to the other sampled populations. The levels of diversity were quite high in both populations, however, the Tåsen (TA) population showed the highest values, e.g. percentage of polymorphic loci, even though the sample size was much smaller than for RO (see Table 1). When sampling at this locality I sampled both fertile individuals and small infertile

individuals. The last ones have probably not contributed seeds to the *ex situ* collection on which the red list individuals are based on, and it is therefore to expect that some genetic diversity has been lost. Another reason for lower genetic diversity in RO may be that some unintentional selection has occurred in the process of propagating plants for the flower bed.

4.1.4 Diversity in the Norwegian populations compared to the Swedish population (Hypothesis 5)

By comparing levels of genetic variation in the rare *D. rupestris* in Norway to the large and more continuous Swedish population, we could understand whether factors associated with small population size are affecting the levels of genetic diversity in the Norwegian populations. If the levels of diversity in the Norwegian populations were lower than the Swedish, this would suggest that factors associated with small populations (e.g., inbreeding, genetic drift or a genetic bottleneck) have negatively affected the Norwegian populations of *D. rupestris*. There was no obvious difference in variation between the Norwegian and Swedish populations. This is in contrast to what Nybom (2004), Edwards et al. (2013) and Cole (2003) reported. They all found that levels of heterozygosity and allelic richness or percentage of polymorphic loci was lower in small and isolated populations compared to widespread congeners. This might suggest that *D. rupestris* in general has low genetic variation, or that the Swedish population sampled is not a fair representation of the southern distribution of the species.

4.1.5 Distinctness of the Norwegian populations (Hypothesis 6)

The genetic structure analyses showed initially two distinct genetic groups among the studied material of *D. rupestris*, respectively singling out the Tåsen population (TA) and the red list flowerbed (RO) as one group, and the rest of the wild populations as another. This means that in Norway we have two genetic groups that are easily detected by structure analyses. Furthermore, genetic relationships among the other populations of *D. rupestris* reflected geographic proximity to a certain extent. When investigating further potential for genetic structuring with five out of eight populations, the population at Hellvik (HE) showed a more complex genetic structure than the rest, placed mostly by itself with some similarities with Esviken (EV) and Munkesletta (MS), which are geographically not too distant. The geographically close MS and EV were grouped together. This could indicate that these populations have in the past been part of a larger, continuous population that has been fragmented in recent times or that people have selectively transferred seeds or individuals from the more remote Munkesletta location to the garden at Esviken. This was also shown by

low F_{ST} values and very close proximity in the PCoA. The population at Jeløya (JO) was placed in a group with the Swedish population (VN). Of the Norwegian populations JO is geographically closest to Sweden. The other Norwegian populations are closer geographically, but the reason for the genetic affinity to the Swedish populations rather than the other Norwegian populations may indicate that the immigration history of the JO population is different from the other Norwegian populations. It is likely that the Norwegian populations rarely exchange genes because of limited seed and pollen dispersal between these relatively isolated populations. Genetic drift will then lead to diverging allele frequencies, increasing levels of differentiation. This was shown in the pairwise F_{ST} values, which showed clearly significant values between most of the different localities. This is similar to the results of Chricton et al. (2016), which showed that populations of a highly selfing plant, *Melampyrum sylvaticum*, had high average differentiation. This was further visualized in the PCoA, where the genetic distance between the different populations also reflected geographic distance.

A note on the F_{ST} values presented in this thesis: Although the F_{ST} values generally represented geographic proximity, and showed low values between populations who are geographically close together (e.g. MS and EV) they where generally higher than would be expected when comparing them to the scale presented in section 2.2.9 of this thesis. There has been much debate as to whether the use of F_{ST} is even useful as a measure of population differentiation and several studies have argued that large sample sizes (n>20) are required to provide reliable F_{ST} estimates (Kalinowski, 2005; Morin, Martien, & Taylor, 2009; Whitlock, 2011). This is very often a problem for conservation genetic analyses of species that are endangered and/or rare. However, Willing, Dreyer and van Oosterhout (2012) compared different F_{ST} estimators and stated that if the number of allelic markers is high, one can get a meaningful F_{ST} value even with small sample sizes. GenAlEx provides three different approaches for calculating F_{ST} between populations, one of the F_{ST} estimators is said to give estimates in line with Weir-Cockerham's (correcting for uneven sample sizes but following a different formula) (Peakall & Smouse, 2006; Peakall, Smouse, & Huff, 1995), which Willing et al. (2012) states overestimated genetic differentiation when the sample size was small and actual differentiation was >0.1. This could indicate that the F_{ST} estimates this study are overestimated, however, it is likely that they are equally scaled and therefore at least are compatible with each other. The amount of missing data may also skew the estimates. When comparing GenAlEx's "simplest" method for estimating F_{ST} (which does not correct for uneven sample sizes) with the one previously described, there was a noticeable difference in

the F_{ST} estimates (see Appendix nr. 5 for additional F_{ST} values). Based on the GenAlEx manufacturer's recommendation for research purposes, I have chosen to present the F_{ST} estimates that are in line with Weir-Cockerham's, although keeping in mind that they may be overestimated.

4.2 Seed Germination and Fruit Set

4.2.1 Effect of light on germination (Hypothesis 7) and percentage of germination between populations (Hypothesis 8)

The results from the seed germination experiment with light- and dark grown seeds showed that the light grown seeds germinated quicker and in a higher percentage that the dark grown (Table 7, Figure 7) . This is partly similar to the results found in Whittington et al. 1988. In contrast to our experiment, they transferred the dark grown seeds to light at day 15 of the experiment and they do not have any results on germination in the dark beyond that time. A certain amount of the dark grown seeds did eventually germinate so the effect of being isolated from light does not seem to be critical for recruitment, but delayed germination may certainly play a role in seedling's competition for space and light. Overgrowth may in other words limit recruitment of *D. rupestris*. When comparing germination percentages between populations (Table 8, Figure 9), seeds from Hellvik showed markedly higher percentages (96 %) than seeds from any other population (56%-65%), indicating that this population is the most vital. Previous seed experiments done in the seedbank of the Natural History Museum reported similar results (Bjureke, 2015).

4.2.2 Unsuccessful fruit set in self-pollinated flowers (Hypothesis 9)

The result from the self-fertility test showed that the self-pollinated flowers did not successfully produce seeds, contrary to the flowers that were left open and accessible to pollinators. This is in contrast to what Wilson et al. (1995) stated for the species without any further reference and what the genetic test may indicate. It is not known to me if there exists any other studies of self-fertility in *D. rupestris*, however, more thorough experiments should be conducted in order to substantiate my findings (this will be discussed further in section 5 of this thesis).

4.3 Conservation management

In light of my findings I believe it is important that as many of the Norwegian populations of *D. rupestris* are conserved, ideally *in situ* but also *ex situ*. On a global scale, the distribution in Norway can be viewed as a curiosity, and only marks *D. rupestris* northernmost occurrence. In Norway, *D. rupestris* may be of highest conservation value when viewed as part of a unique flora associated with calcareous coastal habitats. However, the Norwegian government has decided that we should in fact conserve this species and in agreement with Thylen (2012), I believe the species viability should be monitored closely. Clear evidence of two major genetic groups of *D. rupestris* in Norway were found, and the results indicated that each of the wild populations is genetically distinct and contains unique genetic diversity. However, not all of them showed values of high variation, which can be a warning sign of an unhealthy population. Based on the distribution of genetic variation and spatial genetic relationships among sites, sourcing plant material for reintroduction or reinforcements should, as far as possible, use large healthy local populations. Large populations are generally predicted to have more individual genotypes and greater genetic diversity compared to small populations. However, as mentioned, there was no correlation between large and small populations having, respectively, more or less diversity. The populations with the highest amount of polymorphic loci and allelic richness were Tåsen (TA) and Hellvik (HE), these two also belong to two different genetic groups. Given that the population at Tåsen already has been introduced at Ekeberg in Oslo, a new probable candidate for reintroduction can be the population at Hellvik. However, the population at highest extinction risk and most genetically distinct from the other wild Norwegian populations is the population at Jeløya (JO).

All the populations are relatively small, which might make them especially vulnerable to human disturbance and invasive plants. The potential for successful seed germination may be lowered by the fact that high growing plants are competing for resources with *D. rupestris* in its natural habitat. The increasingly dense vegetation may limit the propagation of seedlings and restrict population growth. The plants growing in the *ex situ* collection in the Botanical Garden in Oslo are prospering when tended to and freed of competitors. We may see that the annual clearings of invasive species, that have been orchestrated at several of the populations, may have a positive effect on the survival of seedlings. This is regardless a positive conservation measure and may be especially important at the genetically diverse populations Tåsen and Hellvik, where the species is growing alongside tall growing and/or invasive plants.

5. Further Investigations and Final Remarks

It would be interesting to use genomic data to infer potential evolutionary processes (e.g. genetic bottleneck) that could have led to present genetic differentiation within and between the Norwegian populations of *D. rupestris*. This has been done by Edwards et al. (2013), who used levels of heterozygosity to infer whether the populations studied had undergone a dramatic reduction in size. The factor that affects the viability of the populations would also be interesting to investigate. Are there any environmental conditions at micro level that play a part in the persistence of this relict species, or is it solely due to habitat fragmentation and its low competitive capabilities that makes it so rare?

A more comprehensive study of within population diversity of the Norwegian populations compared with several widespread populations of the species, could paint a more complete picture of the state of the Norwegian populations. No evidence suggested that the Norwegian populations had lower genetic diversity than the Swedish population. A full report of genetic diversity and statistical differentiation tests (e.g. regional AMOVA) between different regions could indicate to which degree the Norwegian populations differ in genetic diversity compared to other large populations. This could also tell us whether rarity and small population size have negatively affected levels of genetic diversity in the Norwegian populations and lead to answers of how well they will persist in the future. If low genetic variation is normal, this may buffer the populations against inbreeding. Furthermore, a test of self-compatibility, could provide important insight to this question. This can be done by gathering cross- and self-pollinated infruitescences and studying whether there are any differences in pollen tube growth as done by Nowak, Davis, Anthony and Yoder (2011).

A preliminary AMOVA-test to examine whether there was more genetic variation between populations than within was conducted. However, I concluded that my data set consisted of too much missing data to trust the outcome. The manual for the AMOVA function in GenAlEx says that it is very sensitive to missing data. Although not specifically stated I believe GenAlEx counts missing data as a genotype. With 11.5% missing data I believe the results will be skewed and variation over- or underestimated. The results are therefore not included in this thesis, but can be viewed in Appendix nr. 6. This would, however, be an interesting hypothesis to study, but changes and/or improvements need to be made with the SNP data set before conducting the analysis. I also believe that the data set should be checked

for null alleles, as mentioned in section 4.1.2. This was attempted, but I did not succeed due to technical difficulties with handling such a large data set.

To assess whether the populations show a pattern of genetic relatedness correlating to geographic distance (e.g. they show signs of genetic isolation as well as geographic isolation) an isolation by distance (IBD) test can be conducted. This was done by Crichton et al. (2016). I considered doing this for the Norwegian populations of *D. rupestris*, however I concluded that there were not enough populations to compare and that the distribution was too narrow.

6. Conclusion

The goal of this study was to investigate seed germination and production, variation within and between populations, and genetic structure of the perennial plant *D. rupestris*. In Norway, this species is considered to be endangered, and is only found at a few locations. Initial population structure results indicated that there are two major genetic groups within the sampled populations, separating the Tåsen (TA) population and the redlist flowerbed (RO) from the rest of the Norwegian populations. The latter group was further split into three with Munkesletta (MS) and Esviken (ES) in one group and Jeløya (JO) and Hellvik (HE) in two separate groups. This was backed up by the pairwise F_{ST} values and the PCoA. The populations from Esviken (EV) and Munkesletta (MS) showed little distinctness internally (F_{ST} = 0.048), indicating that these may previously have been part of a larger population, or that individuals (or seeds) from Munkesletta (MS) may have been transferred to Esviken (EV) for cultivation. These two populations showed high distinctness from any other sampled population. The population from Jeløya (JO) showed high distinctness from all the other Norwegian populations, but not from the Swedish (VN). There was no indication of higher variability in the Swedish population (VN), compared to the Norwegian populations, indicating that the Norwegian populations may not be genetically exhausted despite being geographically isolated. There was no correlation of large populations having more diversity or being less inbred than small populations, suggesting that also small populations may harbor valuable genetic information. Seed germination was higher for light grown seeds than for dark grown, which means that overgrowth may be a limiting factor for seed germination and recruitment. Germination varied little among populations. One exception was seeds collected from Hellvik, they showed considerably higher germination percentage than any other population. The self-fertility experiment showed unsuccessful seed set in self-pollinated flowers, which may suggest that the species is self-incompatible. As a conservation measure, the population at Hellvik may be a good candidate for reintroduction, as it showed high diversity, genetic distinctness and high levels of seed germination.

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Appendices

Appendix 1

Table showing sampled populations of *Drymocallis rupestris*. Collectors: KB = Kristina Bjureke, LH = Lise Huseby, BS = Brita Stedje, VH = Vojtec Holubec, NA = Not available.

Population abbreviation	Country	County	Municipality	Locality	Collector	Collection date	Coordinates	Ecology
HE	Norway	Akershus	Nesodden	Hellvik	LH & BS	14.07.2016	59.845748N, 10.691443E	East-facing coastal, rocky slope.
EV	Norway	Akershus	Asker	Esviken	LH & BS	15.08.2017	59.834530N, 10.484193E	Garden, dense vegetation.
MS	Norway	Akershus	Asker	Munkesletta	LH & BS	15.08.2017	59.828746N, 10.490425E	Open coastal, rocky slope. Lime-rich soil.
JO	Norway	Østfold	Moss	Jeløya	LH & BS	20.06.2017	59.501693N, 10.647441E	South-facing coastal, rocky slope. Lime-rich soil.
TA	Norway	Oslo	Oslo	Tåsen	LH & BS	29.06.2016	59.943506N, 10.741164E	Open herbaceous hill. Lime-rich soil.
RO	Norway	Oslo	Oslo	Botanical garden, Tøyen	LH & BS	29.06.2016	59.917381N, 10.770921E	Botanical garden flower bed.
VN	Sweden	Västra Götaland	Åmål	Vingnäs	KB, LH & BS	20.06.2017	58.912949N, 12.489589E	Open meadow. Lime-rich soil.
DR	Steen & Wormsen Perennial Nursery			Pamir Mts. & Southern Europe	VH & NA	09.08.2017	NA	NA

Appendix 2

Permits for collecting samples of *Drymocallis rupestris* in Norway.

MILJØ-DIREKTORATET

Naturhistorisk Museum, Universitetet i Oslo
Boks 1172 Blindern
Oslo
Att. Brita Stedje

Trondheim, 05.07.2016

Deres ref.:	Vår ref. (bes oppgitt ved svar):	Saksbehandler:
[Deres ref.]	2016/5511	Sunniva Aagaard

Dispensasjon til innsamling av hvitmure *Drymocallis rupestris* til forskningsformål

Miljødirektoratet gir Naturhistorisk museum, Universitetet i Oslo, ved Brita Stedje og Kristina Bjureke, dispensasjon til innsamling av hvitmure *Drymocallis rupestris* til forskningsformål, jf. forskrift 21. desember 2001 nr. 1525 om fredning av truede arter.

Om søknaden
Vi viser til søknad datert 23.06.2016 samt ettersendt informasjon datert 30.6.2016. I søknaden søkes det om dispensasjons for innsamling av deler av blad samt frø fra hvitmure *Drymocallis rupestris* i følgende områder:

Fylke	Kommune	Sted	Biotop	Forvaltning
Ak	Asker	Løkenes	Åpen grunnlendt kalkmark	Naturreservat
	Nesodden	Hellvik	Sørvendt berg, rikt strandberg	Plantefredning
Oslo	Oslo	Tåsen	Åpen grunnlendt kalkmark	Administrativt vern
		Ekeberg	Åpen grunnlendt kalkmark,	Naturreservat
		Blankvann	Slåttemark / naturbeitemark	Landskapsvernom
Østfold	Moss	Munkestein, Jeløya	Sørvendt berg	-

Det skal samles inn bladprøver og frø fra to blomster fra inntil 15 individer per populasjon for DNA analyse for bruk i populasjonsgenetiske studier for å avklare de ulike hvitmureforekomstenes opprinnelse og slektskap.

Masterstudent på prosjektet vil veiledes og ledsages i felt av Brita Stedje og Kristina Bjureke ved Naturhistorisk museum. Feltarbeidet vil bli utført i 2016 og 2017.

Det rettslige grunnlaget
Etter forskrift 21. desember 2001 nr. 1525 om fredning av truede arter (fredningsforskriften), punkt II, er *Drymocallis rupestris* fredet mot bl.a. innsamling og annen form for direkte etterstrebelse. Etter forskriftens punkt III, kan *"forvaltningsmyndigheten gjøre unntak fra fredningen når formålet med fredningen krever det, samt for vitenskapelige undersøkelser og arbeider, eller tiltak av vesentlig*

Postadresse: Postboks 5672, Sluppen, 7485 Trondheim | **Telefon:** 03400/73 58 05 00 | **Faks:** 73 58 05 01
E-post: post@miljodir.no | **Internett:** www.miljødirektoratet.no | **Organisasjonsnummer:** 999 601 391
Besøksadresser: Brattørkaia 15, 7010 Trondheim | Grensesvingen 7, 0661 Oslo|
Besøksadresser Statens naturoppsyns lokalkontorer: Se www.naturoppsyn.no

samfunnsmessig betydning, og i andre særlige tilfeller, når det ikke strider mot formålet med fredningen." Miljødirektoratet er forvaltningsmyndighet etter forskriften.

Miljødirektoratets vurdering
Den omsøkte innsamlingen skal skje i forbindelse med et forskningsprosjekt som skal utføres av Naturhistorisk museum, Universitetet i Oslo, som direkte relateres til kunnskapsbehov påpekt i det faglige grunnlaget for hvitmure (BioFokusrapport 2012-17). Da søknaden gjelder innsamling som ledd i «vitenskapelige undersøkelser og arbeider» som vil bidra til bedre forvaltning av arten, er vilkåret for å gi dispensasjon oppfylt, jf. fredningsforskriften punkt III.

Selv om ett av dispensasjonsvilkårene i fredningsforskriften er oppfylt, beror det på en skjønnsmessig vurdering om det skal gis dispensasjon i den enkelte sak.

Hvitmure vurderes som sterkt truet på Norsk rødliste 2015, fordi den har en begrenset og fragmentert utbredelse med bare noen få kjente forekomster i Oslofjord-området, i til dels sterkt utsatte og nedbygde områder, hvor innslaget av fremmede arter reduserer habitatkvaliteten (Artsdatabanken). Arten er kjent fra seks lokaliteter, og er estimert til å ha 350 individer. Et faglig grunnlag for hvitmure ble publisert i 2012 (BioFokusrapport 2012-17. Flere av forekomstene i Oslo og Akershus er fulgt opp med skjøtsel de siste årene, og antall individer øker (Blyttia 74, s 27 – 34). Miljødirektoratet vurderer kunnskapsgrunnlaget om hvitmure til å være godt, jf. naturmangfoldloven § 8. Føre-var prinsippet, jf. naturmangfoldloven § 9, vurderes dermed som ikke som relevant i denne saken.

Søker opplyser at det til DNA-analysene skal tas bladprøver på 1 – 2 cm^2 fra 15 individ per populasjon. DNA som blir til overs vil bli deponert i Naturhistorisk museums DNA-bank. Det skal også samles inn frøene produsert av to blomster per individ for estimering og sammenlikning av frøproduksjon og eventuelt spireforsøk. Søknaden gjelder innsamling av et begrenset omfang plantedeler og frø, fra et begrenset antall planter. Etter Miljødirektoratets syn, vil innsamlingsmåtene ikke være destruktive, jf. naturmangfoldloven § 12 (miljøforsvarlige teknikker og driftsmetoder). Den omsøkte innsamlingen vurderes til å ha minimal negativ effekt på de berørte individene, og følgelig også på bestandssituasjonen for hvitmure, og vil heller ikke øke den samlede belastningen på arten, jf. naturmangfoldloven § 10. Innsamlingen vil dermed ikke forringe artens bestandssituasjon eller -utvikling, jf. naturmangfoldloven § 5. På denne bakgrunn er Miljødirektoratet kommet til at det kan gis dispensasjon til innsamling av plantedeler og frø av hvitmure som omsøkt. I denne sammenheng vil vi også peke på at innsamlingen vil kunne bidra til å gi ny kunnskap som kan være nyttig med tanke på framtidig forvaltning av arten.

Miljødirektoratet gjør oppmerksom på at innsamling i verneområder kan kreve egen tillatelse etter verneforskriften. Det vises til at Fylkesmannen i Oslo og Akershus har gitt tillatelse til innsamling av plantemateriale fra lokaliteter i verneområdene Spirodden naturreservat, Asker, Ekebergskråningen naturreservat, Oslo og Hellvik gamle brygge plantefredningsområde, Nesodden, jf. vedtak datert 24.6.2016, ref. 2016/12914-1 M-NA.

Vedtak
Miljødirektoratet gir Naturhistorisk museum, Universitetet i Oslo, ved Brita Stedje og Kristina Bjureke, dispensasjon til innsamling av *Drymocallis rupestris* til forskningsformål, jf. jf. forskrift 21. desember 2001 nr. 1525 om fredning av truede arter, punkt III, på følgende vilkår:

1. Innsamlingen kan skje i sommersesongene 2016 og 2017 på følgende lokaliteter:

Fylke	Kommune	Sted
Ak	Asker	Løkenes
	Nesodden	Hellvik
Oslo	Oslo	Tåsen
		Ekeberg
		Blankvann
Østfold	Moss	Munkestein,

2. Det kan samles inn materiale fra opptil 15 individer på hver lokalitet. Fra hvert individ gis det tillatelse til å ta blodprøver på 1-2 cm^2 samt alle frø produsert av to blomster på hver plante. Innsamlingen skal gjennomføres så skånsomt som mulig og på en måte som ikke reduserer individenes overlevelsesevne.
3. Publiserte rapporter og artikler med data fra innsamlede eksemplar, samt ferdig masteroppgave, oversendes Miljødirektoratet
4. Alle eksemplar rapporteres inn til Artsdatabankens artsobservasjonstjeneste
5. Frø som blir til overs skal deponeres i frøbanken ved Naturhistorisk museum, Universitetet i Oslo.

Denne dispensasjonen skal medbringes og forevises grunneier og oppsynspersonell ved forespørsel.

Dette vedtaket kan påklages til Klima- og miljødepartementet av sakens parter eller andre med rettslig klageinteresse innen tre uker fra det tidspunkt underretning om vedtaket er kommet fram til vedkommende part. Klagen skal sendes til Miljødirektoratet.

Hilsen
Miljødirektoratet

Dette dokumentet er elektronisk godkjent

Bjarte Rambjør Heide
seksjonsleder

Sunniva Aagaard
seniorrådgiver

Tenk miljø - velg digital postkasse fra e-Boks eller Digipost på www.norge.no.

Kopi:
Fylkesmannen i Oslo og Akershus, Miljøvernavdelingen

Fylkesmannen
i Oslo og Akershus

Miljøvernavdelingen

Tordenskioldsgate 12
Postboks 8111 Dep, 0032 OSLO
Telefon 22 00 35 00
fmoapostmottak@fylkesmannen.no
www.fmoa.no
Organisasjonsnummer NO 974 761 319

Naturhistorisk museum, Universitetet i Oslo v/Brita Stedje
Postboks 1172 Blindern
0318 OSLO

Deres ref.:
Deres dato:
Vår ref.: 2016/12914-1 M-NA
Saksbehandler: Øystein Røsok
Direktetelefon: 22 00 36 40

Dato: 24.06.2016

Tillatelse til innsamling av plantemateriale fra verneområdene Spirodden naturreservat, Asker, Ekebergskråningen naturreservat, Oslo og Hellvik gamle brygge, Nesodden - 2016

Forutsatt dispensasjon fra forskrift om fredete arter, gis det dispensasjon til å samle små bladprøver og frø fra den fredete arten hvitmure innenfor verneområdene Spirodden naturreservat, Asker, Ekebergskråningen naturreservat, Oslo og Hellvik gamle brygge plantefredningsområde, Nesodden.

Vi viser til søknad om tillatelse om å ta små bladprøver fra planten hvitmure, datert 23. juni 2016.

Søknaden
Det søkes om tillatelse til å ta små bladprøver til isolering av DNA for analyser av genetisk variasjon, samt frø for estimering og sammenlikning av frøproduksjon og eventuelt spireforsøk. Til DNA-analyse er det nødvendig å ta små bladprøver 1 – 2 cm^2 store. Det søkes om å samle frø av to blomster per individ. For begge formål er det aktuelt å samle inn materiale av inntil 15 individer per populasjon.

Prosjektet er delvis basert på forskningsbehov skissert i handlingsplan for hvitmure (BioFokusrapport 2012-17), der det blant annet pekes på viktigheten av å avklare de ulike hvitmureforekomstenes opprinnelse og slektskap ved en DNA-analyse.

Verneformål og bestemmelser
Fylkesmannen i Oslo og Akershus (FMOA) er forvaltningsmyndighet for verneområdene i Oslo og Akershus. I det følgende presenteres en oversikt over verneformål og utdrag fra vernebestemmelsene der innsamling av planter kan være forbudt i henhold til forskriften.

Ekebergskråningen naturreservat, Oslo:
Formålet med naturreservatet er *å bevare et tilnærmet urørt område med en spesiell naturtype med stor variasjon i vegetasjonstyper og med stort biologisk mangfold.*
I følge vernebestemmelsene, § 3 pkt. 1, er *vegetasjonen, herunder døde busker og trær, er fredet mot skade og ødeleggelse. Det er forbudt å fjerne planter eller plantedeler fra reservatet*.

Hellvik gamle brygge plantefredningsområde, Nesodden:
Formålet med plantefredningsområdet er *å ivareta den sjeldne plantearten hvitmure og dens livsmiljø*.
I følge vernebestemmelsene, § 3 pkt. 1, er *hvitmure og all vegetasjon som er viktig for livsmiljøet til hvitmure er fredet mot skade og ødeleggelse. Det er forbudt å fjerne planter eller plantedeler fra fredningsområdet.*

Spirodden naturreservat, Asker
Formålet med naturreservatet er *å bevare en klassisk lokalitet for forståelsen av Oslofeltets fossilførende bergarter med meget høy vitenskapelig og pedagogisk verdi. Området er botanisk rikt med kalkfuruskog og alm-lindeskog som inneholder flere sårbare og truete arter. Området er også egenartet på grunn av en stor forekomst av den nasjonalt sjeldne arten hvitmure.*
I følge vernebestemmelsene er *vegetasjonen, herunder døde busker og trær, er fredet mot skade og ødeleggelse. Det er forbudt å fjerne planter eller plantedeler fra reservatet.*

Fylkesmannens vurdering
I henhold til naturmangfoldloven (NML) § 7 skal prinsippene i §§ 8-12 legges til grunn ved utøving av offentlig myndighet.

Kunnskapsgrunnlaget (§ 8) om karplanter generelt i indre Oslofjord vurderes som godt, og om hvitmure som svært bra. Et faglig grunnlag for hvitmure ble publisert i 2012. Flere av forekomstene i Oslo og Akershus er fulgt godt opp med skjøtsel de siste årene, og antall individer øker. Dette er beskrevet i tidsskriftet Blyttia i 2016 (Blyttia 74, s 27 – 34). Fylkesmannens kunnskap om verneområdene er basert på informasjon fra Naturbase og Artskart, samt flere rapporter om plantelivet. Opplysninger i søknaden om et begrenset omfang av innsamling av plantedeler samt begrenset innsamling av frø fra hvitmure, i begge tilfeller fra et begrenset antall planter, tilsier at den omsøkte innsamlingen ikke kan ha nevneverdig negativ påvirkning hverken på de individene det samles inn plantedeler fra, eller på bestandssituasjonen av hvitmure. Tiltaket er derfor ikke i strid med forvaltningsmålet for arten, men kan bidra til å gi ny kunnskap som kan være nyttig i langsiktig forvaltningen av arten. Innsamlingsmetodene vurderes som miljøforsvarlige teknikker (§ 12). Kunnskapsgrunnlaget ansees etter dette som tilstrekkelig for vurdering av søknaden og føre-varprinsippet (§ 9) tillegges derfor mindre vekt. Flere påvirkningsfaktorer tilsier at den samlede belastning (§ 10) på disse aktuelle verneområdene er allerede stor. Til tross for dette, vurderes det omsøkte tiltaket på kort sikt å innebære en beskjeden og akseptabel tilleggsbelastning for populasjonen av hvitmure. På lang sikt kan tiltaket gi en positiv effekt i form av kunnskap som kan bidra til bedre forvaltning av arten. Verneformål for de aktuelle verneområdene varierer, men bevaring av naturtyper, vegetasjon og til dels hvitmure, er felles. Innsamling av plantedeler og frø fra hvitmure trenger følgelig dispensasjon fra vernereglene.

Det følger av naturmangfoldloven § 48 at man kan gjøre unntak fra vernevedtaket dersom det ikke strider mot vernevedtakets formål, og ikke kan påvirke verneverdiene nevneverdig. Dette er to alternative vilkår, som begge må være oppfylt for å ha hjemmel til å gi dispensasjon. Naturmangfoldlovens § 48 gjelder fra 2009 istedenfor tidligere generelle dispensasjonsregler i alle verneforskrifter. Fylkesmannen har anledning til å gjøre unntak fra verneforskriften for vitenskapelige undersøkelser. Under forutsetning av at innsamlingen vil ha liten betydning for hvitmurens populasjonsstørrelser i verneområdene, vurderer Fylkesmannen at innsamling av plantedeler og frø av hvitmure ikke strider mot noen av verneområdenes formål eller påvirker

verneverdiene nevneverdig. Fylkesmannen mener derfor det kan gis tillatelse til innsamling av plantedeler og frø av hvitmure i henhold til beskrevet metodikk.

Hvitmure er en fredet art i henhold til forskrift om fredning av truede arter. Miljødirektoratet er forvaltningsmyndighet etter denne forskriften. Det omsøkte tiltaket krever derfor et dispensasjonsvedtak fra fredningsforskiften av Miljødirektoratet. Tiltaket kan ikke settes i gang før det er gitt dispensasjon etter fredningsforskriften.

Vedtak
I medhold av Naturmangfoldlovens § 48, gis det dispensasjon til innsamling av plantedeler og frø av hvitmure i de over nevnte verneområdene i Oslo og Akershus. En vurdering av naturmangfoldlovens §§ 8-12 er langt til grunn for vedtaket. Tillatelsen vil vare fra juni 2016 ut 2014.

Dispensasjonen gis på følgende vilkår:

- Tiltaket skal gjennomføres så skånsomt som mulig og ta hensyn til naturverdiene.
- Denne tillatelsen skal medbringes i felt og kunne forevises oppsyn og politi.
- Fylkesmannen kan trekke dispensasjonen tilbake dersom vilkårene ikke følges eller ved uforutsette negative effekter på verneverdiene.
- Tiltaket kan ikke settes i gang før Miljødirektoratet har gitt dispensasjon etter fredningsforskriften.

Klageadgang
Vedtaket kan påklages til Miljødirektoratet av sakens parter eller andre med rettslig klageinteresse innen 3 uker fra avgjørelsen er mottatt. Eventuell klage skal angi hva det klages over og den eller de endringer som ønskes. Klagen skal begrunnes, og andre opplysninger av betydning for saken bør nevnes. Klagen skal sendes via Fylkesmannen.

Med hilsen

Ellen Lien
seksjonssjef

Øystein Røsok
seniorrådgiver

Dokumentet er elektronisk godkjent.

Kopi til:
Miljødirektoratet Postboks 5672 Sluppen 7485 TRONDHEIM

Appendix 3

Adapters used in ddRADseq library preparation.

All sequences are written from 5' to 3' end.

Amplification primers
Amplification Primer 1 (forward)
AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACG
Amplification Primer 2 (forward) (note: no index included for dual indexing)
AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACG
PCR2_Idx_1_ATCACG
Amplification Primer 2 (Reverse) (Index 1)
CAAGCAGAAGACGGCATACGAGATCGTGATGTGACTGGAGTTCAGACGTGTGC

P1.1 oligos

Name	**Sequence**
ddRAD_P1.1-1	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCTCCTGCA
ddRAD_P1.1-2	ACACTCTTTCCCTACACGACGCTCTTCCGATCTACTATGCA
ddRAD_P1.1-3	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGGTGTTGCA
ddRAD_P1.1-4	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGGAACTGCA
ddRAD_P1.1-5	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTAATATGCA
ddRAD_P1.1-6	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTCGTTTGCA
ddRAD_P1.1-7	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCCAGCTTGCA
ddRAD_P1.1-8	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTTCAGATGCA
ddRAD_P1.1-9	ACACTCTTTCCCTACACGACGCTCTTCCGATCTATCGTATGCA
ddRAD_P1.1-10	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCATCGTTGCA
ddRAD_P1.1-11	ACACTCTTTCCCTACACGACGCTCTTCCGATCTATGAAACTGCA
ddRAD_P1.1-12	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGTCGATTTGCA
ddRAD_P1.1-13	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCATAAGTTGCA
ddRAD_P1.1-14	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTAGCGGATGCA
ddRAD_P1.1-15	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTAGCATGCTGCA
ddRAD_P1.1-16	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGGATTGGTTGCA
ddRAD_P1.1-17	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTACTTGCA
ddRAD_P1.1-18	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCAGATGCA
ddRAD_P1.1-19	ACACTCTTTCCCTACACGACGCTCTTCCGATCTAACTTGCA
ddRAD_P1.1-20	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGCGTTGCA
ddRAD_P1.1-21	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCGATTGCA

ddRAD_P1.1-22	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGTAATGCA
ddRAD_P1.1-23	ACACTCTTTCCCTACACGACGCTCTTCCGATCTAGGCTGCA
ddRAD_P1.1-24	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGATCTGCA
ddRAD_P1.1-25	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTCACTGCA
ddRAD_P1.1-26	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTGCGATGCA
ddRAD_P1.1-27	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCGCTTTGCA
ddRAD_P1.1-28	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTCACCTGCA
ddRAD_P1.1-29	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCTAGCTGCA
ddRAD_P1.1-30	ACACTCTTTCCCTACACGACGCTCTTCCGATCTACAAATGCA
ddRAD_P1.1-31	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTTCTCTGCA
ddRAD_P1.1-32	ACACTCTTTCCCTACACGACGCTCTTCCGATCTAGCCCTGCA
ddRAD_P1.1-33	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGTATTTGCA
ddRAD_P1.1-34	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCTGTATGCA
ddRAD_P1.1-35	ACACTCTTTCCCTACACGACGCTCTTCCGATCTACCGTTGCA
ddRAD_P1.1-36	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGCTTATGCA
ddRAD_P1.1-37	ACACTCTTTCCCTACACGACGCTCTTCCGATCTAGGATTGCA
ddRAD_P1.1-38	ACACTCTTTCCCTACACGACGCTCTTCCGATCTATTGATGCA
ddRAD_P1.1-39	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCATCTTGCA
ddRAD_P1.1-40	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCCTACTGCA
ddRAD_P1.1-41	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGAGGATGCA
ddRAD_P1.1-42	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGTCAATGCA
ddRAD_P1.1-43	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTACATTGCA
ddRAD_P1.1-44	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGGTTGTTGCA
ddRAD_P1.1-45	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTAGGAATGCA
ddRAD_P1.1-46	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGCTCTATGCA
ddRAD_P1.1-47	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCCACAATGCA
ddRAD_P1.1-48	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCTTCCATGCA
ddRAD_P1.1-49	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGAGATATGCA
ddRAD_P1.1-50	ACACTCTTTCCCTACACGACGCTCTTCCGATCTATGCCTTGCA
ddRAD_P1.1-51	ACACTCTTTCCCTACACGACGCTCTTCCGATCTAGTGGATGCA
ddRAD_P1.1-52	ACACTCTTTCCCTACACGACGCTCTTCCGATCTACCTAATGCA
ddRAD_P1.1-53	ACACTCTTTCCCTACACGACGCTCTTCCGATCTATATGTTGCA
ddRAD_P1.1-54	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCGCGGTTGCA
ddRAD_P1.1-55	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCTATTATGCA
ddRAD_P1.1-56	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGCCAGTTGCA
ddRAD_P1.1-57	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGGAAGATGCA
ddRAD_P1.1-58	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGTACTTTGCA
ddRAD_P1.1-59	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGTTGAATGCA
ddRAD_P1.1-60	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTAACGATGCA
ddRAD_P1.1-65	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGAATTCATGCA
ddRAD_P1.1-66	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGAACTTCTGCA
ddRAD_P1.1-67	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGGACCTATGCA

ddRAD_P1.1-68	ACACTCTTTCCCTACACGACGCTCTTCCGATCTAACGCCTTGCA
ddRAD_P1.1-69	ACACTCTTTCCCTACACGACGCTCTTCCGATCTAATATGCTGCA
ddRAD_P1.1-70	ACACTCTTTCCCTACACGACGCTCTTCCGATCTACGTGTTTGCA
ddRAD_P1.1-71	ACACTCTTTCCCTACACGACGCTCTTCCGATCTATTAATTTGCA

P1.2 oligos

Name	Sequence
ddRAD_P1.2-1	/5Phos/GGAGAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
ddRAD_P1.2-2	/5Phos/TAGTAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
ddRAD_P1.2-3	/5Phos/ACACCAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
ddRAD_P1.2-4	/5Phos/GTTCCAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
ddRAD_P1.2-5	/5Phos/TATTAAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
ddRAD_P1.2-6	/5Phos/AACGAAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
ddRAD_P1.2-7	/5Phos/AGCTGGAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
ddRAD_P1.2-8	/5Phos/TCTGAAAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
ddRAD_P1.2-9	/5Phos/TACGATAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
ddRAD_P1.2-10	/5Phos/ACGATGAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
ddRAD_P1.2-11	/5Phos/GTTTCATAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
ddRAD_P1.2-12	/5Phos/AATCGACAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
ddRAD_P1.2-13	/5Phos/ACTTATGAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
ddRAD_P1.2-14	/5Phos/TCCGCTAAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
ddRAD_P1.2-15	/5Phos/GCATGCTAAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
ddRAD_P1.2-16	/5Phos/ACCAATCCAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
ddRAD_P1.2-17	/5Phos/AGTAAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
ddRAD_P1.2-18	/5Phos/TCTGAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
ddRAD_P1.2-19	/5Phos/AGTTAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
ddRAD_P1.2-20	/5Phos/ACGCAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
ddRAD_P1.2-21	/5Phos/ATCGAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
ddRAD_P1.2-22	/5Phos/TTACAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
ddRAD_P1.2-23	/5Phos/GCCTAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
ddRAD_P1.2-24	/5Phos/GATCAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
ddRAD_P1.2-25	/5Phos/GTGAAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
ddRAD_P1.2-26	/5Phos/TCGCAAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
ddRAD_P1.2-27	/5Phos/AAGCGAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
ddRAD_P1.2-28	/5Phos/GGTGAAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
ddRAD_P1.2-29	/5Phos/GCTAGAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
ddRAD_P1.2-30	/5Phos/TTTGTAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
ddRAD_P1.2-31	/5Phos/GAGAAAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
ddRAD_P1.2-32	/5Phos/GGGCTAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
ddRAD_P1.2-33	/5Phos/AATACAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
ddRAD_P1.2-34	/5Phos/TACAGAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
ddRAD_P1.2-35	/5Phos/ACGGTAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
ddRAD_P1.2-36	/5Phos/TAAGCAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT

ddRAD_P1.2-37	/5Phos/ATCCTAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
ddRAD_P1.2-38	/5Phos/TCAATAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
ddRAD_P1.2-39	/5Phos/AGATGAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
ddRAD_P1.2-40	/5Phos/GTAGGAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
ddRAD_P1.2-41	/5Phos/TCCTCAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
ddRAD_P1.2-42	/5Phos/TTGACAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
ddRAD_P1.2-43	/5Phos/ATGTAAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
ddRAD_P1.2-44	/5Phos/ACAACCAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
ddRAD_P1.2-45	/5Phos/TTCCTAAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
ddRAD_P1.2-46	/5Phos/TAGAGCAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
ddRAD_P1.2-47	/5Phos/TTGTGGAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
ddRAD_P1.2-48	/5Phos/TGGAAGAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
ddRAD_P1.2-49	/5Phos/TATCTCAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
ddRAD_P1.2-50	/5Phos/AGGCATAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
ddRAD_P1.2-51	/5Phos/TCCACTAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
ddRAD_P1.2-52	/5Phos/TTAGGTAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
ddRAD_P1.2-53	/5Phos/ACATATAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
ddRAD_P1.2-54	/5Phos/ACCGCGAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
ddRAD_P1.2-55	/5Phos/TAATAGAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
ddRAD_P1.2-56	/5Phos/ACTGGCAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
ddRAD_P1.2-57	/5Phos/TCTTCCAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
ddRAD_P1.2-58	/5Phos/AAGTACAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
ddRAD_P1.2-59	/5Phos/TTCAACAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
ddRAD_P1.2-60	/5Phos/TCGTTAAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
ddRAD_P1.2-65	/5Phos/TGAATTCAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
ddRAD_P1.2-66	/5Phos/GAAGTTCAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
ddRAD_P1.2-67	/5Phos/TAGGTCCAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
ddRAD_P1.2-68	/5Phos/AGGCGTTAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
ddRAD_P1.2-69	/5Phos/GCATATTAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
ddRAD_P1.2-70	/5Phos/AACACGTAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
ddRAD_P1.2-71	/5Phos/AATTAATAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT

P2 adapter
ddRAD_P2.1 bottom Y
GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
ddRAD_P2.2 top
AGATCGGAAGAGCGAGAACAA

Appendix 4

Recipe for agar used in seed germination experiment.

For 1 l Agar

- Heat up 900 ml distilled water to boiling point.
- Mix 10 gr agar-powder and 100 ml distilled water.
- Mix agarmix with the boiling water and stir until the agar is boiling and fully mixed (important for proper hardening of agar).
- Cool agarmix down to approximately 50°C and pour into petri dishes (put lid on).
- Store in refrigerator

Appendix 5

F_{ST} estimates for pairwise test without correction of sample size.

Pairwise Population F_{ST} Values								
	DR	**EV**	**HE**	**JO**	**MS**	**RO**	**TA**	**VN**
DR	0,000							
EV	0,435	0,000						
HE	0,362	0,307	0,000					
JO	0,453	0,620	0,418	0,000				
MS	0,432	0,048	0,299	0,597	0,000			
RO	0,381	0,662	0,549	0,643	0,653	0,000		
TA	0,286	0,512	0,443	0,484	0,508	0,114	0,000	
VN	0,440	0,555	0,391	0,069	0,534	0,615	0,468	0,000

Appendix 6

Analysis of molecular variance (AMOVA) between the 6 Norwegian populations, Hellvik (HE), Tåsen (TA), Munkesletta (MS), Esviken (EV), Jeløya (JO) and the redlist flowerbed (RO).

Number of samples: 48

Number of permutations: 999