The endangered *Dracocephalum ruyschiana*: a study of factors affecting reproduction and recruitment in Norwegian populations

Thorstein Paulsen

Master's in biology: Ecology and Evolution
Department of Biosciences and the Natural History Museum

UNIVERSITY OF OSLO

01.04.2019

The endangered *Dracocephalum ruyschiana*: a study of factors affecting reproduction and recruitment in Norwegian populations

Thorstein Paulsen
thorpau@gmail.com
+47 93 86 32 29

Main supervisor
Brita Stedje
brita.stedje@nhm.uio.no

Co-supervisor
Nina Sletvold

Department of Ecology and Genetics, Uppsala University, Sweden
nina.sletvold@ebc.uu.se

ISOP (Integrative Systematics of Plants and Fungi) group
Natural History Museum

Master's in biology: Ecology and Evolution
Department of Biosciences
University of Oslo

01.04.2019

2019

The endangered *Dracocephalum ruyschiana*: a study of factors affecting reproduction and recruitment in Norwegian populations

Thorstein Paulsen

http://www.duo.uio.no/

Trykk: Reprosentralen, Universitetet i Oslo

Summary

Dracocephalum ruyschiana is a rare species in Europe and its conservation is considered a priority by the Norwegian Environment Agency. Specialist species in fragmented and rare habitats such as dry, calcareous grasslands may be especially vulnerable to changes in the environment. Eutrophication and overgrowth of habitats is thought to favour more competitive species, potentially leaving specialists like *D. ruyschiana* at a disadvantage. Efficient conservation of *D. ruyschiana* calls for more knowledge on the survival and reproduction of the species. This thesis provides a study of factors that may influence survival, seed set and germination in *D. ruyschiana*, including an investigation of the presence of *D. ruyschiana* soil seed banks in Norway. The aim is to illuminate factors that may explain the observed decline of *D. ruyschiana* populations in Europe and to contribute knowledge that may be helpful for conserving and restoring populations of the species. The research questions address (1) the effect of neighbour competition on flower size, seed set and germination, (2) the relationship between population size and genetic diversity, seed set and germination, (3) whether light and scarification promote seed germination, and (4) the representation of *D. ruyschiana* in natural soil seed banks.

The effects of increased competition through habitat overgrowth on *D. ruyschiana* reproduction were investigated by a vegetation removal experiment. Seed set, seed germination percentage, and flower size measurements for 77 individual *D. ruyschiana* were compared to measurements of a control group of 82 individuals. *D. ruyschiana* that had surrounding vegetation trimmed produced significantly more seeds per flower. A small positive effect of vegetation removal was also found on seed germination percentage.

The effects of population size on genetic diversity, seed set, and germination percentage, and the effects of seed set on germination was investigated for 11 Norwegian populations of *D. ruyschiana.* Seed set was a significant predictor of germination percentage. Population size also appeared to have a positive effect on genetic diversity, although this effect was not statistically significant. Population size could not predict variation in seed set or germination.

Seed scarification was found to increase *D. ruyschiana* seed germination percentage. Although not statistically significant, exposure to light seemed to have a small, positive effect on germination. Similarly, the population from which the seeds came had a near-significant influence on seed germination percentage. No interaction between light and scarification could be found.

The presence of *D. ruyschiana* seeds was found in soil from two out of three populations, using two different methods of seed detection, suggesting that *D. ruyschiana* has a soil seed bank.

Preface

This master thesis was written at the Natural History Museum of Oslo, and I am grateful to have been a part of the Integrative Systematics of Plants and Fungi research group (ISOP) there. This thesis is based on and supplements an ongoing study by Stedje *et al.* (in prep.) Thanks to my supervisors for their guidance, Olav Skarpaas for statistical help, Odd Stabbetorp for his help with the study design and fieldwork of the seed soil bank experiment, Anders Often for his help identifying seedlings, and thanks to Cecilie Midtøy et al. for their help with keeping the plants alive through a very hot summer.

Table of Contents

1 INTRODUCTION

Biodiversity loss reduces ecosystem functioning and stability, and has severe implications for the continued prosperity of life on Earth (Cardinale *et al.* 2012). Human activity pressures biodiversity through climate change, habitat conversion, degradation, and fragmentation, overharvesting, pollution and introduction of foreign species (Tittensor *et al.* 2014). Habitat degradation usually leads to reductions in population size and viability and affects both demographic and genetic processes. Small populations are more vulnerable to environmental stochasticity, and genetic drift and inbreeding are likely to further deplete genetic variation.

A form of habitat degradation is increased availability of nutrients in terrestrial and aquatic ecosystems (Vitousek *et al.* 1997). The effect of this eutrophication on plant species diversity is highly variable (Gough *et al.* 2003), rarely resulting in an increase in species diversity, e.g. (Vellend *et al.* 2017). Nitrogen enrichment of soil may have effects on plant community composition (Manning *et al.* 2008) and for the higher trophic levels occurring in a given plant community (Pöyry *et al.* 2016). Loss of plant species diversity caused by addition of nitrogen has been observed in grasslands through soil acidification (Stevens *et al.* 2010) and by favoring nitrophilic species, thus increasing competition for light (Hautier *et al.* 2009). It is generally recognized that the availability of nitrogen and water are the main limiting factors of plant growth. Plant species with limited capacity for vertical growth may not be able to compete in a nitrogen enriched environment and are likely to suffer from a reduced growth and reproductive ability. Tall surrounding vegetation may also cause pollen limitation (Sletvold *et al.* 2013) and influence factors which promote seed germination like light. Scything and grazing are commonly linked to nutrient poor nature types that would not exist without this activity, giving rise to habitats which may support a higher number of plant species than the surrounding vegetation (Kull and Zobel 1991). The effects of overgrowth are not only limited to direct competition over water, nutrients and sunlight, but can also influence indirect biotic factors such as competition for pollinators (Sletvold *et al.* 2013). Hay meadow, one of the important habitats for my study organism, is a semi-natural nature type that is particularly prone to habitat degradation through overgrowing, and is considered a highly endangered habitat in Norway (DN-rapport 2009) and in Europe (Janssen *et al.* 2016). These habitats are rich in plant, fungi and insect species, and are often host to endangered species like *D. ruyschiana,* which occur in calcareous hay meadows. *D. ruyschiana*

also occur in natural habitats like open calcareous ground with shallow soil, another threatened nature type (Wollan *et al.* 2011). Some literature suggests that *D. ruyschiana* moderately benefit from scything (Eken and Hoell 2012). The *D. ruyschiana* populations in Norway occur almost exclusively in open habitats and in nutritionally poor sediment, but the particular effects overgrowth may have on the growth and reproduction in *D. ruyschiana* are unknown. It has been proposed that *D. ruyschiana* benefits little in vertical growth from additional nutrients (Stabbetorp and Endrestøl 2011) and relies heavily on pollinators for seed set (Milberg and Bertilsson 1997). Tall surrounding vegetation may reduce flower visibility and pollinator accessibility in *D. ruyschiana*.

A reduction in reproductive success is likely to reduce the population size over time, possibly self-perpetuating (Allee and Bowen 1932) through a negative influence on seed set (Luijten *et al.* 2000, Hensen and Oberprieler 2005) and population genetic diversity (Frankham 1996, Peterson *et al.* 2008). A reduction in population size also increase susceptibility to stochastic processes and drastically increase the probability of extinction (Matthies *et al.* 2004). The effects of population size on genetic diversity in *D. ruyschiana* are not known, but Milberg and Bertilsson (1997) show that population size does not have an effect on seed set in *D. ruyschiana*. Dostálek *et al.* (2015) found that genetic diversity had a significant effect on seed set in the congeneric *D. austriacum*. Soil seed banks may be a source of recruitment for many species. Revealing if *D. ruyschiana* has a soil seed bank and also knowledge on specific seed germination factors could have important implications for further conservation of this species.

1.1 POPULATION RECRUITMENT AND SEED GERMINATION

Population recruitment is the process in which new individuals are added to the population pool. Recruitment counteracts deaths in the population by replacing lost individuals, and as such is a potential driver of local extinction. The availability of seeds, as well as the availability of microsites, may limit recruitment in plant populations (Eriksson and Ehrlén 1992). Microsites are sub-environments within an environment, and serve as pockets of unique environmental features, conditions or characteristics. As specific microsites may be required for seed germination, their availability can greatly influence recruitment and population survival. Seed germination is commonly known to be influenced by a variety of environmental factors, including light, temperature, pH and humidity (Baskin and Baskin 2001). These environmental factors are sources

of information that give cues about the environment surrounding the seed, and environmental preferences vary widely between species.

1.2 POLLINATION, SEED SET AND GERMINATION

Several factors may affect seed set in *D. ruyschiana*. Among those is the frequency of visits by pollinators, which again is affected by the pollinator's access to the flowers. According to Milberg and Bertilsson (1997) the species is mainly pollinated by bumble bees and though the species is most likely self-fertile, very few seeds are produced in absence of a pollinator. Seed set may be a rough estimate of maternal fitness in plants. Sletvold *et al.* (2013) showed that tall vegetation has a negative effect on female fitness (number of fruits X mean fruit mass) in a population of the orchid *Dactylorhiza lapponica*. Pollen limitation was higher in tall than in short vegetation.

Light is known to induce a multitude of physiological responses in plants. These responses are collectively called photomorphogenesis (Kendrick and Kronenberg 1994), and are induced when specific phytochromes are excited. Light stimulation can influence seed germination in several ways (Baskin and Baskin 2001): Some species have an absolute light requirement to break dormancy and induce germination (positive photoblastism), while others can be neutral or have negative photoblastism, meaning light will prevent seed germination. The importance of light for seed germination is commonly known to be more true for species with smaller seeds, as small seeds hold less endosperm and their seedlings are forced to start photosynthesis earlier. This variation may also be present on an intraspecific level, as Veloso *et al.* (2017) has measured to be true for the shrub *C. oblongifolia*. Puchalski *et al.* (2014) suggest that *D. ruyschiana* seed dormancy is best broken using gibberellic acid, with a low success rate using scarification. There is no available literature on the effects of light on seed germination in *D. ruyschiana* specifically, but some literature exists on other species of genus *Dracocephalum*. Fattahi *et al.* (2010) found that *D. kotschyi* Boiss seeds gathered in Iran had a slightly higher germination percentage when subjected to light, compared to the seeds that germinated in darkness.

1.3 POPULATION SIZE AND FITNESS

Mean fitness in a population is expected to increase with increasing population size and density, especially so for smaller populations. This positive density-dependence is known as the Allee effect (Allee and Bowen 1932). Hensen and Oberprieler (2005) found that both number of seeds

and seed mass was higher in larger than in smaller populations of *Dictamnus albus*. A correlation between population size and seed set has also been shown in *Arnica montana* (Luijten *et al.* 2000). A reduction in population size may result in reduced genetic diversity in the population. In a study of another species of *Dracocephalum* from Central Europe, Dostálek *et al.* (2015), found that seed set was significantly higher in large and genetic diverse populations compared to smaller and less diverse. Genetic heterogeneity within and between populations of *D. ruyschiana* in Norway is largely unknown, although Kleven *et al.* (2018) recently identified 96 novel SNP markers in Norwegian populations of *D. ruyschiana*, markers which could facilitate studies on *D. ruyschiana* population genetic structure. Observed heterozygosity is a common statistic for assessing genetic diversity within populations. Expected heterozygosity is the probability that any two alleles at a single locus, chosen at random from the population, are different from each other. Averaging the observed heterozygosity of all measured loci yields an estimate of genetic variability within the population. Allele frequencies can be used to find the genetic distance between populations and thus the degree of population genetic isolation. Genetic distance can be estimated using Nei's index (Nei 1987) or the fixation index (F_{st}). As well-developed *D. ruyschiana* can have large rhizomes that give rise to new shoots (Stabbetorp and Endrestøl 2011), genetic diversity within a population can be reduced through asexual reproduction. It is unknown what form of reproduction is most prevalent for the various *D. ruyschiana* populations in Norway.

1.4 SOIL SEED BANK

Soil seed banks are commonly thought of as being significantly important for plant population renewal, being a major source of seedling recruitment for some species (Leek *et al.* 1989). Vandvik *et al.* (2016) found that plant species diversity represented in soil seed bank is consistently higher than what is represented in the established community and describes soil seed banks as biodiversity reservoirs that offer plants the ability to disperse through time. In a varying environment, reproduction may be optimized by delaying seed germination until triggered by key germination factors (Cohen 1966).

There are two main methods for estimating seed density and species composition of soil seed banks: Seedling emergence and manual seed extraction. Gonzalez and Ghermandi (2012) compared the methods of seed extraction and seedling emergence and how seed size and mass effects the efficiency of the methods for estimating seed density. The estimated seed density in

their study was four times higher when using the seed extraction method. Gonzalez and Ghermandi (2012) found that the seed extraction method using buoyancy is preferable if the seeds to be found are larger than 0.3mg and longer than 1mm.

In the event of population decline, conservational efforts can be made to reestablish the population. This can be done directly by transplanting seeds, seedlings or soil from other populations or gardens. Alternatively, if the species in question is known to have a soil seed bank, reestablishment can be performed by removal of competing plants and exposing the soil, allowing possible seeds present in the soil to germinate. Using the seeds already in the soil may be preferable as it maintains the genetic composition and possible local adaptations of the original population. The importance of soil seed banks for recruitment and renewal of *D. ruyschiana* populations has thus far not been studied.

1.5 THE STUDY ORGANISM: *DRACOCEPHALUM RUYSCHIANA*

Dracocephalum ruyschiana, or Northern Dragonhead, is a perennial herb in the Lamiaceae family. It is the only indigenous specie of the genus *Dracocephalum* found in Norway. The plant stands 15-50 cm tall, leaves 2-5 cm long. Its flowers are deep blue to purple and pink, with fused petals creating an upper and lower lip resembling the mouth and tongue of a dragon. Species of the family Lamiaceae have schizocarp fruits with 4 nutlets (Mossberg *et al.* 2012).

1.5.1 Ecology and distribution

It is a drought-adapted plant that thrives on nutritionally poor, calcareous (Østhagen 1972) and well-drained soil. In Norway, *D. ruyschiana* is mostly found below the tree line in open, semi-natural habitats such as grazed land and hay meadows, as well as in disturbed land like avalanche slopes, cliffs and rocky ledges.

The geographic distribution of *D. ruyschiana* in Norway is generally limited to the southern parts of the country, with the highest abundance in the Oslo area and Oppland county. Older literature suggests that *D. ruyschiana* is more common in Kazakhstan, Kyrgyzstan, Mongolia, Russia and Turkmenistan, with a relatively minor presence in western Europe (Meusel *et al.* 1978). From data available on GBIF.org it can be derived that *D. ruyschiana* is primarily distributed between 45°N and 60°N (figure S1). *D. ruyschiana* also occurs in China along with 34 other species in genus *Dracocephalum* (Xiwen and Hedge 1994).

1.5.2 Conservation status

Based on an observed reduction in abundance of *D. ruyschiana* in Europe (CoE 1979), it is considered endangered and is on the red list of 15 European countries, including Norway. The Norwegian Environment Agency has proposed an action plan for the continued survival of *D. ruyschiana* in Norway (DN-rapport 2010). This action plan points out the need for more knowledge on demography, population genetics, reproductive biology and dispersal ability for *D. ruyschiana.* The mechanisms responsible of the observed decline of *D. ruyschiana* populations in Norway are thought to be a combination of natural and anthropogenic causes. Conservation of *D. ruyschiana* is particularly important in Norway, as the endemic beetle species *Meligethes norvegicus* rely on *D. ruyschiana* for reproduction.

1.6 AIMS AND RESEARCH QUESTIONS

My thesis will mainly focus on factors which influence the survival of *D. ruyschiana* populations in the wild, answering the following questions:

1. Does neighbor competition reduce flower size, seed set and germination in *D. ruyschiana?*
2. Is there a positive correlation between population size and genetic diversity, seed set and germination in *D. ruyschiana*?
3. Does light and scarification promote germination in *D. ruyschiana* seeds, and are the effects independent?
4. Is *D. ruyschiana* represented in natural soil seed banks in Norway?

The project uses a mix of field and lab experiments and is related to an ongoing project (Stedje *et al.* in prep.) which addresses the relationship between seed set and seed germinability, genetic variation, population size and isolation. My thesis will contribute new data to this ongoing project.

2 MATERIALS AND METHOD

2.1 STUDY SITES

For the study on the effects of population size on genetic diversity, seed set and germination, 11 study sites were selected from Norwegian localities. In 2011 inflorescences with seeds and leaves on silica were collected from all 11 populations (figure 1, table S1) as part of the study of Stedje *et al.* (in prep.). The 11 locations include seven from Oslo, Østfold and Buskerud: Fornebu, Hovedøya, Nakholmen, Svartorsæter, Jeløya, Nordre Hegstad gård and Åserud (figure S2). Three of the sites are in Oppland and Hedmark: Fauske, Lomen, Syltegårdene and Fossum (figure S3). The populations can be roughly divided into those clustered around Oslo and those occurring further north and inland.

The main site at Fornebu, Bærum municipality was chosen and established in 2017 to perform experiments involving manipulation of surrounding vegetation. At this site, flowers were collected and stored in 70% alcohol. When mature, whole inflorescenses with seeds were collected. Field work at Fornebu was carried out during the summer of 2017.

All of the populations studied suffered from drought and high temperature during the summer of 2018 when an additional field season was planned. According to yr.no (2019) the median temperatures of June, July and August 2018 were the highest median temperatures ever measured at the Blindern weather station in Oslo for these three months (table S2; figure S4). The total precipitation for these three months (table S3; figure S5) was the lowest measured at Blindern since 1983, and 8'th lowest overall. Nearly all *D. ruyschiana* in the studied populations died or failed to produce any seeds during 2018. This had negative implications for the vegetation manipulation experiment as no supplementary experiments could be performed, and positive implications for the soil seed bank experiment as it was certain that none of the seeds detected in the seed bank could have been produced that year.

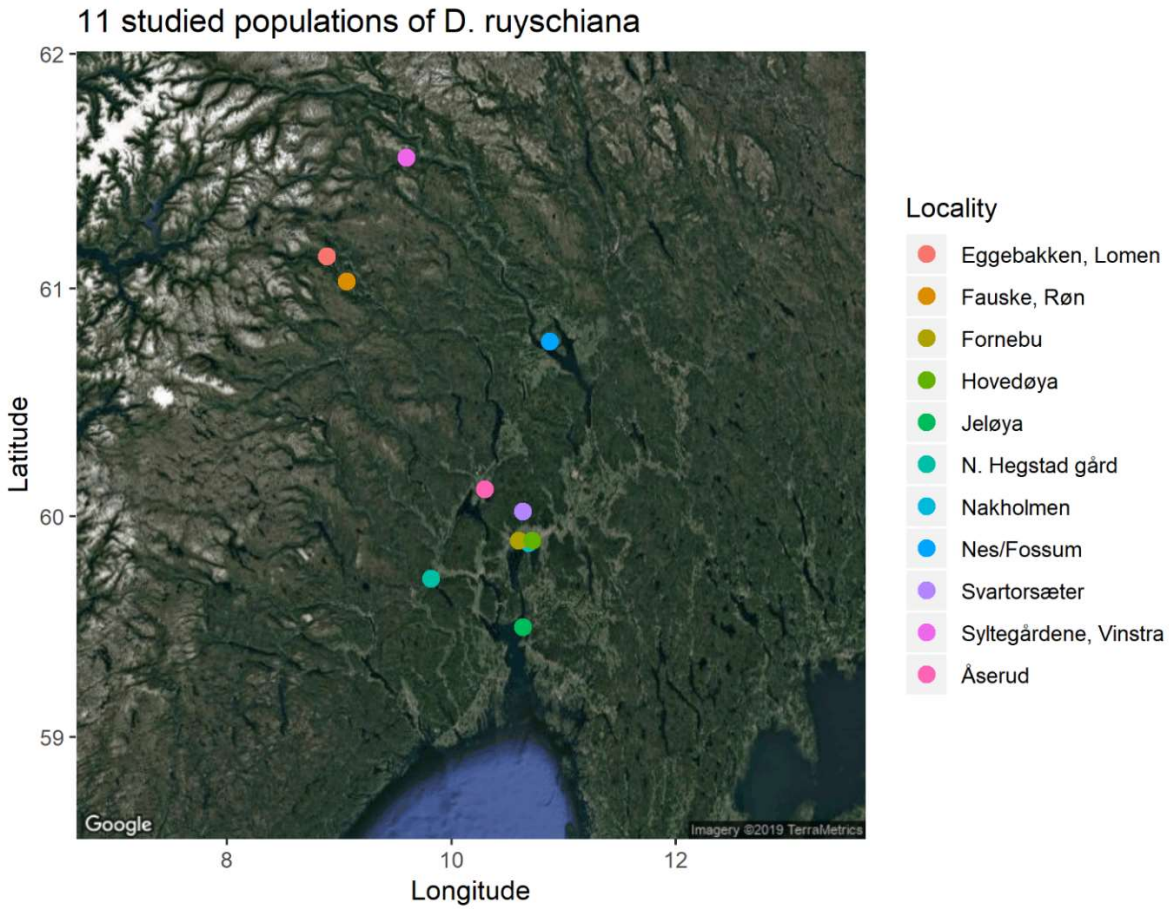

Figure 1: A map of the eleven Norwegian populations of D. ruyschiana included in this study. The symbol for the locality Nakholmen is partly covered by the symbols for Hovedøya and Fornebu. Local maps of Oslo and Oppland are included in the appendix (figure S2.; figure S3). Maps were made in R using the ggmap package (Kahle et al. 2019) and GPS data.

2.1.1 The south eastern populations: Fornebu, Hovedøya, Nakholmen, Svartorsæter, Jeløya, Nordre Hegstad gård and Åserud

Fornebu is a peninsula with a long history of agriculture, up until the airport was built in 1939 and closed in 1998 (Bjørge 2012). Fornebu has undergone extensive residential and industrial development after the construction of the airport, and more development is underway. The northern half of Fornebu, including the study site, is rich in limestone deposited during the Cambro Silurian period . As exposed limestone is preferable for *D. ruyschiana*, much of Fornebu could have been suitable habitat in the past. I spoke to some of the locals, who expressed that they don't want this area to overgrow for esthetic reasons, and that they have been regularly cutting the vegetation. The health of the *D. ruyschiana* population at Fornebu is largely unknown, but some

of the locals living in the area has stated that the population size has appeared to be in decline over recent years.

Hovedøya is an island located close to mainland, but compared to Nakkholmen it is larger, and has a more variable topography and thus also deeper sediment. Hovedøya is home to several rare plant species, some of which may have been established there through a monastery garden that used to be on the island.

Nakkholmen is a small island in Oslo, located close to mainland. It is uninhabited, although there are many summer houses there. The island topography is relatively flat, dominated by exposed rock and mostly shallow sediment. Many other rare plant species thrive there in addition to *D. ruyschiana*.

Jeløya is a peninsula in Moss and is partly covered with residential areas as well as forests and farmland. *D. ruyschiana* is mostly found along the rocky edges where land meets ocean, and the studied population occurs in the northern part of the peninsula.

Svartorsæter is an old farm in the interior of the Nordmarka forest and the area is still used as a heavily grazed pasture. Walking further into Nordmarka, about 22km north-west ones arrives at the population at Åserud near Hønefoss in Buskerud county. This population is in a sloped field with shallow sediment, right next to a road and surrounded by forest. The final site in the Oslo area is nordre Hegstad gård in Buskerud, an area dominated by extensively farmed land.

2.1.2 The Oppland populations: Syltegårdene, Fauske, Lomen & Fossum

Syltegårdene in Oppland county hosts one of the few Norwegian *D. ruyschiana* populations that is not considered threatened. This is likely due to the population being mostly located in a field that is still grazed by around 1000 sheep during spring and autumn (Grimstad and Olsen 2010). This study site is dominated by a geology rich in limestone, and the soil is relatively dry and shallow.

The study sites at Fauske and Lomen are also located in Oppland county. The population at Lomen has *D. ruyschiana* occurring in a sizeable hay meadow and is considerably larger populations than the one at Fauske. At Fauske *D. ruyschiana* mostly occur along a road at a slight elevation. Further south-east from these three sites is the population occurring on a site in Fossum. The site in Fossum

is largely dominated by farmed land, and the population is occurring at the boundary of farmed land and a small forest.

2.2 MANIPULATION OF VEGETATION AT FORNEBU

To examine the effects of vegetation height on individual fitness, I manipulated the vegetation height surrounding 77 *D. ruyschiana* individuals (treatment group), while leaving 82 individuals undisturbed (control group).

Well-developed *D. ruyschiana* can have long rhizomes that give rise to new shoots that can be mistaken for another genetic individual. A minimum distance of 1 meter between individuals was therefore used to reduce the probability of the same genetic individual being included several times. All tall vegetation surrounding members of the treatment group was cut as short as possible (< 5 cm) in a 20-30 cm radius around the plant. The height and density of the natural surrounding vegetation varied considerably between individuals. Treatment was repeated throughout the flowering period to maintain a low vegetation height.

To estimate female reproductive success the number of mature seeds in each fruit was counted. The mature fruits were gathered in early August 2017 and stored in paper bags. The number of infructescences gathered from each plant varied as some of the plants had not yet completely developed all their fruits. Only the most mature were selected, and care was taken to leave some seeds for future generations. Additionally, some of the plants were very small and had less fruits than others, but they were sampled nevertheless to avoid a bias towards the large individuals. Over the summer of 2017 data was collected from 159 plants. As members of family Lamiaceae typically has fruits with four nutlets, counting the number of mature seeds was done by counting the number of aborted seeds and subtracting this number from 4. This method was preferred as some mature seeds could have escaped the fruit before or during storage, contrary to the aborted seeds which stay inside the calyx even after seed release.

One flower was gathered from each of the 173 individuals and stored in 70% alcohol. The size of four flower parts was measured, as indicated in figure 2. The measures are as following: (a) length of the corolla tube, from its base to the start of the upper lip; (b) length of the upper lip, (c) length

of the lower lip and (d) width of the mouth of the corolla tube. Measurement d may be influenced by compression of the flower due to improver storage, introducing random variation to this measurement. Of the 173 flowers collected, 108 were used for comparing flower size with seed set. The remaining 65 lacked seed set data as these plants could not be found at the end of the season.

Figure 2: Flower measurements, a: length of corolla tube; b: length of upper lip; c: length of lower lip; d: width of corolla mouth

2.2.1 Effects of vegetation trimming on seed germination

To quantify the effect of vegetation treatment on germination, 10 individuals were randomly chosen from each of the treatment groups, and the seeds from each of the two treatment groups were germinated to determine germination percentage. 54 seeds from plants in trimmed surrounding vegetation and 38 seeds from plants in natural vegetation were scarified and put on sterile petri dishes with agar gel for germination. The petri dishes were placed in an incubator with 12 hours of light a day, and a stable day and night temperature of 20° C and 10° C, respectively.

The petri-dishes were regularly checked, and number of germinations were noted over a timespan of three months.

2.2.2 Analysis

The programming language R (R Development Core Team 2019) was used for the analysis. In order to avoid a type II error (thinking there isn't an effect when in fact there is one), the effect size was calculated to compliment the p-value. As a measure of effect size, the Cohens' d for each flower part was calculated using the R package effsize V0.7.4 (Torchiano 2018). Cohens' d is defined as $\frac{M_1 - M_2}{\mathrm{SD}}$ where M_1 and M_2 are the group means and SD is the standard deviation common to both groups (Cohen 1988). The effect size indicates the degree to which the null hypothesis is false, while a p-value only tells us if the null hypothesis is rejected or accepted. Cohen's d is thus particularly helpful when attempting to find a weak effect with an insufficient sample size, or to estimate the sample size needed to prove a weak effect.

The average number of seeds per flower and the average number of seeds and flowers per inflorescence of 82 natural vegetation samples and 77 trimmed vegetation samples was compared using a parametric, unpaired two-sample t-test to check for a difference between the natural and trimmed vegetation.

The flower size measurements of plants from both groups were compared using a Welch two sample t-test in R. The measurements of individual flower parts as well as the sum of the measurements per flower were compared. In order to investigate if a larger general size of the flower is correlated with higher seed set, the flower size measurements were added together into a sum that represents the total size of the flower.

For the germination experiment, a Welch two-sample t-test was utilized to compare the mean germination percentage of plants from natural and trimmed vegetation. Cohen's d was calculated to estimate the strength of treatment. The data on germination is binomial (germinated, not-germinated). The seeds consisted of the entire seed collection of 20 randomly selected plants from natural and trimmed vegetation. As the 2x10 plants were randomly selected there is a discrepancy of 10 seeds between the groups, with plants from natural vegetation being represented by only 38 seeds (as opposed to the 48 seeds representing the plants from trimmed vegetation).

To test the hypothesis that large flowers produce more seeds, linear regression models were made in R and put through a model selection process. The average number of seeds per flower was used as the response variable and the flower size measurements as the explanatory variables. Flower measurements and seed count data for plants from both natural and trimmed vegetation was used in the same model. To determine which of the models explained most of the variation in the data, R-squared and adjusted R-squared was calculated for each model. Models using several or all the flower part measurements as independent variables was avoided, as these measurements are moderately correlated with each other but assumed in the regression model not to be.

2.3 EFFECTS OF POPULATION SIZE ON GENETIC DIVERSITY, SEED SET AND SEED GERMINATION

All data included in this section were prepared by Stedje *et al.* (in prep.) in an ongoing project. I was given the estimates of population size and genetic diversity, as well as germination percentage and seed and flower count for each inflorescence collected for further analyses in my study. Population size was estimated by counting the number of what appeared to be distinct individuals. For the largest populations the number of individual *D. ruyschiana* were counted for a given area to estimate population size for the total area.

Amplified Fragment Length Polymorphism (AFLP) data was generated by Stedje *et al.* (in prep.) for 11 populations and analyzed using standard procedures for DNA extraction and for AFLP (Vos *et al.* (1995), Stedje *et al.* (in prep.)). Using the AFLPdat package (Ehrich 2006) in R, the average expected heterozygosity was calculated by Stedje *et al.* (in prep.) as a measure of intrapopulation genetic diversity according to Nei's genetic diversity formula.

2.3.1 Analysis

The dataset used for analysis (table 1) consists of five variables: Estimated population size, estimated genetic diversity, germination percentage and the average number of seeds per flower and inflorescence. One alternative to the latter were also tested: The number of flowers per sampled inflorescence. Univariate linear regression models made in R were used to test the hypotheses, using population size as the independent variable. In one model the average number of seeds per flower was used as the independent variable to explain variation in germination percentage.

2.4 EFFECTS OF LIGHT AND SCARIFICATION ON SEED GERMINATION IN D. RUYSCHIANA

D. ruyschiana seeds that were collected the summer of 2017 in Oppland and Oslo were germinated on agar under four different treatment regimes. The agar was made following the standard operating procedure of the seed conservation department of Kew botanical gardens, using 10 g agar powder per 100 ml tap water.

100 seeds were selected from each of the four populations (Fornebu, Fauske, Nakholmen and Syltegårdene). The seeds were visually inspected to be of the same size, and a high precision weight was used to make sure the seeds had a homogenous weight. Out of the 100 homogenous seeds, 25 were randomly selected for each of the four treatments. The four combinations of treatments listed below were applied to the seeds from all four populations, giving a total of N = 100 for each treatment group. Being a factorial experiment both independent and interactive effects of the two treatment factors were tested.

- No light + No scarification
- Light + No scarification
- No light + Scarification
- Light + Scarification

16 petri dishes (4 populations * 4 treatment combinations) with 25 seeds each were prepared. The petri dishes with seeds were placed in plastic bags that where either transparent or aluminum foil coated. Double bags of the latter type were used to exclude light. Dishes were then placed inside a large incubator that was configured to have a day length of 12 hours and a day-temperature of 20° C.

2.4.1 Analysis

Being a two-way factorial experiment, analysis was done using ANOVA fit with the lm function in R. The model used the germination percentage as the dependent variable and light, scarification, interaction between light and scarification and population as the independent variables. Such analysis assumes a normally distributed dependent variable for the analysis to be valid. Normality was tested using a Shapiro test and visually using a QQ-plot in R.

2.5 INVESTIGATING IF *D. RUYSCHIANA* HAS A SOIL SEED BANK

The presence of *D. ruyschiana* seeds in soil was measured to determine the presence of a soil seed bank for the species. Soil was gathered from three localities (figure S6), two of which being in Ringerike county and one at Fornebu, Oslo. The three populations (Oslo, Haugsbygda and Åserud; table 2) are of varied sizes (large, medium and small), the smallest being the population at Haugsbygda in Ringerike and the largest being the population on Fornebu. The third population is located at Åserud in Ringerike and is considered to have a size in-between the smallest and largest populations.

At each study site, the same procedure for gathering soil was repeated. An area of roughly 5x5m with the presence of *D. ruyschiana* was selected at each site, and 5 soil samples were taken at random from each 5x5m square. Each soil sample was dug out from an area with a radius of roughly 10cm and depth of up to 5cm. Great care was taken to avoid damaging the surrounding vegetation, and most digging was performed without the use of tools. Under normal conditions sampling should be done before any seeds are mature, but in 2018, the year of sampling a severe drought (tables S2 and S3) caused total failure of seed set and the sampling was done in early July.

To quantify the richness of *D. ruyschiana* seeds contained in the different samples of soil, the soil was divided into two portions and the two methods of Gonzalez and Ghermandi (2012) were used. The first of these methods, here to be referred to as "seedling emergence" is simply observing what species of plants will emerge from the soil when subject to light and water. The second method used consists of mixing the soil in a container of salt water to count the number of *D. ruyschiana* seeds that float to the surface.

2.5.1 Seedling emergence

To induce germination of any *D. ruyschiana* seeds that might be present in the soil, the soil was sieved and placed in germination trays and covered with sand. Each tray was split in two equally large parts using a cloth to separate them. Each of the resulting chambers measured about 20x30cm. The sand used to cover the soil was first sieved to remove any large particles. Sand was used for several reasons. Most importantly, the sand creates a sterile barrier that separates the soil from the environment, reducing the possibility of contamination. Any airborne seeds or spores that land on the trays will have to germinate on sand that is poor in both water and nutrients. The layer of sand should also reduce evaporation of water from the soil, as the lighter color of the sand

should reflect more electromagnetic radiation than the darker soil. To further protect against contamination and evaporation, a plastic sheath was used to cover the trays. Due to high ambient temperature throughout the duration of the experiment, the trays had to be watered daily. The trays were placed in a greenhouse, where they remained for roughly two months. To simulate winter and cold stratify any remaining seeds in the soil, the trays were put in a cooling room for two months. The temperature in the room was held stable at 4-5° C. Subsequently both trays were placed in a warm greenhouse to induce germination.

2.5.2 Floating

As a first step, soil was further broken down into its smallest constituents and sieved to remove unnecessary organic material. Using *D. ruyschiana* seeds collected for another project, the ideal sieve hole size could be found, thus only catching supernatant particles that are of the same size as *D. ruyschiana* seeds, or larger. This method uses salt water to increase the buoyancy of the seeds, aid organic material, like seeds, to float to the top of the solution while the densest material still sinks.

The aqueous salt solution was prepared by adding 35 g of sodium chloride to 100 ml of tap water. Roughly 1 L of soil from each location was soaked in the solution in separate containers and allowed to settle for 30 minutes. The supernatant material was collected and filtered using a filter paper, and dried in an oven at 30°C. The dried material was examined using a binocular stereomicroscope, and all seeds were separated out. The *D. ruyschiana* seeds found from floating were visually identified as they have a distinct shape and color (figure 3). Seed coat integrity was visually inspected to estimate viability. With the exception of *D. ruyschiana* seeds, the species of the seeds found using the floating method were not identified.

Among the mature *D. ruyschiana* seeds found during the fieldwork of summer 2017 in Oppland and Fornebu, virtually none were smaller than 1 mm. For the purpose of this study, it is assumed that the *D. ruyschiana* seeds in the soil seed bank of the Ringerike populations are of the same size, and that the seed extraction method will most efficiently estimate *D. ruyschiana* seed density of the soil at these three sites.

Figure 3: Picture of the underside of a D. ruyschiana seed resting on the blade of a scalpel. Note the distinct V-shape going towards the middle of the seed, and the scar tissue to the left in the picture. The seed is approximately 3 millimeter long.

3 RESULTS

From the experiment at Fornebu it was found that trimming of surrounding vegetation had a statistically significant effect on the number of seeds produced per flower, but not on flower size or seed germination percentage. In the analysis on the effects of population size on genetic diversity, seed set and seed germination for 11 populations of *D. ruyschiana*, population size did not explain variation in any of the parameters, but there appeared to be a correlation between population size and genetic diversity. The population average number of seeds per fruit explained variation in germination percentage. Scarification was found to have a statistically significant effect on germination in *D. ruyschiana* seeds, but light alone did not have a significant effect. The presence of *D. ruyschiana* seeds were found in soil from two of three locations studied, and both detection methods used were useful.

3.1 VEGETATION MANIPULATION AT FORNEBU

3.1.1 Effects of vegetation trimming on seed set

A t-test comparing the average number of seeds produced per flower revealed a statistically significant ($P_{df=156}$ = 0.001) difference in mean between plants from natural and trimmed vegetation, based on 82 and 77 individuals respectively. Plants from natural vegetation produced on average 1.19 seeds per flower, while the group with surrounding vegetation trimmed produced on average 1.52 per flower (figure 4). Estimating the effect size using Cohen's d show a medium strength effect (d = 0.52) of vegetation trimming on seed set. In the natural vegetation group, six plants produced zero seeds, while only two did the same in the trimmed vegetation group. Adjusting for this difference by removing all zero valued samples did not change the outcome of the analysis much.

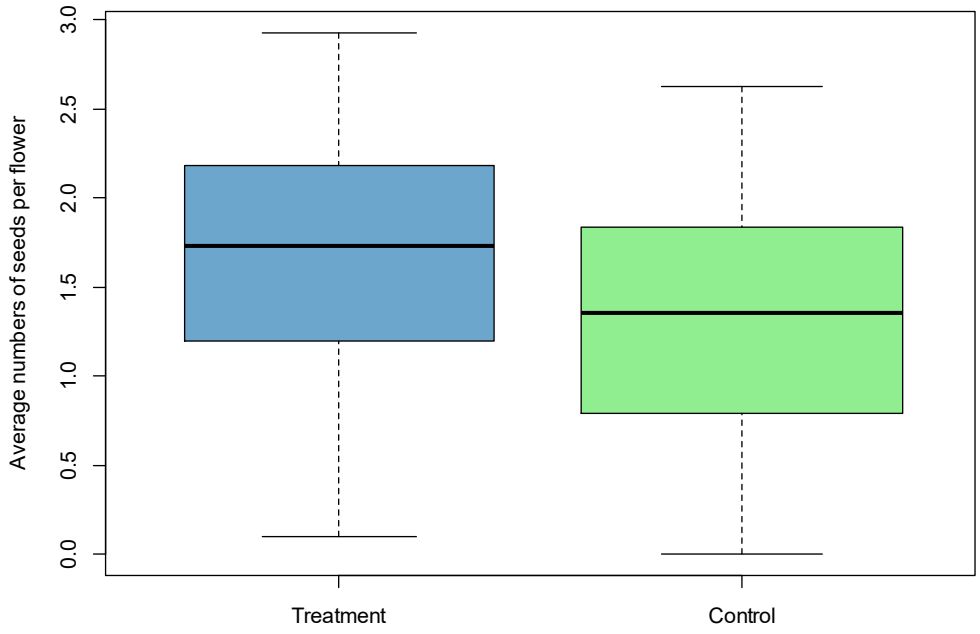

Figure 4: The effects of vegetation trimming on D. ruyschiana seed-set; average number of seeds produced per flower on the y-axis. Plants with surrounding vegetation trimmed (treatment) is shown in blue; plants in natural vegetation (control) is shown in green.

3.1.2 Effects of vegetation trimming on flower size

There were no statistically significant differences in flower size measurements between plants in the natural vegetation and trimmed vegetation (Table 3; figure 5).

Table 3: Welch Two Sample T-test comparing the data on flower size between the natural vegetation and trimmed vegetation group; mean flower size in millimeter for the natural and trimmed vegetation shown in first two columns.

Flower Part	**Mean flower size (Trimmed vegetation)**	**Mean flower size (Natural vegetation)**	**t - value**	**Degrees of freedom**	**p - value**
A: corolla tube	21,76 mm	21,30 mm	1.4025	149	0.1628
B: upper lip	6,74 mm	6,51 mm	1.3708	160	0.1724
C: lower lip	4 mm	3,86 mm	1.5795	165	0.1161
D: corolla mouth	5,40 mm	5,36 mm	0.21723	165	0.8283
sum	37,68 mm	36,94 mm	1.2194	163	0.2244

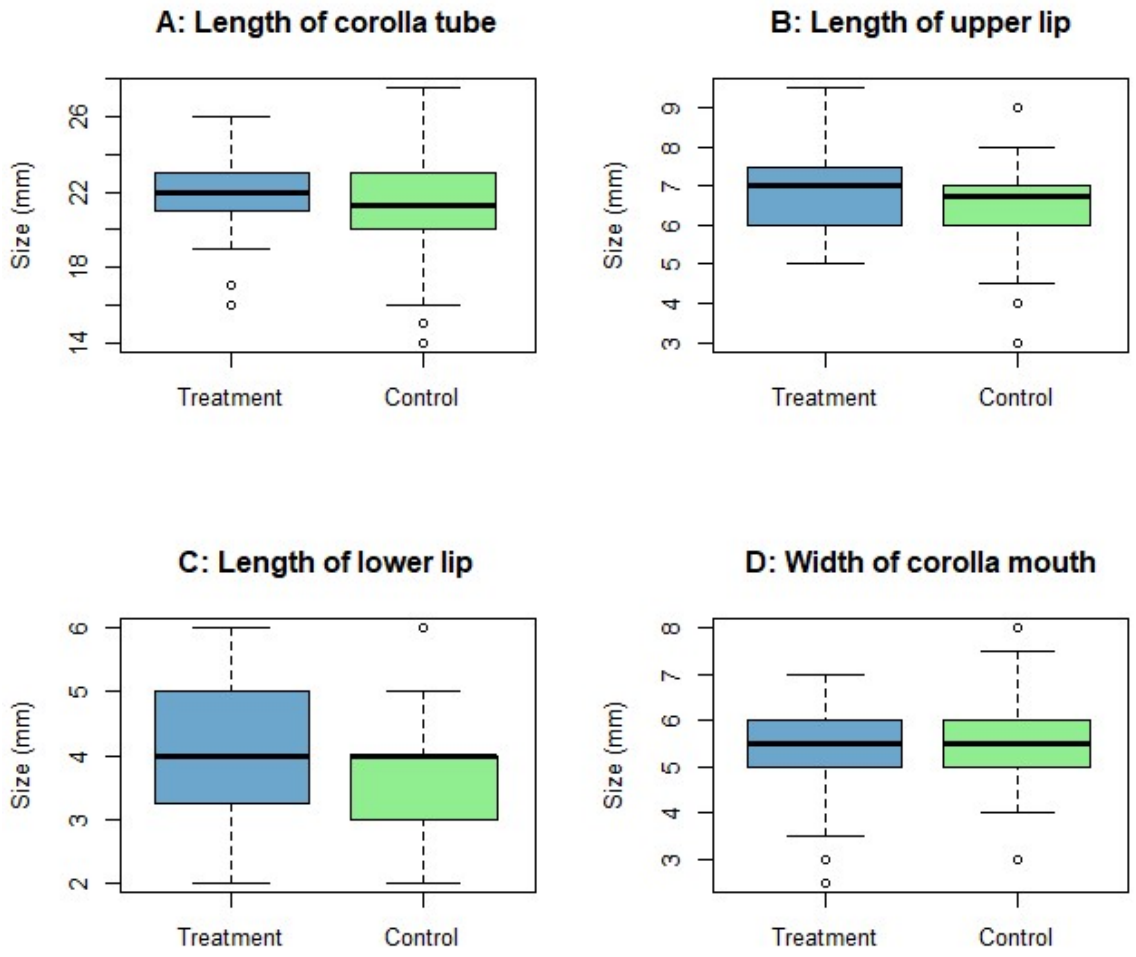

Figure 5: Boxplot of the effects of vegetation trimming on D. ruyschiana flower size. Plants with surrounding vegetation trimmed (treatment) is shown in blue; plants in natural vegetation (control) is shown in green.

On average, the flowers produced by plants with surrounding vegetation trimmed were 2.2% larger than the flowers in plants from natural vegetation. Cohen's d was estimated to find the effect sizes. For the length of the corolla tube there was found a weak effect (d=0.21), as was true with the length of the upper lip (d=0.21) and lower lip (d=0.24). For the width of the corolla mouth only a negligible effect was estimated (d=0.033). No relationship could be determined between the four measures of flower size and seed set ($p > 0.05$).

3.1.3 Effects of vegetation trimming on seed germination percentage

Germination percentage was calculated to be 66% for the trimmed vegetation and 48% for the natural vegetation. A t-test was utilized to compare the mean of the two groups, and no statistically significant difference was found ($P_{df=7}$ = 0.2; figure 6). Both groups had one occurrence each where zero seeds germinated. Estimating the effect size using Cohen's d revealed a small effect (d = 0.35) of vegetation trimming on seed viability.

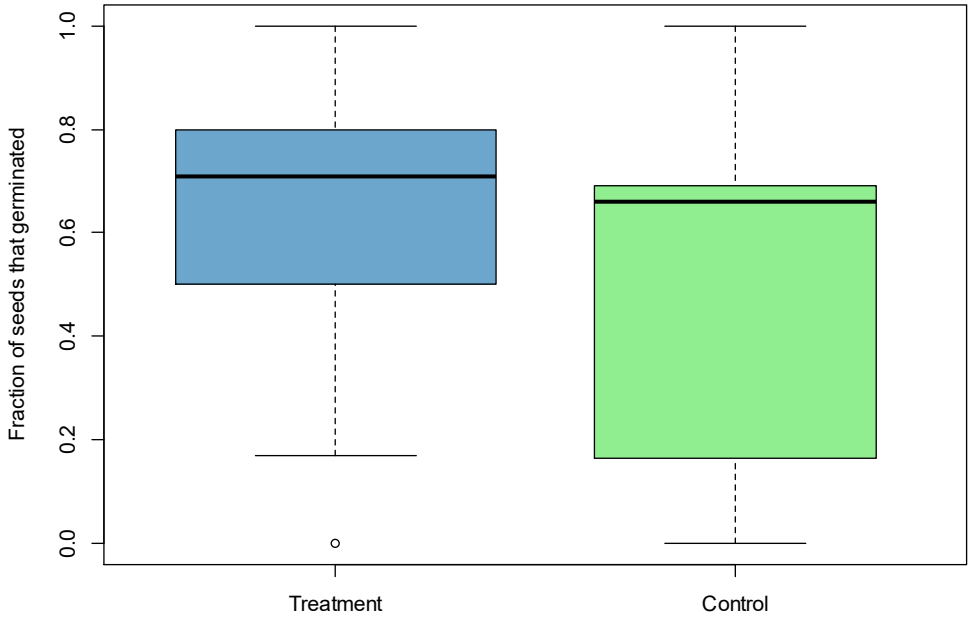

Figure 6: Fraction of seeds germinated for both natural (control) and trimmed (treatment) vegetation

Figure 6: Boxplot of the effects of vegetation trimming on D. ruyschiana germination percentage; seeds from plants with surrounding vegetation trimmed (treatment) is shown in blue; seeds from plants in natural vegetation (control) is shown in green.

To investigate if the randomly chosen samples in the germination experiment were representative of their respective groups, the mean number of seeds produced by the individuals used in the germination experiment was compared with the overall group mean. The randomly chosen plants from the natural vegetation produced on average 2 seeds per flower, which is 160% of the mean of the natural vegetation.

3.2 EFFECTS OF POPULATION SIZE ON GENETIC DIVERSITY, SEED SET AND SEED GERMINATION

The dataset used for analysis is shown in table 1. Variation in the average number of seeds produced per flower explain variation in germination percentage (Beta $_{(SE = 0.07)}$ = 0.62, p = 0.04, adjusted R^2 = 0.38; figure 7). Variation in average expected heterozygosity is not significantly explained by variation in population size (Beta $_{(SE = 2.699*10^{-5})}$ = 0.54, p = 0.09, R^2 = 0.29; figure 8). The average number of seeds produced per flower is not explained by population size (Beta $_{(SE =0.00054)}$ = 0.30, p = 0.36; figure 9), although more strongly associated with population size than the total number of seeds per inflorescence (Beta $_{(SE =0.02)}$ = 0.1, p = 0.82) or the total number of flowers per inflorescence (Beta $_{(SE =0.01)}$ = -0.1, p = 0.76). However, these models explained none of the variation in the data (R^2 close to zero). No statistically significant relationship between population size and germination rates was detected.

Table 1: The dataset used; showing the average number of seeds pr. Flower (SD = 0.50), average number of seeds per inflorescence, population size estimates, genetic diversity estimates and germination percentage (SD =0.13) for each of the 11 Norwegian populations of D. ruyschiana

Population	Estimated population size	Genetic diversity (Nei's index)	Average number of seeds per inflorescence	Average number of seeds per flower	Germination percentage
Jeløya	870	0.13	50	2.27	76
Fornebu	850	0.17	31	1.71	73
Åserud	300	0.12	9	1.09	61
Syltegårdene	250	0.16	21	0.84	53
Lomen	200	0.13	30	1.53	70
N. Hegstad gård	200	0.08	68	2.49	75
Nes	150	0.08	10	1.00	60
Hovedøya	130	0.11	51	1.40	48
Nakholmen	100	0.10	61	1.77	93
Fauske	30	0.13	37	1.72	73
Svartorsæter	23	0.11	16	1.58	54

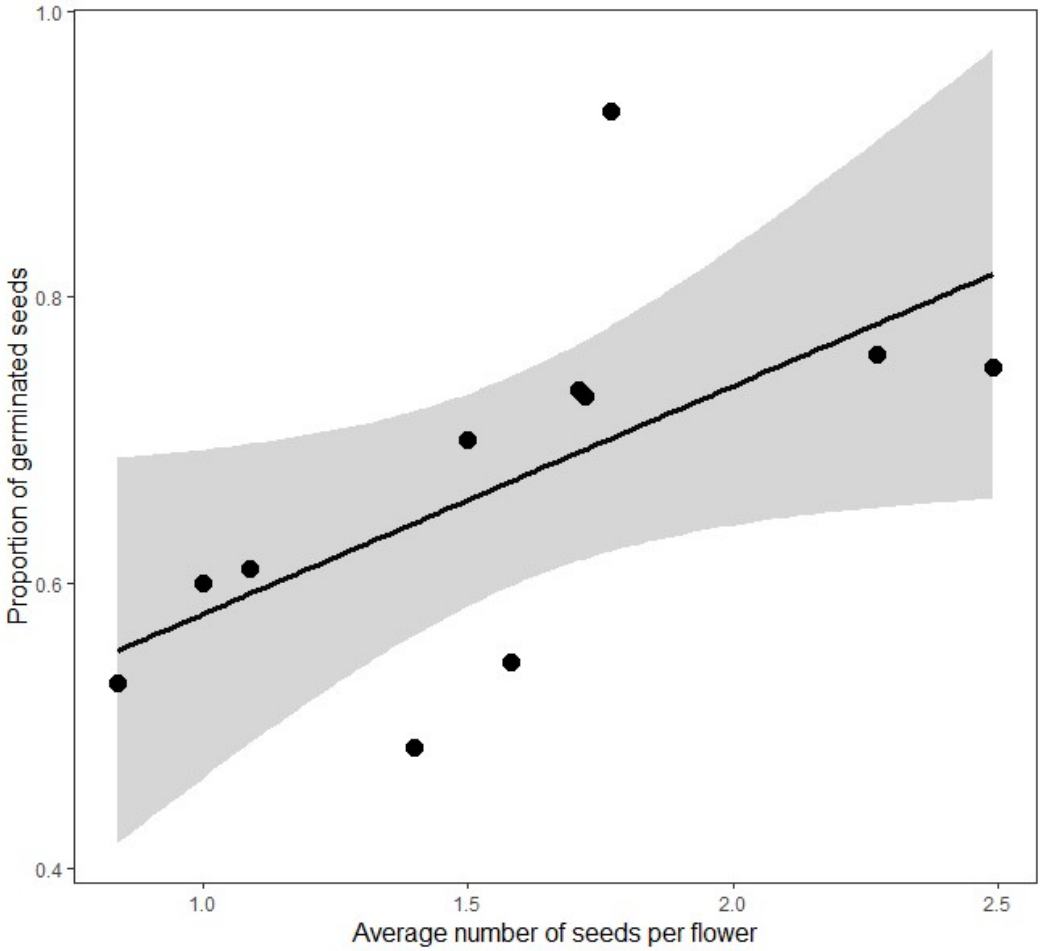

Figure 7: The relationship between seed set and germination percentage for 11 Norwegian populations of D. ruyschiana. Lm, Beta = 0.62, p = 0.04, R^2 = 0.38. The effect strength of each datapoint on the regression line is shown in gray

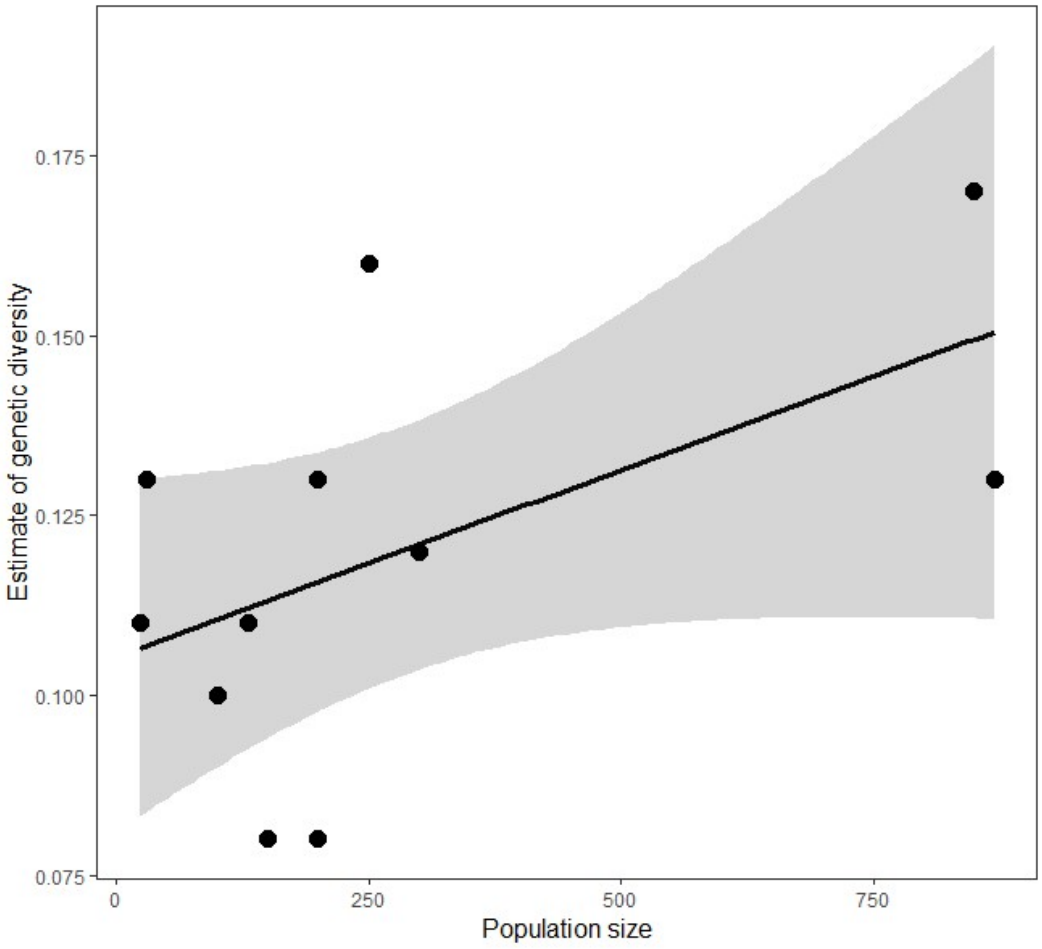

Figure 8: The relationship between estimated population size and estimated genetic diversity for 11 Norwegian populations of D. ruyschiana. Lm, Beta = 0.54, p = 0.09. The effect strength of each datapoint on the regression line is shown in gray

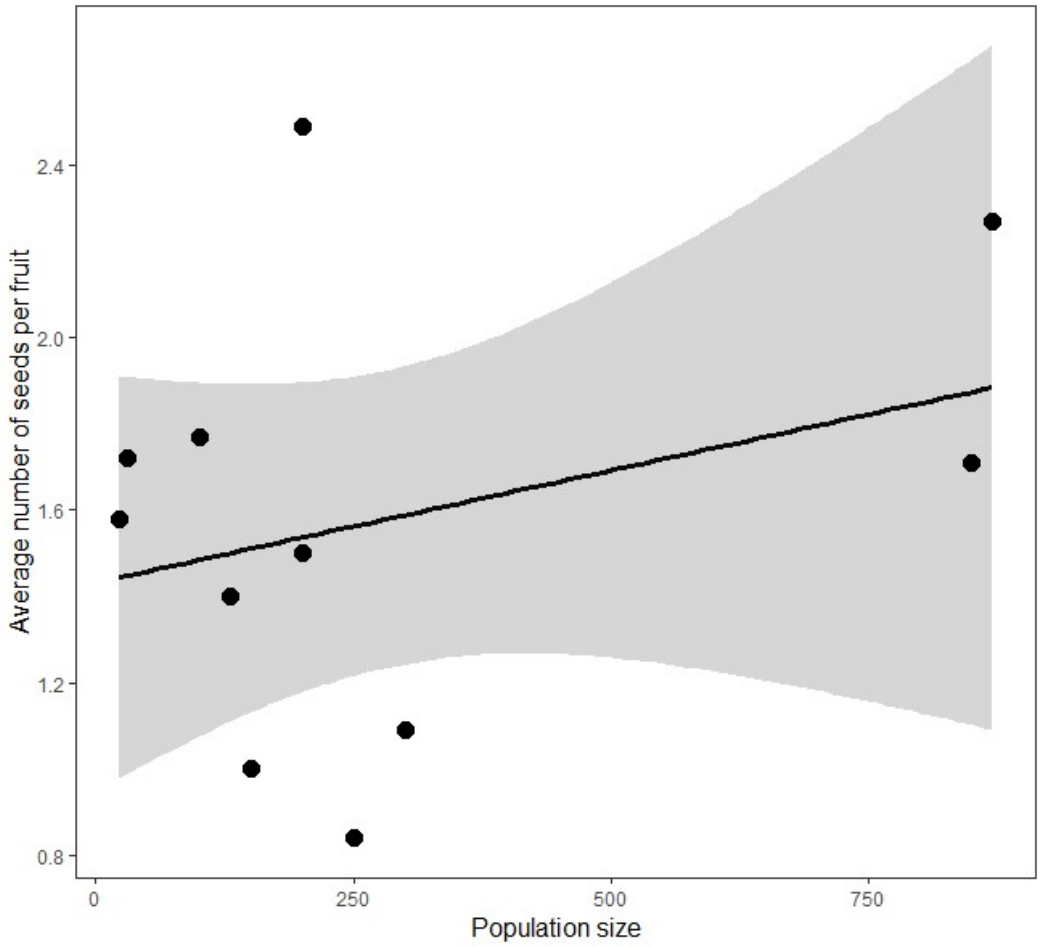

Figure 9: The relationship between estimated population size and the average number of seeds per fruit for 11 Norwegian populations of D. ruyschiana. Lm, Beta = 0.30, p = 0.36. The effect strength of each datapoint on the regression line is shown in gray

3.3 EFFECTS OF LIGHT AND SCARIFICATION ON SEED GERMINATION IN *D. RUYSCHIANA*

Scarification had a significant effect (F-value = 51.33, $p < 0.05$; Table 4) on *D. ruyschiana* seed germination. Light alone was not significantly correlated with germination (F-value = 2.4, p = 0.15). The overall model fit was adjusted $R^2 = 0.80$. Seeds from three of the four populations benefited the most from a combination of light and scarification, with 68% germination on average for the seeds that received both treatments (figure 10). Of the seeds that were only scarified, 59% germinated within two months. The seeds that only received light germinated at nearly twice the rate of the seeds that received no treatment (23% v. 12%, respectively). The population from which the seeds came had some effect on the germination percentage (F-value = 2.5, p = 0.12). No interaction between the treatments could be detected (F-value = 0.02, p = 0.9), suggesting light and scarification had additive effects. The number of germinated seeds for each treatment group and population is shown in table 5 and visualized in figure 11.

Table 4: Showing the results of ANOVA analysis with variables studied, sum og squares, degrees of freedom, F- and P-value.

Source	SS	Df	F-value	P-value
Scarification	529	1	51	< 0.05
Light	25	1	2.4	0.15
Population	77	3	2.5	0.12
Scarification : Light	0.25	1	0.02	0.9
Residuals	93	9		

Table 5: The individual and combined effects of light and scarification on D. ruyschiana seed germination; showing number of germinated seeds for all treatment groups for each population

Population	**Light**	**Scarification**	**Count**
Syltegårdene	Yes	No	3
Syltegårdene	Yes	Yes	19
Syltegårdene	No	Yes	18
Syltegårdene	No	No	4
Fauske	Yes	No	4
Fauske	Yes	Yes	17
Fauske	No	Yes	7
Fauske	No	No	0
Nakholmen	Yes	No	12
Nakholmen	Yes	Yes	15
Nakholmen	No	Yes	19
Nakholmen	No	No	6
Fornebu	Yes	No	4
Fornebu	Yes	Yes	17
Fornebu	No	Yes	15
Fornebu	No	No	2

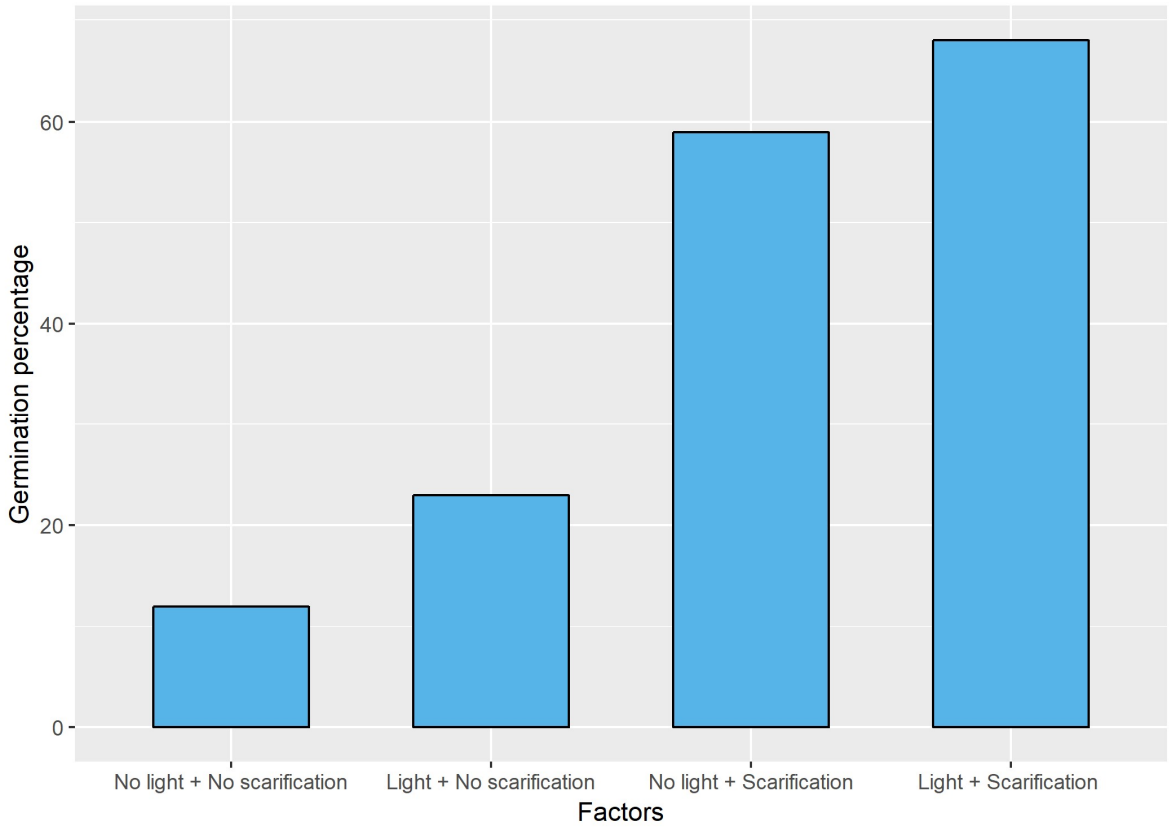

Figure 10: Showing the effects of each treatment combination on the x-axis and the average germination percentage on the y-axis

The population from which the seeds were collected was not found to have a statistically significant effect on the germination percentage. Seeds from the populations at Fauske and Nakholmen differed most from the overall mean germination percentage, differing with 28% and 52%, respectively. The seeds from Fauske and Nakholmen also differed the most in response to treatment. The seeds from Fauske benefitted greatly from a combination of scarification and light, while the seeds from Nakholmen benefitted the most from scarification only. Seeds from all populations suffered a low germination rate when not subject to either treatment, with the exception of the seeds from Syltegård, which had the lowest germination rate in the group that only received light.

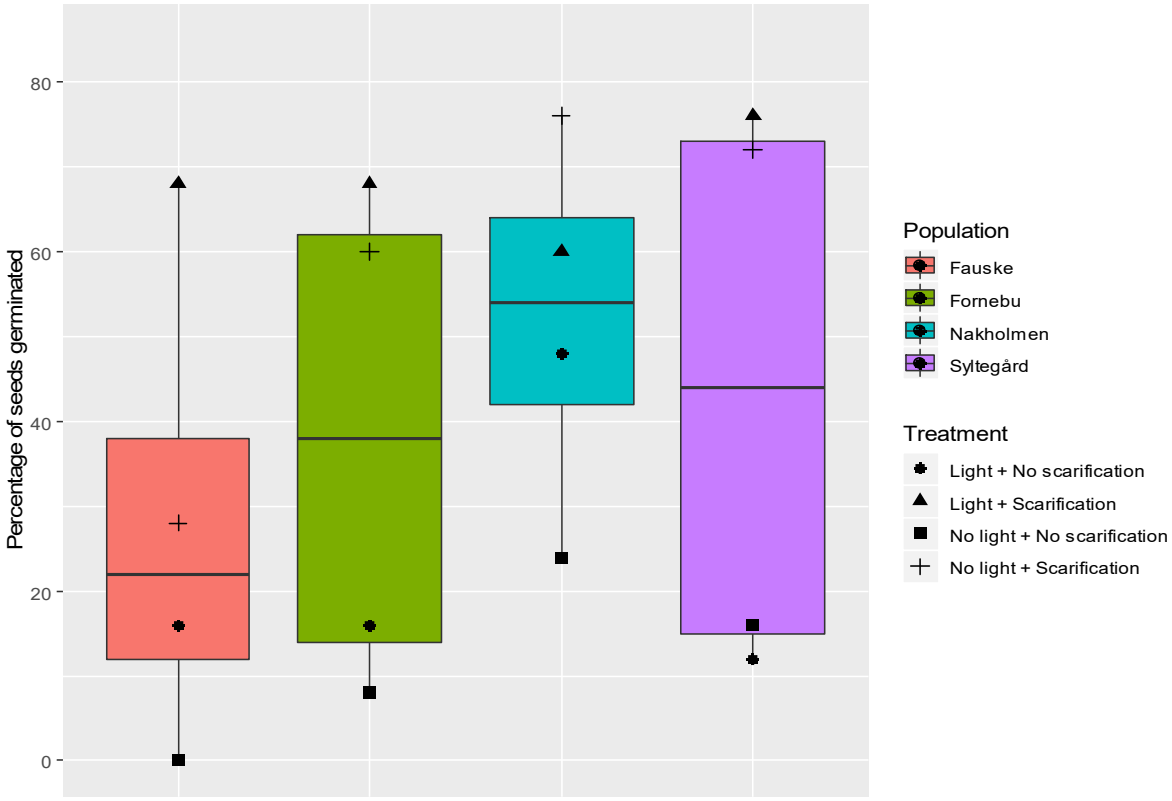

Figure 11: Combined boxplot and scatterplot showing the mean germination percentage on the y-axis within each treatment group, across all four populations).

The positive effect of scarification on germination was fairly constant across all populations, but the effect of light was not. The seeds from Syltegård had approximately the same number of germinations in the group that was only exposed to light (12/25 germinations) as in the group that received no treatment (16/25 germinations).

3.4 INVESTIGATING IF *D. RUYSCHIANA* HAS A SOIL SEED BANK

The presence of seeds in soil was detected using both described methods, with floating yielding 79 seeds in total (table 2) and emergence 118 seedlings (table 6). With the emergence method 10 additional species were identified, whereas 8 species could not be accurately identified as this would require flowering. 15 of the 118 seedlings were identified to be *D. ruyschiana*. The soil from the smallest population at Haugsbygda only produced 5 seedlings, and all of these were one or several unidentified species of grass.

Only large seeds can be found and were extracted using the floating method. Of the large seeds found in my samples, 60% of the seeds found in the soil from the medium-sized population at Åserud and 33% of the seeds found in the soil from the large population at Fornebu were identified as being *D. ruyschiana* seeds. No *D. ruyschiana* seeds could be found in the soil from the smallest population at Haugsbygda. The viability of the seeds found by floating was not tested, and fragmented but identifiable seeds were included in the final count. Visual inspection of the seeds found by floating indicate that a total of 18 *D. ruyschiana* seeds had an intact seed coat, although sometimes heavily eroded (figure 12).

Table 2: Results of the floating method for soil from three locations with a presence of D. ruyschiana. Shown is the number of seeds found for D. ruyschiana and unidentified species

Place	**# Other seeds**	**# *D. ruyschiana* seeds**	**Total**	***D. ruyschiana***
Åserud	12	18	30	60%
Haugsbygda	7	0	7	0%
Fornebu	28	14	42	33%

Table 6: Results of experiment using emergence method. Shown is the species name, if known, and the respective number of occurrences for soil from all three locations.

Species	**# Haugsbygda**	**# Åserud**	**# Fornebu**	**# Total**
Dracocephalum ruyschiana		7	8	15
Potentilla argentea L.		33	7	40
Stellaria graminea		8		8
Hypericum maculatum		7		7
Trifolium arvense			6	6
Arenaria serpyllifolia L			6	6
Betulaceae sp.	1		1	2
Acinos arvensis			1	1
Arabis hirsuta		1		1
Veronica officinalis			1	1
Leucanthemum vulgare			1	1
Stellaria media			1	1
Viola tricolor		1		1
Lamiaceae sp.		1		1
Caryophyllaceae sp.		11		11
Unidentified grass specie	5	2	3	10
Unidentified Galium		2	3	5
Unidentified Species 1			2	2
Unidentified Species 3		1		1
Unidentified Species 4		1		1
Unidentified Species 5		1		1

Figure 12: Nine D. ruyschiana seeds found in 1 liter of soil from Fornebu using the floating method of seed extraction; seed coats are relatively intact, although some are heavily eroded

4 DISCUSSION

My results suggests that neighboring vegetation reduces seed set in *D. ruyschiana*, and that variation in population size may explain variation in genetic diversity, with small populations being less genetically variable than larger ones. Variation in seed set between 11 populations of *D. ruyschiana* explain variation in germination percentage, i.e. populations with high seed set also have a high germination percentage. *D. ruyschiana* seeds collected from four populations were found to be highly viable, and light and scarification had a statistically significant positive effect on seed germination. I discovered the presence of a *D. ruyschiana* soil seed bank using two methods of seed detection. Flower size did not predict seed number per flower. There was no statistically significant association between population size and seed number per flower or germination percentage.

4.1 EFFECTS OF VEGETATION TRIMMING ON SEED SET, FLOWER SIZE AND GERMINATION

4.1.1 Effect of vegetation trimming on seed set

Positive effects found of vegetation trimming on reproduction can be both a result of increased access to resources through reduced competition, and increased pollinator attraction through increased visibility. The observed effect of vegetation trimming on reproduction is in accordance with the results of a similar study by Sletvold *et al.* (2013) who showed that tall vegetation has a negative effect on female fitness. (Ågren *et al.* 2006) found that vegetation trimming via selective grazing increased seed set in *Primula farinosa* and credited this effect to the increased resource status of the plants that were not grazed, suggesting that increased pollinator accessibility had a minimal effect. The observed difference in my study in seed set between the plants with surrounding vegetation trimmed and the plants with natural surrounding vegetation is assumed to be largely credited to an increase in pollination events due to increased flower visibility, as *D. ruyschiana* is thought to produce few seeds in absence of pollinators (Milberg and Bertilsson 1997). Some effect, assumed to be of a lesser importance, could be credited to increased resource acquisition due to vegetation trimming. However, to truly separate between the effects of increased resources and increased pollinator attraction it is necessary to perform an experiment where one

group of individuals are given excess pollen by hand pollination and one is left to be open pollinated.

The comparison of seed set in the trimmed and natural vegetation was not severely afflicted by a low sample size, as data from all 82 individuals with natural vegetation and 77 individuals with trimmed vegetation could be used for this analysis.

4.1.2 Effect of vegetation trimming on flower size

The small observed effect of vegetation trimming on flower size suggest that flower growth and thus size is not strongly limited by surrounding vegetation. There was however a small difference between the groups. For all four flower parts (therefore also including the sum), the vegetation trimming group had on average larger flower parts than the individuals in natural vegetation. This measured difference in size between the groups (2.2%) is so small that it appears insignificant. The Cohen's d estimate of effect size (D = 0.21) suggest that the vegetation trimming did have an effect, but that the effect is weak. A weak effect could partly be due to the fact that vegetation trimming started slightly after onset of flowering. If it had started earlier the effect may have been stronger. Also, *D. ruyschiana* is known to benefit little from excess nutrients (Stabbetorp and Endrestøl 2011), and the very narrow leaves of the species suggest that it is more adapted to retaining water than to maximize photosynthesis and grow rapidly. There is also surely a physiological limit to how big a *D. ruyschiana* flower can be even under ideal conditions. Knowing this physiological limit may be necessary to determine what my results imply.

4.1.3 Effect of vegetation trimming on seed germination

The average germination percentage was 66% for the plants that had surrounding vegetation trimmed and 48% for the plants in natural vegetation. Although there is not a statistically significant difference between the groups, there appear to be a trend in the data as shown by figure 6. If this apparent trend is a true trend, there could be several underlying explanations. Earlier pollination may have provided more time for seed maturation. Trimming of surrounding vegetation may have increased resource acquisition, providing more resources for seed maturation.

The Cohen's d estimate suggest that the effect of vegetation trimming was between small and medium. If this is the case, a larger sample size would be likely to yield a smaller p-value. Additionally, the randomly picked seeds that were to be representative of the individuals in natural vegetation came from individuals that produced 60% more seeds per flower than the natural

vegetation average. In my study comparing data on 11 populations of *D. ruyschiana* I found a positive correlation between seed set and germination percentage. The natural vegetation was thus likely overrepresented in this germination experiment, as the seeds from natural vegetation came from fruits that were exceptionally well-developed. Adjusting for this would further increase the significance of the observed effect. Therefore, it seems likely that a new study using average seeds and a larger sample size would find that vegetation trimming does have an effect on *D. ruyschiana* seed germination.

A mismatch of data and low germination percentage was the cause of the low sample size for the germination experiment of seeds from Fornebu. As the number of samples is low, randomness could greatly influence the results (type II error). There are several sources of variation in seed germinability that were not accounted for in the study design. Variations in the scarification process as well as variations in the germination chamber may explain some of the variation in germination results, as conditions will never be exactly the same throughout the chamber. There was also some variation in how the scarification was done, primarily because it was done by hand and it was difficult to cut all the seeds to the same depth. A seed cut too deep may not survive, and a seed not cut deep enough will take longer to germinate. A standardized method of scarification would be preferred, but none is known to me.

4.1.4 Effect of flower size on seed set

It cannot be concluded from my data whether there is a correlation between flower size and seed set in *D. ruyschiana*, but my results indicate that flower size does not have an effect on seed set per flower in *D. ruyschiana,* which is also what Milberg and Bertilsson (1997) found in their study on *D. ruyschiana* using the same measure of seed set as I did. A weakness in both their and my study may be using the average number of seeds per flower as a measure of reproduction. As *D. ruyschiana* only has four ovules per flower, and the average number of seeds per flower for a given inflorescence will only be maximized if every single ovule is pollinated, one cannot expect a great variation in this data. In the study of Milberg and Bertilsson (1997) the proportion of seeds per flower varied between 0.027 and 0.402, averaging at 0.26, and in my data between 0 and 0.73, averaging at 0.23. Using the total number of seeds produced per individual would have been preferable, but this is difficult to estimate as differentiating individual *D. ruyschiana* from each other requires digging up and potentially damaging the rhizome. It may be possible that the

estimate of seed set used in my study is sufficient for predicting how flower size effects seed set, but that the relationship is not linear. A bigger flower is not necessarily better, e.g. a narrow flower may have a higher pollination efficiency as they attract specialized pollinators. The ideal flower size largely depends on the pollinator, and the ideal flower size for *D. ruyschiana* may neither be "small" or "large" but somewhere in between. A correlation between flower size and seed set may therefore gravitate around an intermediate flower size, with a positive correlation observed in the smaller flowers and a negative correlation in the larger flowers.

4.1.5 What could be done differently?

It should be taken into consideration that the treatment involving removal of vegetation may have a somewhat limited effect as the treatment started slightly after the onset of flowering. If vegetative growth and accumulation of sugar has been impaired over time before the start of flowering, production during the flowering period may also be impaired. The treatment may also have had effects that were not considered during the design phase of the study. One of these unwanted effects of treatment was the potential for inducing physiological shock and stress, which is known to happen to most plants when key environmental factors change too rapidly. For most of the *D. ruyschiana* that underwent treatment at Fornebu, no obvious ill effects were observed. But several of the individuals were observed not being able to stand on their own after the surrounding vegetation had been trimmed and spent an unknown length of time lying horizontally on the ground. At least two of these spent the rest of the season lying horizontally, which is likely to have reduced the productivity of the plant due to reduced sun and wind exposure and increased susceptibility to diseases and pests. Starting treatment earlier in the growth season would remedy these problems.

In order to not attract unwanted attention to the study site at Fornebu, inconspicuous markers were used to identify the studied plants. The flower size measurements were produced during peak flowering, while the seed count data was produced after flowering had ended. This meant that all 200 individuals had to be rediscovered after a period of several weeks, which proved to be more difficult than anticipated, and a total of 31 plants were not found. The experiment was supposed to continue the subsequent summer of 2018, but a record drought (table S3; figure S5) and high temperatures (table S2; figure S4) killed most above ground parts or deflowered virtually all *D. ruyschiana* individuals found at Fornebu. For the study on the effects of flower size on seed set

there was a mismatch of data that further reduced the sample size by 30. Mismatch was caused by a great difficulty identifying some of the individuals at the end of the season, as some of the identification labels were too worn to be readable.

4.2 EFFECTS OF POPULATION SIZE ON GENETIC DIVERSITY, SEED SET AND GERMINATION

4.2.1 Effect of population size on genetic diversity

My results on the effects of populations size on genetic diversity suggests that there is not a statistically significant relationship (Beta $_{(SE = 2.699*10^{-5})} = 0.54$, $p = 0.09$, $R^2 = 0.29$). However, these results are not conclusive as the nearly significant p-value of 0.09 and relatively good fit of the model suggests that there is indeed an association between population size and genetic diversity. My results therefore appear to be in agreement with the general assumption that genetic diversity is reduced in small populations (Frankham 1996). The fit of the model could surely be improved, as the relationship between population size and genetic diversity may not be linear. To avoid overfitting the model the most conservative models were preferred. Including data on more than 11 populations seems to be necessary to properly determine this relationship without risking overfitting the model. It should also be considered that there may not be a strong relationship between population size and genetic diversity for *D. ruyschiana,* even though this has been shown for other plant species, e.g. *Anthericum liliago* (Peterson *et al.* 2008). *D. ruyschiana* produces long lived rhizomes and can clonally reproduce, but the potential size of these genetic individuals is not known. Some populations may be dominated by a few, very large individuals, and could thus appear to have a relatively large population size yet low genetic diversity. Differentiation *D. ruyschiana* individuals from each other is difficult without digging up and examining the rhizome, and even then, it can be difficult to estimate the population size without confirming this with genetic data.

4.2.2 Effect of population size on seed set

Population size was not found to have an effect on any of the estimates of female fitness used in my analysis, average number of seeds per fruit/inflorescence or average number of flowers per inflorescence. My results suggests the same relationship found by Milberg and Bertilsson (1997) in their study on *D. ruyschiana,* but are in disagreement with a similar study on the congeneric *D. austriacum* (Dostálek *et al.* 2015), a study in which the proportion of developed seeds and seeds

per plant were used a measures of seed set in a similar manner as in my study. This difference in results hints to a difference in the effects of population size, and that *D. ruyschiana* is less susceptible to these effects. Milberg and Bertilsson (1997) discusses this in their paper and suggests that since *D. ruyschiana* is known to be long-lived, many of the currently living *D. ruyschiana* may be individuals that were once part of a larger cohesive population and have survived primarily asexually until the present. Populations of *D. ruyschiana* may thus not suffer the same inbreeding depression and effects thereof which is observed in other species.

A simple comparison of the estimates of population size and the measure of seed set in table 1 show that the three smallest populations (Svartorsæter, Fauske & Nakholmen) were among the top six seed producers, together with the two largest populations and another relatively small population (N. Hegstad gård). The population at N. Hegstad gård had an estimated size of 200, which is less than ¼ of the size of the largest population yet produced 57% more seeds per fruit on average (2.49 seeds per fruit) than the overall average (1.58 seeds per fruit). From this is seems obvious that there is no relationship in my data between population size and seed set. My results suggests that the studied populations are sufficiently large enough to not be influenced by pollen limitation or inbreeding depression, as is found for other species like *Dictamnus albus* (Hensen and Oberprieler 2005).

All but two of the populations have low population sizes (≤ 300), with the remaining two estimated at 850 (Fornebu) and 870 (Jeløya) (Table2). This gap in the population size data from 300 to 850 leaves room for inaccuracy in the model, as does the low number of large populations. This suggests that the true relationship between population size and seed set for *D. ruyschiana* is yet to be determined, as this cannot be done conclusively with my data.

There are several possible sources of variation in the estimates of female fitness that are not accounted for. Estimates of population density were not recorded or included in the analysis and were thus assumed to be uniform across all the populations. Variation in the distance from the populations to other resource patches for pollinators were not accounted for. A population being far away from other resource patches may be visited less frequently than populations that are closer to other resource patches. There may also be effects of community co-flowering that are not known for *D. ruyschiana.* The seeds were all collected during the same season, but there could have been variation in the local weather and climatic conditions of each population. The populations in

Oppland county are further north and inland, while most of the populations from the Oslo area are in close proximity to the ocean. Thanks to the high heat capacity of water, land areas close to the ocean have an additional source of heat. Combined with being further south, the Oslo populations also benefited from faster thawing and an earlier spring, providing an advantage due to an increased length of the growth season.

I used three measures of female fitness in my study: Average number of seeds produced per flower, average number of seeds produced per inflorescence and average number of flowers produced per inflorescence. The golden standard of female fitness in plants is arguably the number of viable seeds produced per individual. Measuring seeds per plant was avoided in my experiment as it is difficult to differentiate individual *D. ruyschiana* from each other without digging up the rhizome. This may be a weakness of this study design as one would expect stronger effects of population size on total seed set rather than on the number of seeds per flower.

4.2.3 Effect of population size and seed set on germination

Seed set had a statistically significant effect on germination percentage (Beta $_{(SE = 0.07)} = 0.62$, $p = 0.04$, adjusted $R^2 = 0.38$). Germination percentage did not appear to be influenced by population size. The observed effect of seed set on germination indicate that *D. ruyschiana* with more seeds also have higher quality seeds. There is high variation in germination rate (48-93%) across the smaller populations. This may be indicative of inbreeding having an effect on germination for some of the smaller populations. The observed variation in overall germination percentage may be due to different environmental stimuli between populations, which may have triggered epigenetic effects in relation to seed germination factors and the strength of which that is required to break seed dormancy. *D. ruyschiana* is a long-lived species that can reproduce clonally, and there may be a high variation in the age of the individuals included in the study, which is likely to influence seed set and germination to some degree. When germinating seeds over a limited span of time, one may end up measuring germination rate rather than germination percentage, as only a fraction of the viable seeds germinates within that window of time. A variety of factors are known to hasten or delay seed germination (Baskin and Baskin 2001), and some of these factors may be unaccounted for if the seeds are not given enough time to germinate. Differences in the required seed germination factors between the populations could thus explain some of the observed variation, as seeds from all the populations received the same treatment. The observed variation in germination percentage could also be explained by differences in conditions during the seed

germination experiment, as small differences in humidity, light, temperature or in the scarification process may have noticeable effects on germination. Seeds were harvested in accordance with the populations stage in the growth season, and seeds from the populations further north were harvested a bit later than the rest. It is therefore unlikely that seeds from some of the populations were harvested prematurely.

4.2.4 Estimates of population size

The estimate of population sizes used in my analyses deserves discussion, as it is central to my study and it is generally difficult to estimate the population size of species that propagate clonally. Milberg and Bertilsson (1997) mention in their paper how one of the *D. ruyschiana* populations they studied consisted of a single individual, and that the number of inflorescences per individual varied considerably.

4.3 EFFECTS OF LIGHT AND SCARIFICATION ON SEED GERMINATION IN *D. RUYSCHIANA*

Scarification appear to decisively influence germination percentage in *D. ruyschiana*. The seeds that only received light were observed to germinate at twice the frequency of those that did not receive neither light nor scarification, although this effect was not statistically significant. It is nevertheless clear that light does have some effect on germination for *D. ruyschiana* seeds, but other germination factors may be more important. There may also be several germination factors in addition to light that are involved in breaking dormancy in *D. ruyschiana*, factors that work together either in synergy or with additive effects. As light appear to increase the germination percentage of *D. ruyschiana* seeds, natural populations of *D. ruyschiana* may suffer a recruitment limitation if there is a lack of disturbances and gaps in the vegetation. This may be particularly problematic in habitats that used to be scythed or grazed but are now left to overgrow. A reduction in sexual reproduction could therefore be expected in overgrowing habitats, as micro-climatic conditions would increasingly become more hostile to the establishment of new *D. ruyschiana* genets.

My results are not in accordance with the results of Puchalski *et al.* (2014) who did not find that *D. ruyschiana* dormancy is broken by scarification using a scalpel, and suggest that the use of gibberellic acid is preferred. Puchalski *et al.* (2014) do however not mention the germination percentage of *D. ruyschiana* in their experiment, making a comparison difficult.

Scarification is a common method for breaking seed dormancy and involves mechanically weakening or opening the seed coat to make the seed permeable to water (Baskin and Baskin 2001). Scarification may serve as a substitute for natural processes such as mechanical or chemical erosion of the seed coat in plant species that have water-impermeable seeds. However, an observed increase in germination success due to scarification does not necessarily mean that the specie relies on seed coat erosion to break dormancy. Examples of this are *Ocimum americanum,* which has a light requirement for germination which can be overcome through scarification (Varshney 1968). *Cucumis anguria* seeds require darkness to germinate, but will germinate in light if scarified (Cardoso and Felippe 1988). Scarification may nevertheless be necessary if the seeds are to germinate within the limited timeframe of the study or to perform a quick seed viability test.

A source of unexplained variation may be small differences in humidity between petri dishes. None of the agar in any of the petri dishes dried out, but minute differences in humidity were observed. This may explain some inconsistencies in the results, such as the seeds from Syltegård suffering the lowest germination rate when only subject to light, while having the highest germination rate when subject to both light and scarification.

4.4 SOIL SEED BANK

To my knowledge extraction of *D. ruyschiana* seeds by floating has never been tested before so my results cannot be compared with earlier results, but it seems like a quick and simple method to get an indication of the presence of the species in the soil. The emergence method of seed extraction is most useful for detecting the presence of any seeds in the soil and could be useful when researching the ecology and biodiversity of the site. Floating is most useful for finding seeds of a particular size, here the size of *D. ruyschiana* seeds or larger is ideal.

Cohen proposed that a "bet-hedging" reproductive strategy explain the existence of soil seed banks, and that they are essential for survival in environments with a high probability of total failure (Cohen 1966). The observed presence of a *D. ruyschiana* soil seed bank therefore suggests that *D. ruyschiana* relies on a bet-hedging strategy that prevent germination under inhospitable conditions.

4.5 CONSERVATIONAL IMPLICATIONS & SUGGESTIONS FOR FURTHER STUDY

D. ruyschiana seeds from previous generations were found to be present in soil from two of the three locations studied. This suggests that population restoration could be done by using the local seeds present in the soil. My results suggests that *D. ruyschiana* seeds are induced to germinate by light. Population restoration must therefore involve regularly removing vegetation to expose the soil to sunlight and potentially other germination factors that are inhibited by increasing vegetation density. Regularly removing vegetation, either by scything, mowing or grazing, will also over time decrease the amount of organic matter in the soil, thus restoring the habitat in which *D. ruyschiana* is more likely to survive. Continuous human involvement appear to be a necessity for the continued survival of *D. ruyschiana* in Norway.

Nitrogen enrichment of *D. ruyschiana* habitats has been proposed to be a threat to the species (Stabbetorp and Endrestøl 2011), and restoration of *D. ruyschiana* habitats must therefore involve removal of plant nutrients from the soil. Traditional scything and removal of the cut vegetation has been proposed as a method of removing nutrients from the soil, but (Eken and Hoell 2012) suggest that *D. ruyschiana* may only benefit from this if the scything is done in a way or at a time that minimizes harm to the *D. ruyschiana* present. Stabbetorp and Endrestøl (2011) do not recommend scything as a method of restoring and maintaining *D. ruyschiana* habitats. Conservation will therefore be a cumbersome process involving selectively removing vegetation and that must continue indefinitely, unless alternative methods are proposed.

A traditional method of removing organic matter from soil is using controlled fire. This method is not as commonly used today as it once was, but several plant species are known to rely on fire for seed germination (Baskin and Baskin 2001), including *Dracocephalum parviflorum* (Lee 2004). Fire as a *D. ruyschiana* germination factor is therefore a logical topic of further study of the species.

4.6 CONCLUSION

My results are congruent with previous work (Stabbetorp and Endrestøl 2011) that suggest a correlation between *D. ruyschiana* population decline and habitat degradation due to overgrowth, as I have found that surrounding vegetation negatively influence seed set in *D. ruyschiana.* In Norway *D. ruyschiana* occur most frequently in habitats that are periodically harvested by scything or animal grazing, and a cessation of these activities appear likely to negatively influence seed set

in *D. ruyschiana*. To ensure a continued presence of this species in Norway, maintenance of their habitats need to continue indefinitely. Norway should follow the precautionary principle as it is the only country known to have a presence of the beetle *Meligethes norvegicus*.

5 REFERENCES

Allee, W. C. and E. S. Bowen (1932). "Studies in animal aggregations: Mass protection against colloidal silver among goldfishes." Journal of Experimental Zoology **61**(2): 185-207.

Baskin, C. C. and J. M. Baskin (2001). Seeds: ecology, biogeography, and evolution of dormancy and germination. San Diego, Academic Press.

Berthelsen, A., S. Olerud and E. M. O. Sigmond (1996). Geologisk kart over Norge, berggrunnskart OSLO 1:250 000. Trondheim, Norges Geologiske Undersøkelse (NGU).

Bjørge, A. S. (2012). Rik på historie : et riss av kulturhistoriens fysiske spor i Bærum. Sandvika, Bærum kommune, Regulering, Natur og idrett.

Cardinale, B. J., J. E. Duffy, A. Gonzalez, D. U. Hooper, C. Perrings, P. Venail, A. Narwani, G. M. Mace, D. Tilman, D. A. Wardle, A. P. Kinzig, G. C. Daily, M. Loreau, J. B. Grace, A. Larigauderie, D. S. Srivastava and S. Naeem (2012). "Biodiversity loss and its impact on humanity." Nature **486**: 59–67.

Cardoso, V. J. M. and G. M. Felippe (1988). "The action of the testa upon the germination of seeds of Cucumis anguria L." Biologia Plantarum **30**(1): 48-52.

CoE (1979). Convention on the Conservation of European Wildlife and Natural Habitats. 104. C. o. Europe. Bern.

Cohen, D. (1966). "Optimizing reproduction in a randomly varying environment." Journal of Theoretical Biology **12**(1): 119-129.

Cohen, J. (1988). Statistical Power Analysis for the Behavioral Sciences. New York, Lawrence Erlbaum associates.

DN-rapport (2009). Handlingsplan for slåttemark, Direktoratet for naturforvaltning. **6**.

DN-rapport (2010). "Handlingsplan for dragehode (Dracocephalum ruyschiana) og dragehodeglansbille (Meligethes norvegicus)." **5**.

Dostálek, T., S. Castro, S. v. d. Meer, G. Oostermeijer and Z. Münzbergová (2015). "Does pollen limitation affect population growth of the endangered Dracocephalum austriacum L.?" Popul. Ecol. **57**: 105-116.

Ehrich, D. (2006). " AFLPdat: a collection of R functions for convenient handling of AFLP data." Mol. Ecol. Notes **6**: 603-604.

Eken, M. and G. S. Hoell (2012). "Rapport fra fagdag om dragehode Dracocephalum ruyschiana og rød skogfrue Cephalanthera rubra i Modum kommune." Blyttia **70**(3): 156.

Eriksson, O. and J. Ehrlén (1992). "Seed and microsite limitation of recruitment in plant populations." Oecologia **91**(3): 360-364.

Fattahi, M., V. Nazeri, F. Sefidkon, Z. Zamani and J. Palazon (2010). "The Effect of Pre-sowing Treatments and Light on Seed Germination of Dracocephalum kotschyi Boiss: An Endangered Medicinal Plant in Iran." Hort. Environ. Biotechnol. **52**(6): 559-566.

Frankham, R. (1996). "Relationship of Genetic Variation to Population Size in Wildlife." Conservation Biology **10**(6).

GBIF.org. (2018). from http://gbif.org.

Gonzalez, S. and L. Ghermandi (2012). "Comparison of methods to estimate soil seed banks: The role of seed size and mass." Community Ecology **13**(2): 238-242.

Gough, L., C. W. Osenberg, K. L. Gross and S. L. Collins (2003). "Fertilization effects on species density and primary productivity in herbaceous plant communities." OIKOS **89**(3): 428-439.

Grimstad, K. J. and O. Olsen (2010). "Kartlegging /reinventering av dragehode (Dracocephalum ruyschiana) i Ringebu, Sør–Fron og Nord-Fron kommuner Oppland fylke." ØkoSøk rapport 2010:3.

Hautier, Y., P. A. Niklaus and A. Hector (2009). "Competition for Light Causes Plant Biodiversity Loss After Eutrophication." Science **324**(5927): 636-638.

Hensen, I. and C. Oberprieler (2005). "Effects of population size on genetic diversity and seed production in the rare Dictamnus albus (Rutaceae) in central Germany." Conservation Genetics **6**: 67-73.

Hortal, J., J. M. Lobo and A. Jimenez-Valverde (2007). "Limitations of biodiversity databases: case study on seed-plant diversity in Tenerife, Canary Islands." Conserv. Biol. **21**: 853–863.

Janssen, J. A. M., J. S. Rodwell, M. García Criado, S. Gubbay, T. Haynes, A. Nieto, N. Sanders, F. Landucci, ... and M. Valachovič (2016). Terrestrial and freshwater habitats. European Red List of Habitats, European Commission.

Kahle, D., H. Wickham, S. Jackson and M. Korpela (2019). ggmap: Spatial Visualization with ggplot2.

Kendrick, R. E. and G. H. M. Kronenberg (1994). Photomorphogenesis in Plants, Kluwer Academic Publishers.

Kleven, O., A. Endrestøl, M. Evju, O. E. Stabbetorp and K. B. Westergaard (2018). "SNP discovery in the northern dragonhead Dracocephalum ruyschiana." Conservation Genet Resour

Kull, K. and M. Zobel (1991). "High species richness in an Estonian wooded meadow." Journal of Vegetation Science **2**(5): 715-718.

Lee, P. (2004). "The impact of burn intensity from wildfires on seed and vegetative banks, and emergent understory in aspen-dominated boreal forests." Canadian Journal of Botany **82**(10): 1468-1480.

Leek, M. A., V. Parker and R. Simpson (1989). Ecology of Soil Seed Banks. London, Academic Press, Inc.

Lomolino, M. V. and R. H. Lawrence (2004). Frontiers of Biogeography: New Directions in the Geography of Nature, Sinauer Associates.

Luijten, S. H., A. Dierick, J. Gerard, B. Oostermeijer, L. E. L. Raijmann and H. C. M. Den Nijs (2000). "Population Size, Genetic Variation, and Reproductive Success in a Rapidly Declining, Self-Incompatible Perennial (Arnica montana) in The Netherlands." Conservation Biology **14**(6): 1776-1787.

Manning, P., S. A. Morrison, M. Bonkowski and D. Bardgett (2008). "Nitrogen enrichment modifies plant community structure via changes to plant–soil feedback." Oecologia **157**(4): 661–673.

Matthies, D., I. Bräuer, W. Maibom and T. Tscharntke (2004). "Population size and the risk of local extinction: empirical evidence from rare plants." Oikos **105**(3): 481-488.

Meusel, H., E. Jäger, S. Rauschert and E. Weinert (1978). Vergleichende Chorologie der Zentraleuropäischen Flora, Karten, Gustav fischer verlag.

Milberg, P. and A. Bertilsson (1997). "What determines seed set in Dracocephalum ruyschiana L. an endangered grassland plant." Flora(192): 361-367.

Mossberg, B., L. Stenberg, S. Båtvik, S. Moen and T. Karlsson (2012). Gyldendals store nordiske flora. Oslo, Gyldendal.

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

Peterson, A., I. V. Bartish and J. Peterson (2008). "Effects of population size on genetic diversity, fitness and pollinator community composition in fragmented populations of Anthericum liliago L." Plant Ecology **198**(1): 101–110.

Puchalski, J., M. Niemczyk, P. Walerowski, W. Podyma and A. Kapler (2014). "Seed banking of Polish endangered plants – the FlorNatur Project." Biodiv. Res. Conserv **34**: 65-72.

Pöyry, J., L. G. Carvalheiro, R. K. Heikkinen, I. Kühn, M. Kuussaari, O. Schweiger, A. Valtonen, P. M. Bodegom and M. Franzén (2016). "The effects of soil eutrophication propagate to higher trophic levels." Global Ecology and Biogeography **26**(1): 18-30.

R Development Core Team (2019). R: A Language and Environment for Statistical Computing, R Foundation for Statistical Computing.

Sletvold, N., J. M. Grindeland and J. Ågren (2013). "Vegetation context influences the strength and targets of pollinator-mediated selection in a deceptive orchid." Ecology **94**(6): 1236–1242.

Stabbetorp, O. E. and A. Endrestøl (2011). Faglig grunnlag for handlingsplanen for dragehode Dracocephalum ruyschiana og dragehodeglansbille Meligethes norvegicus. Trondheim, Norsk institutt for naturforskning. **766**.

Stedje, B., K. E. Bjureke and G. Gusarova (in prep.). The effect of population size on genetic diversity and reproduction of the endangered Dracocephalum ruyschiana in Norway.

Stevens, C. J., C. Duprè, E. Dorland, C. Gaudnike, D. J. G. Gowing, A. Bleeker, M. Diekmann, D. Alard, R. Bobbink, D. Fowler, E. Corcket, J. O. Mountford, V. Vandvik, P. A. Aarrestad, S. Muller and N. B. Dise (2010). "Nitrogen deposition threatens species richness of grasslands across Europe." Environmental Pollution **158**(9): 2940-2945.

Tittensor, D. P., M. Walpole, S. L. Hill, D. G. Boyce, G. L. Britten, N. D. Burgess, ... and Y. Ye (2014). "A mid-term analysis of progress toward international biodiversity targets." Science **346**(6206): 241-244.

Torchiano, M. (2018). effsize: Efficient Effect Size Computation.

Vandvik, V., K. Klanderud, E. Meineri, I. E. Måren and J. Töpper (2016). "Seed banks are biodiversity reservoirs: species–area relationships above versus below ground." Oikos **125**(2): 218-228.

Varshney, C. K. (1968). "Germination of the light-sensitive seeds of ocimum americanum Linn." New phytologist **67**(1): 125-129.

Vellend, M., L. Baeten, A. Becker-Scarpitta, V. Boucher-Lalonde, J. L. McCune, J. Messier, I. H. Myers-Smith and D. F. Sax (2017). "Plant Biodiversity Change Across Scales During the Anthropocene." Annual Review of Plant Biology **68**: 563-586.

Veloso, A. C. R., P. S. Silva, W. K. Siqueira, K. L. R. Duarte, I. L. V. Gomes, H. T. Santos and M. Fagundes (2017). "Intraspecific variation in seed size and light intensity affect seed germination and initial seedling growth of a tropical shrub." Acta Botanica Brasilica **31**(4): 736-741.

Vitousek, P. M., J. D. Aber, R. W. Howarth, G. E. Likens, P. A. Matson, D. W. Schindler, W. H. Schlesinger and D. Tilman (1997). "Human alteration of the global nitrogen cycle: Sources and consequences." Ecological Applications **7**(3): 737-750.

Vos, P., R. Hogers, M. Bleeker, M. Reijans, T. Lee, M. Hornes, A. Frijters, J. Pot, J. Peleman and M. Kuiper (1995). "AFLP: a new technique for DNA fingerprinting." Nucleic Acids Res **23**(21): 4407-4414.

Wollan, A. K., V. Bakkestuen, K. Bjureke, H. Bratli, A. Endrestøl, O. E. Stabbetorp, A. Sverdrup-Thygeson and R. Halvorsen (2011). Åpen grunnlendt kalkmark i Oslofjordområdet – et hotspot-habitat. NINA Rapport Oslo, Norsk institutt for naturforskning. **713**.

Xiwen, L. and I. C. Hedge (1994). Flora of China, Science Press (Beijing) & Missouri Botanical Garden (St. Louis).

yr.no. (2019).

Østhagen, H. (1972). Flora og vegetasjon på Ringerike, Buskerud. En floristisk-økologisk undersøkelse med hovedvekt på den xeroterme vegetasjonen, samt en oversikt over verneverdige områder. Hovedfagsoppgave. University of Oslo**:** 111.

Ågren, J., C. Fortunel and J. Ehrlén (2006). "Selection on Floral Display in Insect-Pollinated Primula farinosa: Effects of Vegetation Height and Litter Accumulation." Oecologia **150**(2): 225-232.

6 APPENDIX

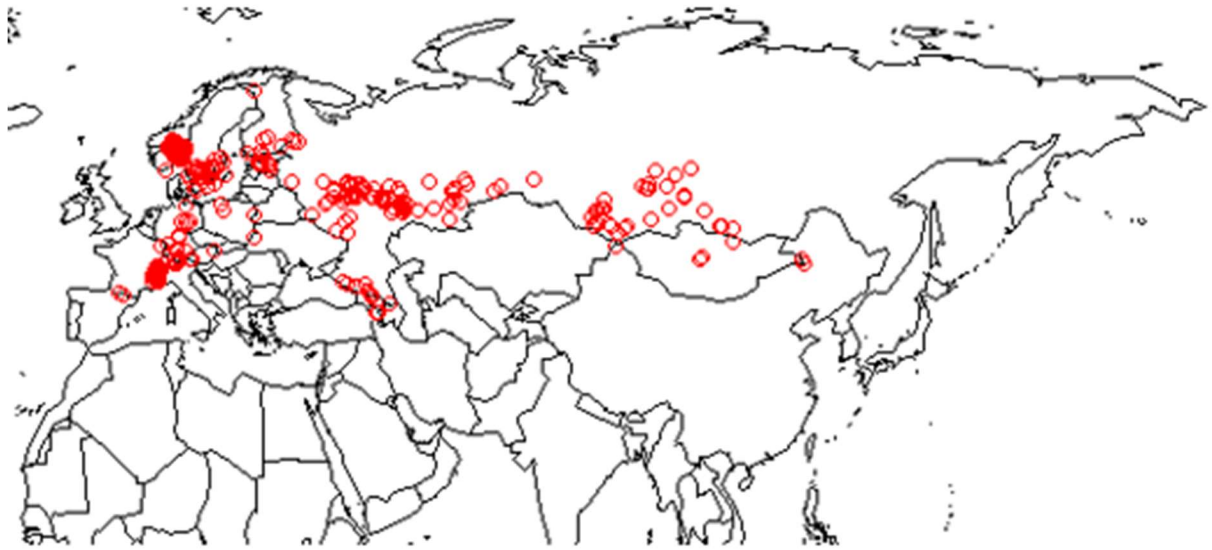

Figure S1: Map of D. ruyschiana distribution, made in R using data from gbif.org. The true distribution of D. ruyschiana is difficult to estimate using species observation databases, as survey of D. ruyschiana is a priority in western Europe but not as much in Russia and Asia. On GBIF.org (2018) half of the occurrences of D. ruyschiana were registered after 2010, and 80% of these were occurrences observed in Norway. This data is thus more useful as a measure of surveying effort rather than the actual distribution of D. ruyschiana, a Wallacean Shortfall (Lomolino and Lawrence 2004, Hortal et al. 2007).

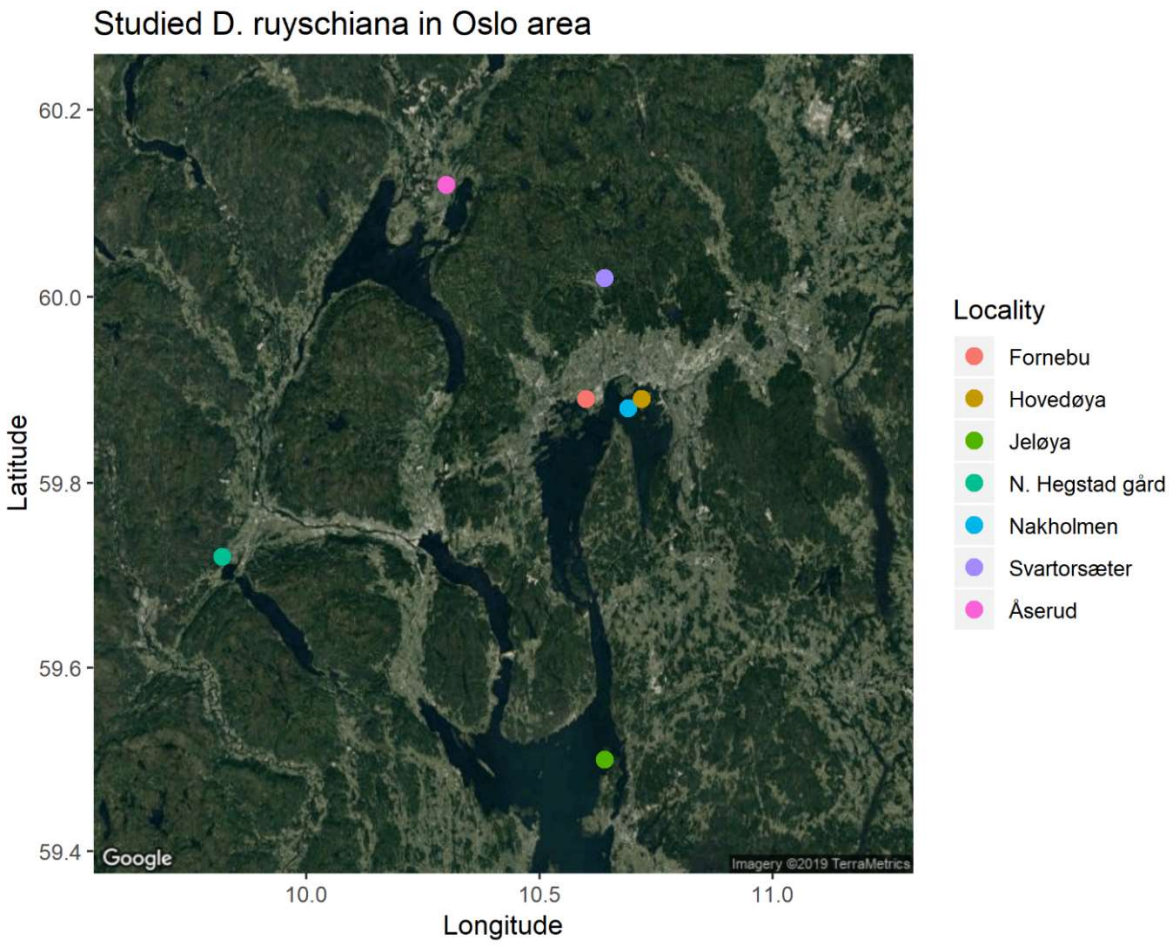

Figure S2: Map made in R using GPS data; showing the location of the seven studied populations in the general Oslo area

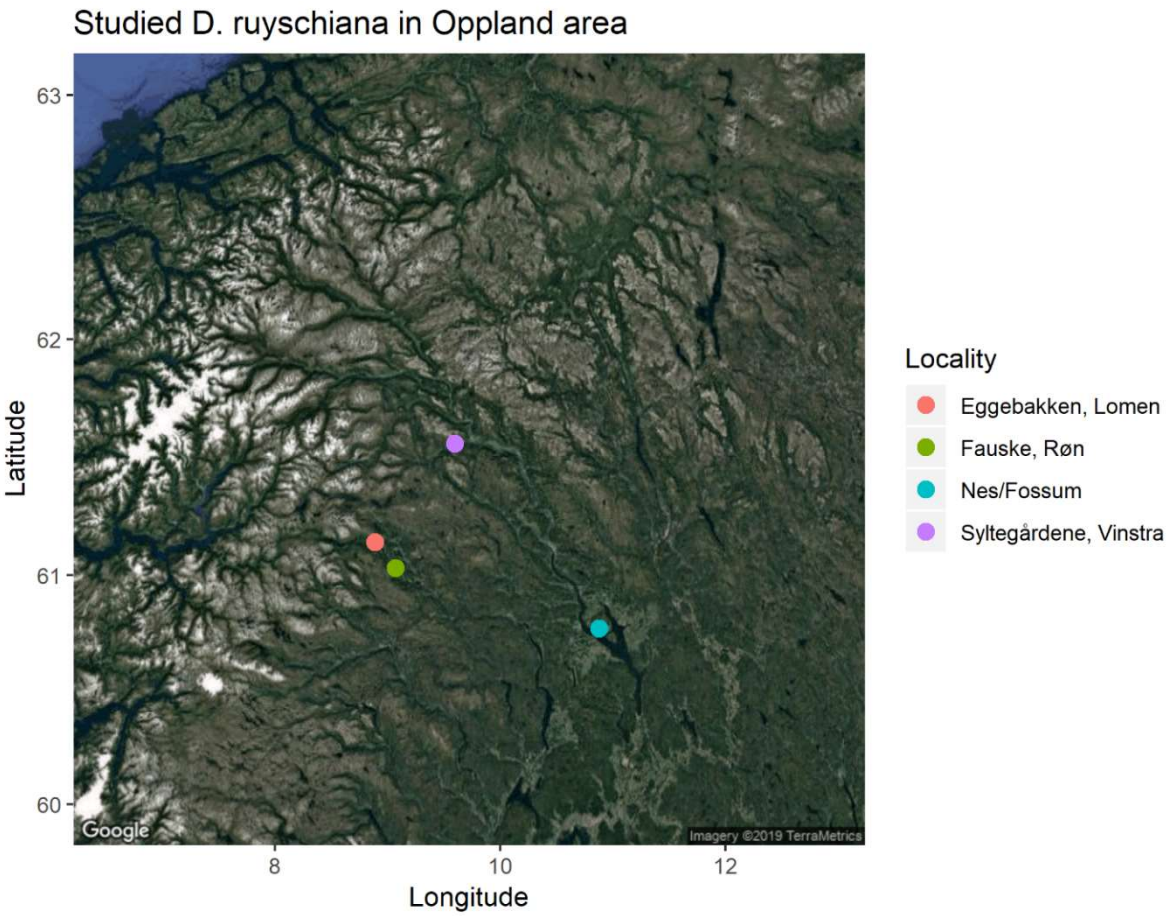

Figure S3: Map made in R using GPS data; showing the location of the four studied populations in the general Oppland area

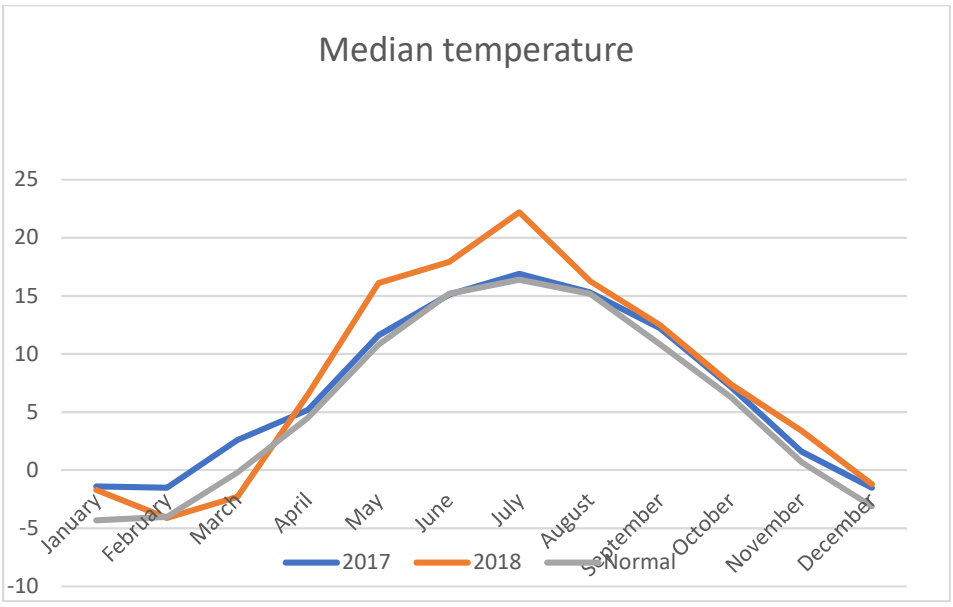

Figure S4: Graph of monthly mean temperature in Oslo for 2017 (blue), 2018 (orange) and normal (gray). Data from yr.no

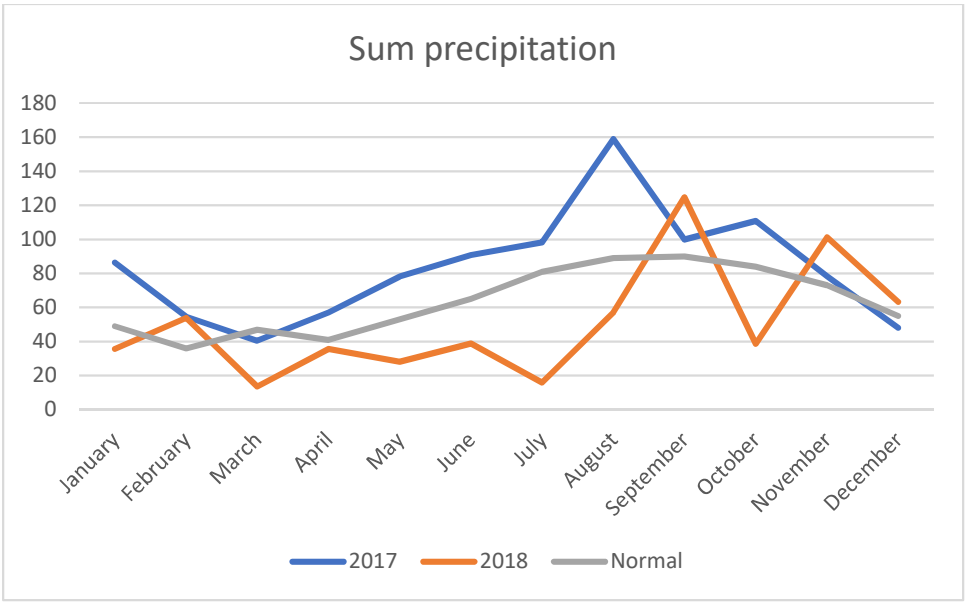

Figure S5: Graph of monthly precipitation in Oslo for 2017 (blue), 2018 (orange) and normal (gray). Data from yr.no

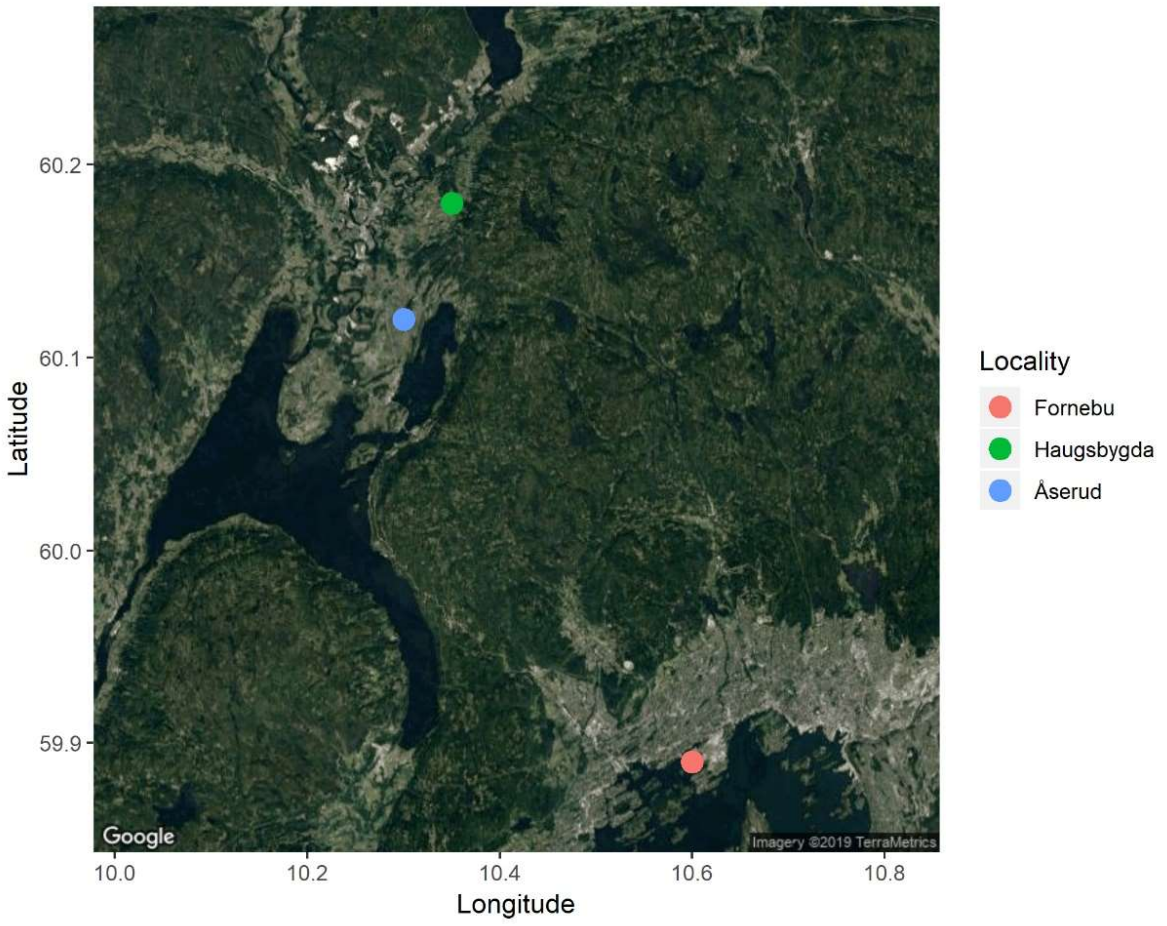

Figure S6: Map of the three populations of D. ruyschiana examined for the presence of a seed soil bank. Haugsbygda (green) and Åserud (blue) are in the Hønefoss area, while Fornebu is in Oslo

Table S1: Location and date of collection of seed and leaves on silica. BS = Brita Stedje; KB = Kristina Bjureke; HE = Hallvard Elven

County	Municipality	Locality	Collector	Collection date	Collection date	Coordinates
				Seeds	Silica	
Buskerud	Hole	Åserud	KB	Mid August	08.jul	60.12N, 10.30E
Oslo	Oslo	Nakholmen	BS	Mid August	21.jul	59.88N, 10.69E
Østfold	Moss	Jeløya	KB	02.aug	12.jul	59.50N, 10.64E
Asker	Bærum	Fornebu	BS & KB	09.aug	15.jun	59.89N, 10.60E
Oslo	Oslo	Svartorsæter	KB	Mid August	19.jun	60.02N, 10.64E
Buskerud	Øvre Eiker	N. Hegstad gård	BS & KB	Mid August	08.jun	59.72N, 9.82E
Hedmark	Ringsaker	Nes/Fossum	HE	26.aug	03.jun	60.77N, 10.88E
Oppland	Nord-Fron	Syltegårdene, Vinstra	HE	27.aug	04.jun	61.56N, 9.60E
Oppland	Vestre Slidre	Fauske, Røn	BS	16.sep	29.jul	61.03N, 9.07E
Oppland	Vestre Slidre	Eggebakken, Lomen	BS	16.sep	29. juli & 16. sept	61.14N, 8.89E
Oslo	Oslo	Hovedøya	BS	Mid August	26.jul	59.89N, 10.72E

Table S2: The median value temperature for each month of 2017, 2018 & mean (normal). Data from yr.no

		Median temperature		
	Month	**2017**	**2018**	**Normal**
1	**January**	-1.4	-1.7	-4.3
2	**February**	-1.5	-4.1	-4
3	**March**	2.6	-2.3	-0.2
4	**April**	5.2	6.5	4.5
5	**May**	11.6	16.1	10.8
6	**June**	15.1	17.9	15.2
7	**July**	16.9	22.2	16.4
8	**August**	15.3	16.3	15.2
9	**September**	12.2	12.5	10.8
10	**October**	7.2	7.4	6.3
11	**November**	1.6	3.4	0.7
12	**December**	-1.5	-1.2	-3.1

Table S3: The sum of precipitation for each month of 2017, 2018 & mean (normal). Data from yr.no

		Sum precipitation (mm)		
	Month	**2017**	**2018**	**Normal**
1	**January**	86.3	35.6	49
2	**February**	54.6	53.9	36
3	**March**	40.5	13.6	47
4	**April**	57	35.6	41
5	**May**	78.2	28.1	53
6	**June**	90.7	38.8	65
7	**July**	98.2	15.9	81
8	**August**	158.9	57.1	89
9	**September**	99.9	124.7	90
10	**October**	110.8	38.6	84
11	**November**	78.4	101.3	73
12	**December**	48.1	63.2	55