Flower visitation and seed set in *Melampyrum*; Effects of elevation and *Rubus idaeus*

Solveig Haug

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Faculty of Mathematical and Natural Sciences

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

Trykk: Reprosentralen, Universitetet i Oslo

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Abstract

Insect pollination is an important contribution to wild ecosystems in Norway and 80 % of plants seem to benefit from this important ecosystem service. During the last century, there has been an overall decline in pollinators, and climate change is one of the possible explanations to this trend. How will Norwegian plant-pollinator communities respond to climate change? By using a space for time substitute, I have observed pollinator visitation along an elevation gradient to see if the flower visitation rate to and pollinator community of the focal plants *Melampyrum pratense* and *Melampyrum sylvaticum* change under different climate conditions. I found no change in flower visitation rate or pollinator community, suggesting that climate change might not have that much impact on the future pollinator community around *M. sylvaticum* and *M. pratense*. One explanation could be the robustness of this plant-pollinator interaction, as both the pollinators and the plants are already adapted to unstable weather conditions and otherwise harsh enviroment. In addition I studied if elevation and flower visitation rate had an effect on seed set, and found a higher number of seeds per capsules at higher elevations and further that bumblebee visitation lead to higher seed set than fly visitation, suggesting that bumblebees are better pollinators then flies. I also looked at the magnet species effect of *Rubus idaeus* to see if the attractive flowers of *R. idaeus* might fascilitate or compete with *M. sylvaticum* and *M. pratense* when it comes to flower visitors and whether this could be reflected in seed set. The flower visitation rate did not change, but the seed set (and number of seeds per capsule) of *M. sylvaticum* and *M. pratense* increased when growing in proximity to *R. idaeus*.

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1 Introduction

Insect pollination is an important contribution to wild ecosystems and to food production for humans and livestock (Bartomeus, et al., 2014). Approximately 70% of crop species worldwide depend to some degree on insect pollination (Klein, et al., 2007) and 87.5 % of all angiosperms are estimated to depend, at least partly, on animal-pollination to increase their seed quality and quantity (Ollerton, et al., 2011). Yield improvement stemming from animal pollination are of substantial value to humans and as shown in the latest report from the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services (IPBS2016) insect contribution to pollinator dependent crops had a worldwide estimated economic value of $235 billion-$577 billion. Unlike tropical ecosystems where birds, bats and other vertebrates form parts of the pollinator community, insect pollination is the only type of pollination that occur in Norway and 80 % of wild plants seem to benefit from this (Totland, et al., 2013). Despite the high percentage of plants depending or partly depending on insects, pollination services from insects are often unstable and it is not uncommon for plants to be partly autogamous. Plants that are partly autogamous can still benefit from pollination services because pollen limitation decreases and outcrossing is promoted (Totland & Schulte-Herbrüggen, 2003).

Northern ecosystems are often considered species poor and are shaped by unstable weather and short growing seasons. Insects are annual because they cannot survive the cold winter and plants often start their growing season as soon as the snow has melted to make sure that they have time to finish seed production (Lütz, 2012). In addition to a high number of autogamous or partly autogamous plants in Norway, many plants have also generalists flowers because the availability of pollinators are unreliable and it is favorable to attract different pollinators to avoid pollen limitation. Pollinators are often generalists as well because the ability to switch between plant species is important to ensure access to nectar and pollen though the season. Bumblebees cope well with these conditions and the density and diversity of bumblebees are high at northern latitudes compared to southern latitudes (Totland, et al., 2013). Their big hairy bodies with the ability to thermoregulate by using flight muscles to warm up when needed (Esch , et al., 1991) are good adaptations to the Norwegian climate. Buzz-pollination, long tongues and high floral fidelity make bumblebees good pollinators because the pollen grains have a higher chance of being transferred to a flower from the same species (Willmer, et al., 1994) (Goulson, 2003) (Dasgan, et al., 2004).

There are 208 species of bees found in Norway that can be divided into solitary bees and social bees, out of which 35 are bumblebees in addition to domesticated honeybees (Totland, et al., 2013). Another important group in Norwegian pollinator communities are flies. This big group of insects is suggested to be poor pollinators on a per flower visit basis, but because of large numbers of individuals and presence where other pollinators may lack, they can to be of importance (Elberling & Olesen, 1999) (Tiusanen, et al., 2016). It is important to note that *syrphids* in some studies are considered to be better pollinators than other flies because they show some degree of flower fidelity (Jauker & Wolters, 2008).

During the last century there has been an overall decline in bumblebees (Potts, et al., 2010) (Cameron, et al., 2010) (Potts, et al., 2016). According to (Nieto, et al., 2014), 23.6% of all European bumblebee species have status threatened with extinction and 45.6 % are in decline. Although flies have not been studied as much as bumblebees, lately some concerns have been raised. A study from (Biesmeijer, et al., 2006) states that the species diversity of *syrphids* seems to have been declining in Britain and the Netherlands since 1980 and another a study (Loboda, et al., 2017) on *Muscids* in Greenland indicates that the general abundance of flies is declining in the higher arctic regions.

Why are bumblebees, flies and other pollinators declining all over the world? Numerous reasons have been suggested and investigated, but there seem to be a high degree of complexity and it looks like it is impossible to single out one leading cause. In addition, the importance of different factors affecting pollinator decline may vary in space and time. A review by (Potts, et al., 2010) summarizes trends, impacts and drivers of global pollinators declines, including habitat fragmentation (Rathcke & Jules, 1993) (Goulson, 2003) (Winfree, et al., 2011), agriculture intensification (Goulson, 2006) (Kleijn & Langevelde, 2006), pesticides (Morandin, et al., 2005) (Whitehorn, et al., 2012), competition between invasive and native plants (Moron, et al., 2009), increased use of imported bumblebees (Kraus, et al., 2011) and spillover pathogen effect from greenhouses to the wild species (Colla, et al., 2006). Climate change is also on the list of possible explanations and this is what my project will focus on.

Climate change is considered to be a major challenge in the future (Parmesan & Yohe, 2003) where temperature increase and extreme weather variation are expected to be the main influencers on pollinator-plant interactions (Hegland, et al., 2009). Phenology, distribution and abundance of plants and pollinators are changing, but the links between recent biological trends and climate change can be difficult to disentangle because non-climatic influences dominate local, short-term variation in ecological patterns and processes (Parmesan & Yohe, 2003).

How will Norwegian plant-pollinator communities respond to these future climate changes? Global warming has caused a range expansion of species toward the poles and higher elevations (Parmesan & Yohe, 2003) (Chen, et al., 2011), but there are concerns that not all species will be able to follow this spatial change in climate conditions. (Kerr, et al., 2015) showed that in contrast to expectations, northern limits of bumblebee distribution in North America and Europe have not changed considerably, while the southern range limits continue to move northwards, causing a contraction of the species' range. This raises an important question about how plant-pollinator interactions are going to look in the future. Are the bumblebees going to decrease in abundance and distribution because the climate is too warm? Are they going to move north or towards higher altitudes because the climate here is closer to their optimum temperature? Plants and pollinators may react differently to climate change which have led to concerns about spatial mismatches where geographic distributions of pollinators and plants do not overlap. Alternatively, it could lead to temporal mismatches where plants and pollinators are separated in time because their life cycles are controlled by different environmental cues (Hegland, et al., 2009). Climate change can lead to direct changes in pollinator behavior such as altered foraging activity (Scaven & Rafferty, 2013). At high temperatures, thermoregulatory limits decide if and when a pollinator can be active (Willmer & Stone, 2004). Large insects have a greater ability to thermoregulate than smaller insects, and bumblebees seem to be even better than their large body size would predict (Bishop & Armbruster, 1999). The downside of being large and furry is that heat is often retained, and is released more slowly because the surface area from where heat is released is small compared to the body size (Peat, et al., 2005). This could result in overheating. Climate change could lead to bumblebees moving their active foraging to a different time of day and take shorter trips to avoid overheating (Scaven & Rafferty, 2013) which again could lead to reduced outcrossing (Waser , 1982), pollen limitation and reduced seed set in the plants they pollinate (Wilcock & Neiland, 2002). Climate change may also change today's pollinator community composition (i.e. the relative dominance of important pollinator groups such as bumblebees, solitary bees and flies), which might again result in cascading effects throughout the ecosystems, like changes in plant community composition because of changes in visitation rate to different type of plant species.

In this study, I focus on the possible transitions in pollinator communities as a result of climate change by looking at flower visitation and seed sets in the herbaceous species *Melampyrum sylvaticum* and *Melampyrum pratense* along an elevation gradient. As the species are very similar in physiology and ecology and often intermingled, I chose to treat the two species as one group hereby referred to as *Melampyrum.*

Elevation gradients are used to achieve a space for time substitute, which is a method to predict the future by comparing to areas at different elevations with contrasting climate condition, temperature regimes in particular. A main assumption is that drivers along the spatial gradients resemble those expected as temporal changes in the future (Bloisa, et al., 2013). Consequently, it is possible to compare plant-pollinator interactions at different elevations to see how the interactions are affected by current climate conditions and make predictions for future climate change. In addition to observe flower visitation along an elevation gradient, I also looked at facilitation and competition between my focal plant species and *R. idaeus*, a species highly attractive to pollinators. Plants that flower at the same time often share pollinators and the presence of a plant with abundant attractive flowers, a magnet species (Lammi & Kuitunen, 1995), might affect the visitation rates to other plants nearby. The effect may lead to facilitation, which means that the presence of one plant species increases pollinator visitation in another plant species because pollinators might show site-fidelity and flexibility in their forage choices (Ogilvie & Thomson, 2016). On the other hand, it could lead to competition, which means that one plant species attracts pollinators away from the other plant, as shown in (Totland, et al., 2006) where they studied competition between the exotic plant *Phacelia tanacetifolia* and the native plant *Melampyrum pratense*. Another type of competition is interference competition where frequently switching between plants might lead to interference in each species pollen flow, which could lead to pollen limitation because stigmas are clogged by heterospesific pollen (Kwak & Jennersten, 1991). It is predicted that competition may arise at times when there are fewer pollinators like in unstable mountain environments, which in turn affect the time of flowering because it is favorable to flower when other plants are not, to attract more pollinators (Pleasants, 1980).

Here I aim to test the following hypothesis: (1) The pollinator community and number of visits to *Melampyrum* change along an elevation gradient. (2) Elevation and flower visitation affect seed set in *Melampyrum*. (3) Flower visitation and seed set in *Melampyrum* is affected by proximity to *R. idaeus*.

2 Materials and methods

Study area

The fieldwork was conducted during June-September 2016 at Norefjell, a mountain range that covers parts of the municipalities Flå, Sigdal and Krødsherad in Buskerud county (SE Norway). The mountain range stretches between the valleys Eggedal in the west and Hallingdal in the east. The highest point is Gråfjelltoppen, 1466 m a.s.l. and at the base of the mountain is lake Krøderen at 133 m a.s.l. To access how elevation, a proxy for climate conditions, affected plant-pollinator interactions and seed set in the focal plant species *Melampyrum*, I selected four different elevations along the slopes of Norefjell ski resort ~200 m a.s.l (low), 425 m a.s.l (mid-low), ~640 m a.s.l (mid-high) and 850 m a.s.l (high). At each elevation; I selected three study sites at least 100 m apart based on the presence of the study species, giving a total of 12 study sites (Fig. 1).

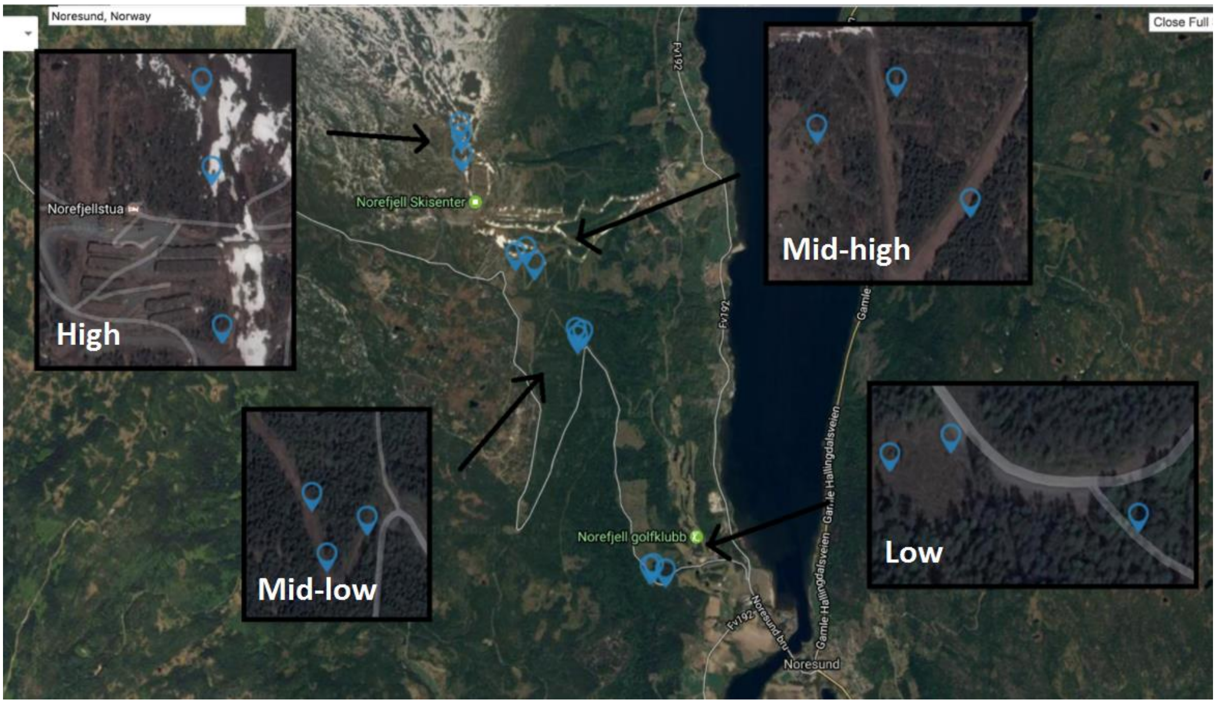

Figure 1: A satellite overview of the fieldwork location at Norefjell. The framed satellite photos show the three study sites at each elevation in more detail. The map was obtained from https://www.freemaptools.com/elevation-finder.htm

At each study site I selected a patch with *Rubus idaeus* and two patches with *Melampyrum*; (one closer than 3 m from *R. idaeus*; hereby referred to as "*Melampyrum* near" and one farther than 10 m away from *R. idaeus*; hereby referred to as "*Melampyrum* far"*)* to assess whether a close-by floral resource hotspot (*R. idaeus*) affected the flower visitation to *Melampyrum* (Fig. 2). At two study sites, one at the elevation mid-high and one at the elevation high, I could not find any *Melampyrum* near, giving in total 22 patches of *Melampyrum* in 12 study sites.

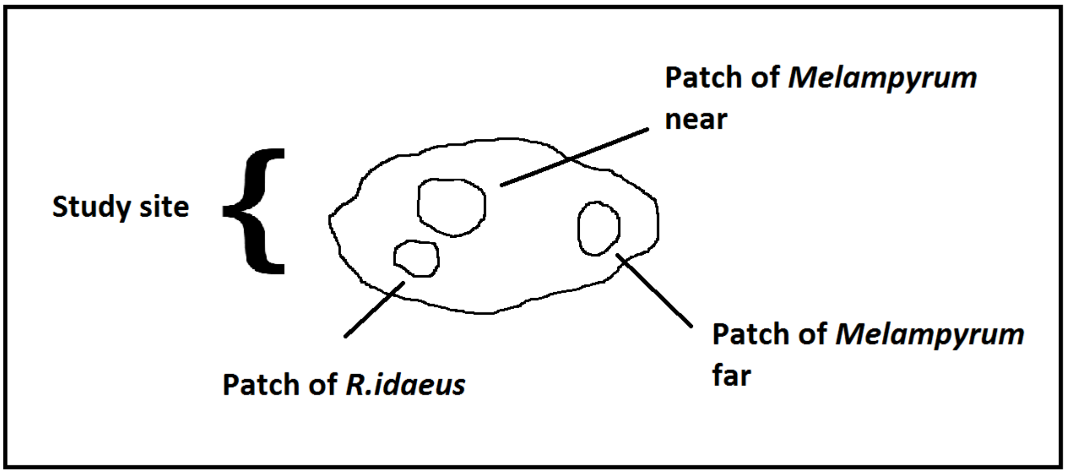

Figure 2: A graphic illustration of a study site with a patch of *Melampyrum* near and a patch of *Melampyrum* far and their relation to the floral hot-spot.(*R. idaeus*)

The mountainside faces south-east, which means that all study sites are exposed to the sun during the day. The study sites were mainly found in forest clearcuts, but some were found in areas with yearly maintenance of ski slopes, or they were next to forest roads with little traffic. Boreal woodland consisting of *Picea abies* dominated the lower elevations while slowly taken over by *Betula pubescens* at the higher elevations. Other surrounding species were *Sorbus aucuparia, Vaccinium myrtillus, V. vitis-idaea* and different ferns. Various herbs like *Potentilla erecta, Geranium sylvaticum, Maianthemum bifolium, Lotus corniculatus, Centaurea montana and Campanula rotundifolia* were also common.

Study species

2.1.1 *Melampyrum pratense* and *Melampyrum sylvaticum*

Melampyrum pratense and *M. sylvaticum* are annual hemiparasitic herbs that belong to the Orobanchaceae family. They are both widespread through most of central and north Europe and grow throughout Norway except in high mountain ranges. They thrive in boreal forest where the soil is acidic and there is accessible shade and water. They extract additional water and nutrients from other plants through haustorial connections between their roots. As they have lower water potential than the host and maintain a high transpiration rate, they are able to translocate resources (Dalrymple, 2007). This high transpiration rate means that they often rely on moist and shady environments and grow close to water sources like lochs, rivers and streams. The need of coverage from canopy usually decreases with altitude. They can use many different hosts, but studies have shown that some are preferred before others. They often prefer woody plant hosts like e.g. *Betula, Quercus, Pinus sylvestris, Sorbus aucuparia,* but also different species of *Vicia* (Smith, 1963) (Dalrymple, 2007). The flowering period of *M. pratense* and *M. sylvaticum* are roughly from June to August and they set seeds from late July to early September. They have zygomorphic flowers with pale yellow corolla which bloom consecutively, each flower for about four days, but with big intraspecies differences dependent of time and place. Solitary plants will for instance have larger horizontal, lateral branches with more flowers, while plants in denser populations have shorter and fewer branches (Winkler & Heinken, 2007). The seed capsule contains 0-4 large seeds with elaiosome as an adaption to dispersal by ants (myrmecochory). Gaps in the forest where ants often build their anthill provide favorable growing conditions for *M. pratense* and *M. sylvaticum.* They are autogamous, but absence of pollinators has been shown to result in lower seed and fruit set (Kwak & Jennersten, 1991).

Pollinators

Bumblebees are common in Norway and they can be found throughout the country, from the coast to the high mountains. There are 35 species registered and 26 of these are social bumblebees that build nest with workers. They have yearly lifecycles where only the fertilized queen overwinters burrowed underground and establishes a new nest and develop a new colony the next spring. The development from egg to adult bumblebee is 3-4 weeks depending on species, food supply and temperature.

Another important group of pollinators in Norway is true flies and where the three families Syrphidae, Muscidae and Empididae has been shown to be the most important (Kevan, et al., 1993) (Totland, et al., 2013) Adult Syrphidae, (Hoverflies) exclusively feed on nectar and pollen from plants (Gilbert, 1981), while Muscidae can be predatory, hematophagous or saprophagous (Malmqvist, et al., 2004). Empididae are small slim flies found in many types of vegetation and are mainly predatory, but some adult may visit flowers for nectar (Courtney, et al., 2009).

Data collection

Flower visitation observations were conducted in 32 days during the period June14th to July 28th between 8am and 8pm. The order of which the study sites were observed during the day was randomized.

I started observing at the lowest elevation where the *R. idaeus* flowering started first. Since the relationship between flower visits to *R. idaeus* and *Melampyrum* was part of the study, most of the observations at a study site was conducted when *R. idaeus* was in flowering. Since *Melampyrum* flowered earlier than *R. idaeus* at all elevations and was in flower until late July when the *R. idaeus* flowering season ended, I did several observations in patches with *Melampyrum* both before and after the *R. idaeus* flowering.

At each study site, the three patches of *Melampyrum* near, *Melampyrum* far and *R. idaeus* itself were observed simultaneously. For each observation, I selected and counted a manageable number of flowers within the patch, ensuring that all flowers could be observed simultaneously so that no visits were missed. The selected flowers were divided further into three sections to gather information about small scale variation between our observations. I observed the selected flowers for 10 min period and recorded all visits to flowers. I defined a flower visit as when an insect was in contact with the flower. Individual pollinators were not taken into account when visitation data were gathered so a single pollinator individual could be recorded several times if it visited several flowers or conducted more than one visit to the same flower. Visits were assigned to taxonomic groups such as "bumblebees", "hoverflies" and "other flies", but because of few flower visits by hoverflies, both groups containing flies were assigned to a group called "flies".

When available, I collected mature seeds from 30 plants from all patches. For each individual plant, the number of capsules and the seeds inside each capsules were counted (For a list of plants, capsules and seeds collected, see Appendix 2).

Enviromental variables

Weather variables (temperature, humidity and wind) were recorded prior to the observations by a hand-held weather recorder. (Weather Hawk SM-28 Skymaster). In addition, I used weather loggers (iButton Hydrocron Temperature/Humidity loggers from maxim Integrated) which measured temperature and humidity every hour. I used linear interpolation between the two recordings closest in time to obtain temperature and humidity measures at each time of observation. The loggers were attached to wooden stakes about 30 cm above ground and one recorder was placed at each of the four elevations. A tinfoil top was used to protect the logger from rain and direct sunlight. I also observed ambient weather conditions during each observation period and categorized it as sunny, cloudy or rainy. I did not do observations in heavy rain, which means that the category "rainy" refers to light rain/drizzle.

Statistical methods

Seed set and flower visits by flies and bumblebees were used as response variables in three separate generalized linear mixed models (GLMM) using the R-package lme4 (Bates, et al., 2015). As both seeds and flower visits represent counts, I used Poisson distribution in both models (Reitan & Nielsen, 2016).

Temperature from Weather Hawk, mean and local temperature from iButton Hydrocron, elevation as category, meters above sea-level (m a.s.l), distance from patches of *Melampyrum* to *R. idaeus*, humidity from Weather Hawk, mean and local humidity from iButton Hydrocon, wind and weather were tested as separate covariates in the flower visitation model (For a full list of covariates included in the model selection, see Appendix 1). In addition, interactions between temperature, humidity and elevation were tested to see if the effect of temperature and humidity changed along the elevation gradient. The interaction between "humidity" and" weather" were also tested because it was important to see if rain had an effect on humidity.

Site ID, observer, time, section ID, sectionNum and ID were included as random factors in the flower visitation model because these are assumed to explain unknown random variations in my observations (For a full list of covariates included in the model selection, see Appendix 1). Number of selected flowers in an observation was included as an offset variable. The flower visitation models were run on bumblebee visitation and fly visitation separately.

Elevation as category, meters above sea-level (m a.s.l.), distance from patches of *Melampyrum* to *R. idaeus*, average fly visitation per 10 min observation and average bumblebee visitation per 10 min observation were tested as separate covariates in the seed set model.

Study sites was included as random factor in the seed set model and the number of capsules from which seed were counted was included as an offset variable.

To find the best model explaining the variation in the different response variables, I ran an automated model selection procedure were all combinations of covariates and predefined interactions were tested. The best model was selected based on the Bayesian Information Criterion(BIC) (Aho, et al., 2014).

All statistical analyses for this thesis were conducted in R version 3.3.2 (R Core Team, 2016).

3 Results

All together 1710 10 min flower visit observation periods were conducted; with 937 observations in patches with *Melampyrum* far and 773 observations in patches with *Melampyrum* near (for which I have two less study sites). In addition 764 10 min observations of flower visits to *R. idaeus* was recorded simultaneously with the *Melampyrum* observations to assess whether flower visitation to *R. idaeus* affected flower visitation to *Melampyrum*. In patches of *melampyrum*, 407 bumblebees visits, 632 fly visits and 10 visits from other insects were recorded as shown in figure 3.

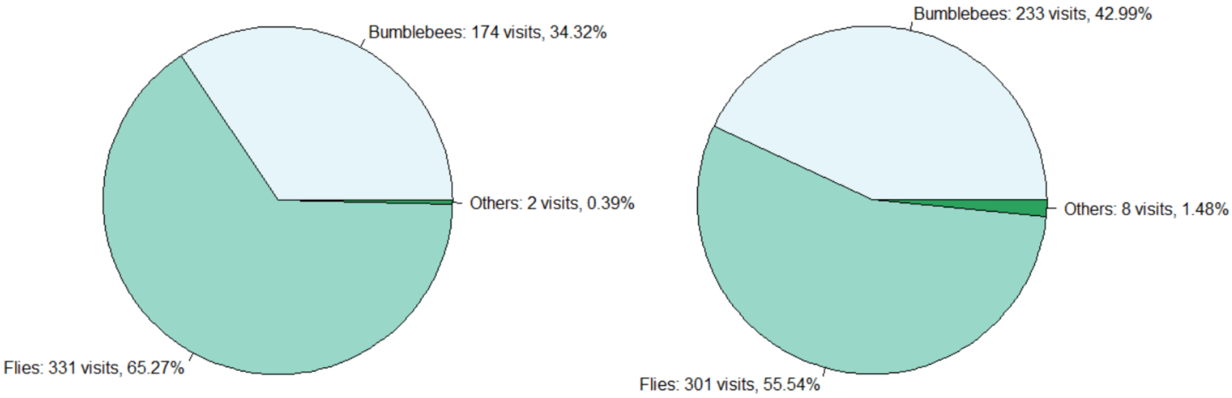

Figure 3: Proportion of flower visits to patches of *Melampyrum* performed by bumblebees, flies and other pollinators, respectively. Left: *Melampyrum* near. Right: *Melampyrum* far. In total 1049 visits.

Flies were the group of flower visitors that conducted the most visits in both type of patches. In patches of *Melampyrum* near, each flower was visited on average 0,0268 times per 10 min by flies and 0,0141 times by bumblebees during 10 min observation. In patches of *Melampyrum* far, each flower was visited on average 0.0157 times by flies and 0.0121 times by bumblebees during a 10 min observation.

When considering only observations for which flower visits were recorded, bumblebees conducted on average 3.164 visits and flies visited on average 2.135 times in patches of *Melampyrum* near during a 10 min observation period. In patches of *Melampyrum* far, bumblebees conducted on average 3,641 visits and flies on average 1,942 visits.

Factors affecting flower visits to *Melampyrum*

3.1.1 Bumblebees

My model selection procedure revealed that the best model explaining bumblebee visits to *Melampyrum* flowers contained no fixed effects. However, the random factors “time” and “ID” were included. This suggests that bumblebee visits to *Melampyrum* were not affected by any of the environmental factors (temperature, humidity, distance to *R. idaeus*, wind or weather). The “time” factor explained 88.4% of the variation and suggests that the two observations conducted at the same time (near *R. idaeus* and further away) were more similar than would be expected by chance. The ”ID” factor explained 11.6% and quantifies the unexplained random variation between *Melampyrum* near and far.

3.1.2 Flies:

The model selection procedure revealed that the best model explaining fly visitation to *Melampyrum* flowers contained the factor “air humidity” calculated from the weatherloggers at every elevation as fixed factor and variation “ID” as random factor. The model shows that increase in air humidity had a negative effect on flower visits (fig.4) (table 1). A relatively small part, 1.8%, of the model was explained by the factor “air humidity”, while 98,2 % were explained by the random factor “ID”, suggesting that most of the variation in flower visits by flies were unexplained random variation.

Table 1: Factors explaining variables chosen for visitation model focusing on flies. Intercept is the random factor “ID”.

	Estimate	Std. Error	Z value	Pr(>|z|)
(Intercept)	-5.4172617	0.0005590	-9691	<0.001
Local humidity	-0.0274972	0.0005302	-52	<0.001

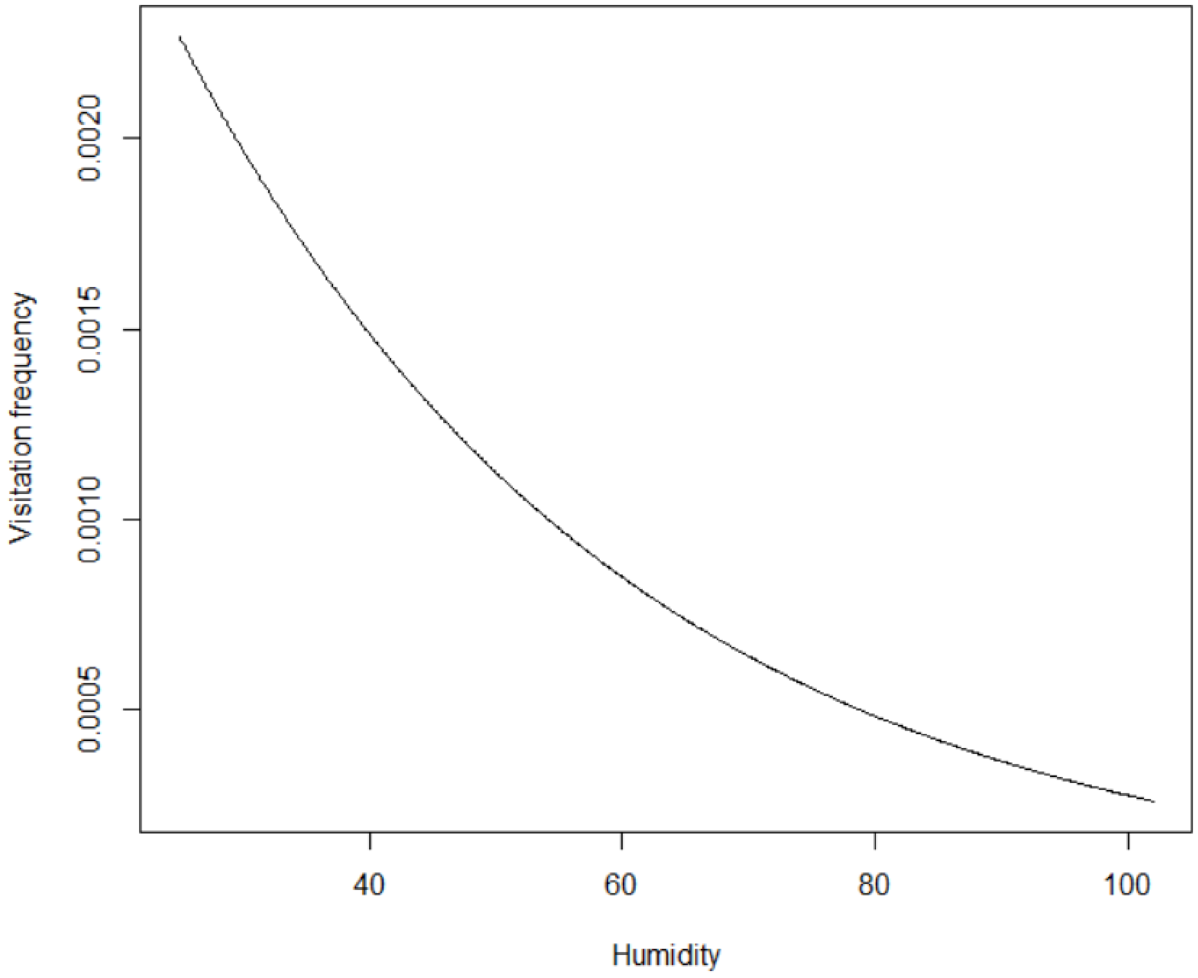

Figure 4: Visitation frequency decrease when humidity increases in the visitation model focusing on flies.

Variation in seed set in *Melampyrum*

I collected seeds from 360 plants on patches of *Melampyrum* far and 266 plants on patches of *Melampyrum* near. Each capsule contained in average 1.40 seeds. The average seed content in capsules at the different elevation was 1.2 seeds per capsule at the lowest elevation, 1.3 seeds per capsule at mid-low, 1.2 seeds per capsule at mid-high and 1.9 seeds per capsule at the highest elevation. The average seed per capsule in *Melampyrum* near and far is seen in (Fig. 5).

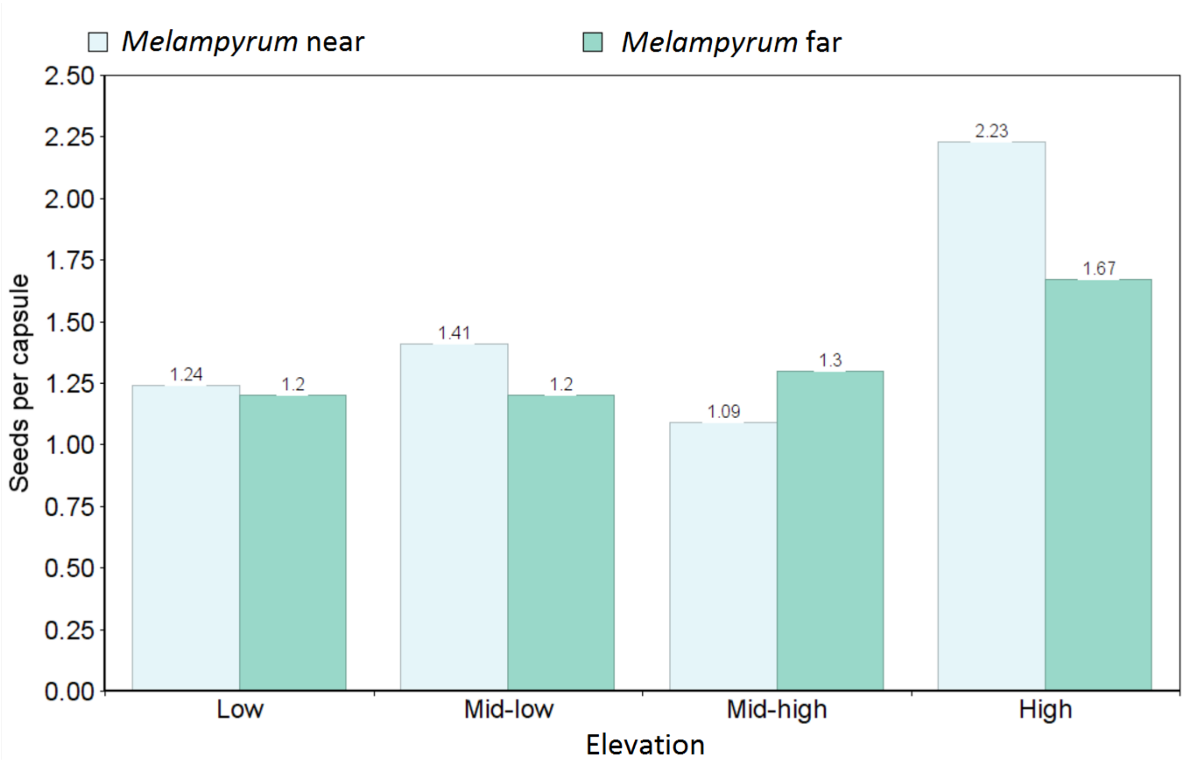

Figure 5: The average number of seeds per capsule with distinction between patches of *Melampyrum* near and far given for elevation as category.

The best model explaining seed set in *Melampyrum*. contained the fixed effects "Near or far from *R. idaeus"*, "Elevation as category", "Average fly visitation", "Average bumblebee visitation" and the random factor "study site" i.e. a factor distinguishing among the 12 study sites (table 2).

Table 2: Factors explaining variables chosen for seed set model. Intercept is the elevation «High»

	Estimate	Std. Error	Z value	P-value
(Intercept)	0.56294	0.05777	9.745	<0.001 ***
Low	-0.39133	0.07378	-5.304	0.001 ***
Mid-high	-0.30659	0.07585	-4.042	0.001 ***
Mid-low	-0.30567	0.06497	-4.705	0.001 ***
Melampyrum near/far	0.13893	0.03542	3.922	0.001 ***
Average fly visitation	-4.52449	1.944757	-2.323	0.05 *

Average bumblebee visitation	2.74345	1.20519	2.276	0.05 *

Melampyrum had higher seed set when growing close to *R. idaeus* and this variable explained 13.5% of the variation in seed set. Elevation as category explained 59.6 % of the variation in seed set and indicated that there is a positive correlation between elevation and seeds per capsule, where "High" had a distinguishable higher seed set than the other three categories. "Mid-high" and "Mid-low" were nearly the same while "Low" had even less seeds per capsule. Average bumblebee visits per flower observed for 10 minutes explained 7.2 % of the variation and had a positive effect on seed set in *Melampyrum*, while average fly visitation per flower observed for 10 minutes explained 9.9 % and had a negative effect on *Melampyrum* seed set. The random factor "study sites" explained 9.8 % of the model suggesting that there is a significant non-linear variation among the different study sites. (Fig.6)

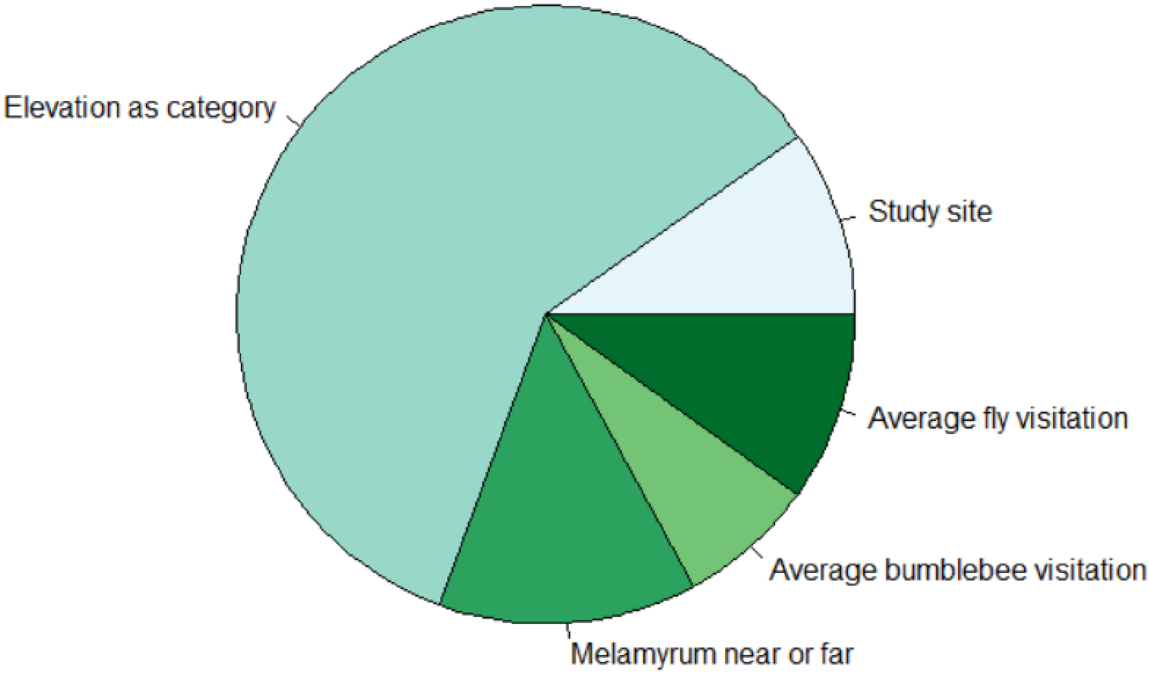

Figure 6: Proportion of variance in seed sets explained by the fixed effect elevation as category, *Melampyrum* near or far, average bumblebee visitation, average fly visitation and the random factor study site, which means variation between study sites.

4 Discussion

In this thesis, I investigated if the pollinator community and visits per flower change along an elevation gradient, if elevation or the number of visits leads to a higher seed set in *melampyrum* and whether presence of *R. idaeus* affect pollination visitation and seed set in *melampyrum*.

The first hypothesis, stating that flower visitation frequency by different groups changed along an elevation gradient was not supported as I found no effect of elevation on visitation frequency by neither bumblebees nor flies. The best model explaining variation in flower visits by bumblebees did not include any environmental variables, while the best model explaining flower visits by flies included only a negative effect of "air humidity". These results indicate that flower visitation frequency is not affected by weather or climate.

The second hypothesis, elevation and number of flower visits will affect the seed set in *Melampyrum*, receive support because there was a positive effect of elevation where the elevation "high" contained on average more seeds per capsules then the three other elevations. It was also a positive correlation between bumblebees visits and seeds per capsules which means that increased bumblebee visitation resulted in more seeds per capsule. Finally, there was a negative correlation between fly visitation and seeds per capsule which means that increased fly visitation resulted in less seeds per capsule.

Proximity to patches of *R. idaeus* did not affect flower visitation, but it did affect seed set to *Melampyrum* giving the third partly support. *Melampyrum* growing in proximity to *R. idaeus* had increased seed set suggesting facilitative

Flower visitations along a 650 M elevation gradient

Bumblebees and flies were the most abundant groups of pollinators at my study sites. This is not surprising, since bumblebees are considered to be important pollinators in Norway (Totland, et al., 2013) and flies is an abundant pollinator group where importance seems to increase with elevation and unstable conditions. (Larson, et al., 2001) (Primack, 1983) (Kudo, 2016).

The observations were carried out along an elevation gradient with 650 M difference between the study sites at the lowest and the highest elevations. The abiotic factors along this large gradient were thought to show enough variation to detect differences in pollinator visitation triggered by fluctuations in abiotic factors as seen in other studies (Malo & Baonza, 2002) (Jiliberto, et al., 2010) (Hoiss, et al., 2012). Surprisingly, even with this large elevation gradient (Average temperature during observations were 4 °C lower at the high elevation compared to the low) (For a list of average temperatures at every study site and elevation, see Appendix 3), none of the factors measured affected flower visitation, except a weak effect of air humidity for fly visitation.

Despite the recent concerns about changed pollinator forage activity as a result of climate warming (Scaven & Rafferty, 2013), no such signal was detected in my data. The flower visitation did not change when temperature decreased at higher elevations and my model selection procedure did not detect an interaction between temperature and elevation which otherwise could have suggested that changes in temperature affected flower visitors differently at different elevations. A plausible explanation for this is that bumblebees and flies in Norway are well adapted to changing weather conditions since a large part of Norway are characterized by a climate that is periodically harsh and highly variable (Deutsch, et al., 2008) (Totland, et al., 2013). Alpine and arctic ecosystems are often considered to be vulnerable and susceptible to climate change because their ecosystems consist of endemic species with poor dispersal abilities (Beniston, 2003). The highest elevation in my study was below the tree line at 856.5 m a.s.l. and it is therefore possible that my study sites did not reach the point of elevation where changes in abiotic factors resulted in changes in flower visitation. Bumblebees and flies were present along the whole elevation gradient which indicate that they can handle different temperatures and the weather conditions. In addition, I did not differentiate between bumblebee species, which means that different species could be visiting flowers at different elevations or temperature. In general, it seemed like the variation in temperature was not large enough to affect the robust plant-pollinator interactions taking place at my study sites.

There was less visitation from flies, when the humidity increased. According to table 5, an increase from 50% to 70 % humidity would lead to exp(-0.027*20) =0.58, which means that when the air humidity reach 70 %, there would be 42 % less fly visits per flower per 10 min then it would be with 50 % humidity. There is no obvious explanation for this, considering that (Devoto, et al., 2005) found that flies often prefer humid habitats, but it could be that the combination of biotic and abiotic factors at Norefjell result in too humid conditions for flies to be active. Increased humidity when it is raining, would usually lower flower visitation (Primack, 1983) (Inouye, et al., 2015) , but neither "weather" or the interaction between "weather" and "local humidity" explained variation in flower visitation.

Wind did not seem to have any effect on flower visitation, which seem plausible since the wind speed was not particularly high. In addition, accurate wind speed was difficult to capture because it was highly unstable during measuring.

The limited extent to which environmental factors where explaining variation in flower visitation indicates that the difference between the elevations are very small. One of the aims of this study was to investigate if there are any signals indicating that climate change might affect plant-pollinator interactions in the future. Neither for bumblebee nor fly visitation I found detectable patterns, therefore one can assume that the flower visitor community to *M. pratense* and *M. sylvaticum* is robust to increased temperatures under future climatic change scenarios. As (Totland, et al., 2013) described in their evaluation of insect pollination in Norway, the pollinators are used to changing conditions and a tough climate. In addition pollinator-plant interactions often have a buffering capacity when abiotic and biotic conditions change. (Potts, et al., 2010)

Plant-pollinator interactions in the wild are very complex and it is impossible to consider and detect all possible influencers and interactions, which could be affecting the system. It is still possible that there are other factors than those recorded here that could affect the system when the climate changes and as the old saying goes: Absence of evidence is not evidence of absence.

Variation in seed set

I found a positive correlation between seeds per capsule and elevation, indicating that there were higher seed set at the highest elevation. This could have been because of more favorable conditions, since *Melampyrum* thrives in cold and wet areas which are moist, but freely draining (Dalrymple, 2007). The study sites at the highest elevation were in steep skislopes, where there was a lot of water streaming from the higher parts of the mountain.

There was a positive correlation between bumblebee visitation and seeds per capsule and a negative correlation between fly visitation and seeds per capsule. This result confirm with other studies suggesting that bumblebees are more efficient pollinators than flies (Goulson, 2003) (Dasgan, et al., 2004) because they show a high flower fidelity (Heinrich, et al., 1977) and are large and hairy which means that they most likely will be in contact with the raised anthers of *Melampyrum* which could brush pollen onto the bumblebees head or thorax (Dalrymple, 2007) when they forage. *Melampyrum* are by several studies considered to be typical bumblebee pollinated species (Jennersten & Kwak, 1991) (Kwak & Jennersten, 1991) (Totland, et al., 2006). Flies on the other hand are not as efficient as bumblebees, they spend a lot of time just "hanging out" inside or on top of the flowers, basking in the sun or using the corollas as shelter (Larson, et al., 2001) (Inouye, et al., 2015). In addition, flies can be small enough to crawl into the corolla without necessarily touching the raised anthers (Carvalheiro, et al., 2014). The pollen that do touch their body is less likely to attach because most flies have very little hair (Wilmer, 2011). The combination of flies being poor pollinators and eating pollen could have lead to decreased seed set as a result of fly visitation.

Effects of *R. idaeus* on *Melampyrum*

Rubus idaeus received on average 0.766 visits per flower per 10 min (Arrian Karbassion *unpublished data*) while *Melampyrum* received only 0.033 visits. This large difference in visitation suggests that *R. idaeus* qualifies as a magnet species because it attracts a lot more flower visitors than *Melampyrum*. I did not however detect any variation in flower visitation between *Melampyrum* near and far, which means that *R. idaeus* did not facilitate or compete for flower visitation for *Melampyrum*. Effects on flower visitation has been shown in other studies e.g (Molina-Montenegro, et al., 2008) where the pollinator service and seed set of the less attractive species *Carduus pycnocephalus* was enhanced when the more attractive

Lupinus arboreus grew nearby and in (Totland, et al., 2006) where the competition between the more attractive *Phacelia tanacetifolia Bentham* and the less attractive *Melampyrum pratense* lead to less flower visitation to the latter, but not to lower seed set. A possible explanation to the lack of facilitation and competition between *Melampyrum* and *R. idaeus* could be bumblebees' high fidelity when they are foraging (Wilmer, 2011). According to the search image hypothesis (Goulson , 2000), pollinators uses search images to look for flowers, which means that they will pass on flowers that do not fit the image they are looking for. This means that if a bumblebee is already looking for *R. idaeus*, it will pass *Melampyrum* and vice versa. Another important factor is the different anatomy between R. *idaeus* and *Melampyrum*. The flowers of *R. idaeus* are white and "open" while *Melampyrum* have tubular zygomorphic yellow flowers. It is more likely that pollinators switch between flowers that have similar morphology (Carvalheiro, et al., 2014). The flower visitation of flies on *Melampyrum* near and far did not change either, which could be explained by the short flying range and low flower fidelity shown by most flies (Inouye, et al., 2015). They usually visit nearby flowers and it is possible that the abundance of flies where the same between patches of *Melampyrum* as in *R. idaeus*, especially since the percentage of fly visitors in *R. idaeus* were very low; 11.34 % compared to bumblebees 87.37% (Arrian Karbassion *unpublished data*).

The number of seeds per capsules varied between *Melampyrum* near and far. It turned out to be a negative effect of distance between *R. idaeus* and *Melampyrum* which means that the flowers growing close to *R. idaeus* had more seeds per capsule than *Melampyrum* far away from *R. idaeus*. This suggests that *R. idaeus* had a facilitating effect on seed set in *Melampyum* that could not be explained by flower visitation. An explanation for the observed pattern could be that *Melampyrum* are hemiparasites and uses *R. idaeus* as a host. The variation in seed set between patches of *Melampyrum* near and far away from *R. idaeus* seemed to be particularly large at high elevations, where the species diversity where lower and there could be less suitable hosts to draw nutrients from.

5 Conclusion

By using the space for time approach, my aim has been to predict how climate change in the future might affect the pollinator community of, flower visitation frequency to and seed set in *Melampyrum*. Simultaneously I have investigated how a floral resource hotspot (*R. idaeus*) might affect flower visitation and seed set in my focal plants.
The pollinator community do not seem to change and flower visitation in *Melampyrum* do not seem to be affected by variation in abiotic factors or elevation. (the climate gradient). This is probably a result of adaption to harsh and unstable environments where high tolerance of different abiotic factors is favored. High elevation seem to have a positive effect on the seed set in *Melampyrum* which could be explained by some of *Melampyrums* abiotic preferences. They are often seen in nutrient poor, cold wet areas which could have been provided by the highest elevation. There was a positive correlation between bumblebee visitation and seed set which could indicate that bumblebees are good pollinators of *Melampyrum*. Bumblebees show high floral fidelity, have hairy bodies and *Melampyrum* physiological traits like zygomorphic tubular flowers and placement of pollen indicate that bumblebees would be suitable pollinators. Fly visitation showed a negative correlation which could be linked to flies eating pollen and their low ability to pollinate due to their small hairless bodies, short flying range and opportunistic behavior. The magnet species *R. idaeus* did not seem to facilitate or compete with *Melampyrum,* because the flower visitation did not change in proximity to *R. idaeus*. The seed set on the other hand, showed a positive correlation close to *R. idaeus*. This might be a result of *Melampyrums* hemiparasitic nature where *R. idaeus* could be a good host.

As a final conclusion, my study suggests that the pollinator community of *Melampyrum* are resistant to future climate change within the extent of climatic variation I have been investigating. Elevation have a positive effect on seed set in *Melampyrum* and bumblebees are probably better pollinators than flies. *R. idaeus* do not effect flower visitation in *Melampyrum,* but it does have a positive effect on seed set.

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Appendix 1

List of covariates tested in the model selection procedures for flower visitation and seed set. In addition to name of the covariates, the left column contain which model the covariates were tested in. If there are no information, the covariate where only tested in the flower visitation model.

Covariates tested in the flower visitation model and the seed set model.	**Definition**
Elevation as category Also used in the seed set analyses	Categorical classification of the elevations; "low", "mid-low", mid-high" and "high".
Elevation as m a. s. l. Also used in the seed set analyses	Numeric measure of the four elevations, in meters above sea-level, for each of the 12 study sites.
Temperature from Weather Hawk SM-28 Skymaster	Temperature from a handheld weather logger measured before each observation period. (*Melampyrum* far and near was observed simultaneously during an observation period)
Local temperature from iButton Hydrocron Temperature/Humidity loggers from maxim Integrated	Temperature from the weather logger placed at each site("low, "mid-low, "mid-high" and "high") interpolatedto the time each observation period started from the two nearest recordings in time (recordings were taken every hour).
Mean temperature from iButton Hydrocron	Average temperature from all the weather loggers at all elevations interpolated to the time each observation period

Temperature/Humidity loggers from maxim Integrated	started from the two nearest recordings in time(recordings were taken every hour).
Air humidity from Weather Hawk SM-28 Skymaster	Air humidity handheld weather logger measured before each observation period. (*Melampyrum* far and near was observed simultaneously during an observation period).
Local humidity from iButton Hydrocron Temperature/Humidity loggers from maxim Integrated	Air humidity from the weather logger placed at each site ("low", "mid-low", "mid-high" and "high") interpolated to the time each observation period started from the two nearest recordings in time (recordings were taken every hour).
Mean humidity from iButton Hydrocron Temperature/Humidity loggers from maxim Integrated	Average air humidity from all the weather loggers at all elevations interpolated to the time each observation period started from the two nearest recordings in time (recordings were taken every hour).
Wind from Weather Hawk SM-28 Skymaster	Wind speed measured by hand held weather logger.
Weather	Categorical classification of weather into three categories; Sunny, cloudy and light rain/drizzle.
linday	Day of the season as a linear variable
lintime	Time of day as a linear variable
"sin1", "cos1", "sin2", "cos2"	Variables that generates daily rhythms in flower visits
"daysin1", "daycos1", "daysin2", "daycos2"	

Melampyrum near and far	Categorical classification containing "*Melampyrum* closer than 3 M from *R. idaeus*" or "*Melampyrum* farther away than 10 M from *R. idaeus*".
Average bumblebee visitation Only used in the seed set analyses	Average bumblebee visitation to *Melampyrum*(total number of flower visits recorded/total number of flowers observed) calculated for all 22 observational units (*Melampyrum* near and far in the 12 study sites)
Average fly visitation Only used in the seed set analysis	Average fly visitation to *Melampyrum* (total number of fly visits recorded/total number of flowers observed) calculated for all 22 observational units (*Melampyrum* near and far in the 12 study sites).
Random effects	
Site ID	Categorical variable distinguishing among the four study 12 study sites
Observer	The person recording flower visitation during an observation
Time	The time the observation was conducted combining date and time of day
SectionID	Observation group including all observations conducted at a specific date and time
SectionNum	Variable distinguishing among the three sections of flowers observed simultaneously on *Melampyrum* near or far at a specific date and time.
ID	The individual observation at a specific time and place(differentiating between *Melampyrum* far and near)
Offset	

Secflowers Only used in the flower visitation model	Total number of flowers observed during an observation (one number per section observed by each observer at each point in time).
Seeds per capsule Only used in the seed set model	Average number of seeds per capsule at either *Melampyrum* near or far at a study site.

Appendix 2

List of the number of plants, seeds and capsules collected at each study site. 2

Elevation	**Study site**	**Plants collected**	**Capsules**	**Seeds**
Low	1 (near)	30	256	263
Low	1 (far)	30	133	151
Low	2 (near)	30	360	374
Low	2 (far)	30	285	272
Low	3 (near)	19	160	265
Low	3 (far)	30	247	370
Mid-low	4 (near)	30	320	477
Mid-low	4 (far)	30	150	204
Mid-low	5 (near)	30	665	980
Mid-low	5 (far)	30	419	479
Mid-low	6 (near)	30	217	276
Mid-low	6 (far)	30	262	295
Mid-high	7 (near)	30	150	152
Mid-high	7 (far)	30	175	289
Mid-high	8 (near)	30	93	109
Mid-high	8 (far)	30	126	146
Mid-high	9 (near)	No seeds collected	No seeds collected	No seeds collected
Mid-high	9 (far)	30	91	100
High	10 (near)	No seeds collected	No seeds collected	No seeds collected
High	10 (far)	30	122	204
High	11 (near)	30	190	368

High	11 (far)	30	169	287
High	12 (near)	7	21	53
High	12 (far)	30	53	87

Appendix 3

List of average temperatures at every study site and elevation.

	Study site 1	Study site 2	Study site 3	Average
Low 1	19,6	20.15	19.73	19.83
Mid-low	15.63	15.57	15.63	15.61
Mid-high	17.21	16.84	16.86	16.97
High	15.15	15.62	15.26	15.37