(Un)safety in numbers?

Spatial variations in plant – pollinator interactions on two volcanic islands

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Master of Science Thesis

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"You just sit and stare and think, and search randomly for new information, and go away and come back again, and after a while the unseen factors start to emerge."

Robert M. Pirsig, *Zen and the Art of Motorcycle Maintenance,* 1974

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Acknowledgements

When I stood at the foot of the M.Sc. hill in May 2020, I was, according to myself, an independent young scientist ready to do research on her own. Stepping now down the other side, it seems appropriate to thank the people without whom this project had not been doable.

First and foremost, a capital letter THANK YOU to my main supervisor, Anders Nielsen. Thanks for all scientific support, but perhaps even more for the encouragement to think freely and to be curious. You create a platform from which students can explore and problem solve independently – that is invaluable. Thank you to my second supervisor, Anne Krag Brysting, for always being ready with clear, wise advices, thorough answers, great pedagogical skills – and contagious interest in botanical life. Any student to cooperate with you is fortunate. To my final supervisor Dave Goulson, thank you for answering long mails in a busy life and for inspiring exploration of scientific communication. To read your numerous histories on plant-pollinator interactions made my study an adventure.

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Stepping now down the other side of the M.Sc. hill, I am convinced that cross field cooperation (i.e. cooperation between adventurers, artists, biologists, sociologists, carpenters, farmers, programmers, writers - and the list could go on) will make projects not only more fun, but also more functional.

Abstract

Habitat fragmentation, causing smaller populations, smaller patches and increased isolation, may affect reproductive success for animal pollinated plants. The general assumptions state that pollinator numbers – and therefore also plant seed set – increase in dense flower aggregations and large patches. Yet, after a certain threshold, an increase in flower number (i.e. increasing patch size or higher flower density) will presumably lead to *decrease* in seed set due to lower relative pollinator visitation. Hence, there may exist an optimal patch size or optimal flower density for every plant species. The aim of this study was to assess spatial variations in plant-pollinator interactions for the herbaceous plant *Viscaria vulgaris*, and, based on the result, evaluate the plants optimal flower density for maximum seed set.

The focal area included two islands in the outer Oslo fiord, southeast Norway. I examined whether flower visitation and seed set of *V. vulgaris* varied between the islands (i.e. on area level), between populations of different flower density within the islands (i.e. on population level) and between patches of different size within the populations (i.e. on patch level). In addition, I analyzed *V. vulgaris* breeding system (i.e. the relative effect of self-pollination, optimal pollination and natural pollination on seed set). This was done by flower treatments (including bagging and hand pollination) and by a germination experiment.

The major finding was the relationship between seed set, legitimate pollinators and plant population density. In dense plant populations, seed set was as expected higher than in sparse. However, seed set in dense populations did not change with increasing visitation frequency, indicating a saturation point for plant reproductive success. For sparse populations, on the other hand, pollinator visitation had a significant, positive effect, and seed set was predicted to surpass that in dense populations for frequencies > 0.2 (bees per flower per min).

My results indicate that *V. vulgaris* optimal flower density depends upon pollinator visitation frequency, and that, *given enough efficient pollinators,* plant reproductive success has potential to be *higher* in sparse than in dense populations. This might be due to elevated levels of geitonogamy in dense populations. My findings highlight the importance of maintaining viable pollinator communities in areas where conservation targeted plant species are sparse or patchily distributed.

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1. Introduction

Pollination, perhaps the best-studied and certainly among the most beautiful interactions in the world, is inevitably driven by self-interest. While plants aim to augment pollen transfer between conspecifics, pollinators forage for maximized nutrition gain to minimal energy cost. In any given landscape, flower visitors will therefore make decisions in how to efficiently exploit the flower rewards present. For example, some flower visitors have adopted a "robbing" forage behaviour, in which primary robbers bite holes in the flower corolla tube to access nectar, and secondary robbers use holes that are already made. In either case, flower reproductive organs are often left untouched, and the nectar robbers will therefore not partake in actual pollination (Irwin et al., 2010). Other "decisions" a flower visitor must make include degree of flower constancy (i.e. how many species of flower to visit in one forage bout), and number of flowers visited within an inflorescence or patch before moving on (Fenster et al., 2004; Willmer, 2011). Clearly, spatial variations (such as plant density and flower patch sizes) will affect pollinator forage behaviour. A change in pollinator forage behaviour will again affect pollen transfer between inflorescences and thus the reproductive success for the plants with which the pollinators interact (Dauber et al., 2010).

For plants that do not self-pollinate (due to e.g. dichogamy in the form of protandry or protogyny), one would anticipate a reduction in pollinator visitation to result in reduced reproductive success. Many plants exhibit a natural, patchy distribution (Watt, 1947). By drawing parallels to the Theory of Island Biogeography, patches of a specific plant species can be viewed as "islands", separated by a "sea" of in-between-vegetation (Jennersten et al., 1983; McArthur & Wilson, 1967; Sodhi & Ehrlich, 2010). In this context, one would expect fragmented or less dense plant populations in general (population level) and small, distant patches in particular (patch level) to receive reduced pollinator visitation due to lower floral display size and reduced attractiveness (Klinkhamer et al., 1989; Ramsey & Vaughyon, 2000). Following this logic, animal pollinated plants in fragmented areas or small patches would achieve a lower seed set than those in dense areas and large patches.

Indeed, several studies have reported reduced pollinator abundance in sparse or fragmented compared to dense flower areas (Jennersten, 1988; Dauber et al., 2010; Nielsen et al., 2012;

Rathcke & Jules, 1993) and in small compared to big flower patches (Dauber et al., 2010; Johnson et al., 2012; Nielsen & Ims, 2000; Sih, 1987). At the same time, there seems to be a general agreement that insects forage *more efficiently* (i.e. visit a higher proportion of available flowers) in small compared to large patches (e.g. Brys et al., 2008; Goulson 2000). Also, while an increase in pollinator abundance (as seen in dense areas and big patches) often is coupled with increase in plant seed set (e.g. Aguilar et al., 2006; Dauber et al., 2010; Jennersten, 1988), seed set might be constrained in large patches or dense plant populations due to intra-specific competition for pollinators (Hegland, 2014; Ward et al., 2013), for resources (Zhao et al., 2014), or elevated levels of geitonogamy (i.e. self-pollination between flowers on the same plant individual) (Finer & Morgan, 2003; Klinkhamer & Jong, 1993; Ramsey & Vaughyon, 2000). Indeed, high levels of geitonogamy may lead to a "saturation point" in plant reproductive success, above which increased visitation frequency does not result in increased seed set (Hegland, 2014).

Uniting the theories, general pollinator abundance (and therefore also seed set in animal pollinated plants) seem to increase with increasing patch size or flower density until a certain threshold (i.e. a concentration effect (Hegland, 2014)). Above that threshold, an increase in flower number (i.e. increasing patch size or higher flower density) will lead to *decrease* in seed set due to decrease in relative pollinator visitation (that is, although insect number may continue to increase, each insect will visit a lower proportion of the available flowers), increased intraspecific competition between plants for pollinators and resources (i.e. a dilution effect (Brys et al., 2008; Hegland, 2014; Johnson et al., 2012; Nielsen et al., 2012)), and higher levels of geitonogamy (Klinkhamer & Jong, 1993).

Ratche (1983) proposed that there exists an "optimal patch size" for every plant species. Similarly, there might exist an optimal number of flowers or "optimal flower density" (figure 1) for a given plant species, both within a patch (number of inflorescences) or within a population (number of patches) (see e.g. Hegland, 2014; Klinkhamer & Jong, 1993). Naturally, the "optimal" patch size or flower density for one particular plant species will vary between areas, depending upon e.g. the presence of other plant species and of available, efficient pollinators. Presence of efficient pollinators may in turn (and somewhat like a catch-22) depend upon patch size of flower density, and probably differ between groups or species.

These theoretical variations underline the complexity of plant-pollinator interactions, as well as the limitations of generalization (Hobbs & Yates, 2003).

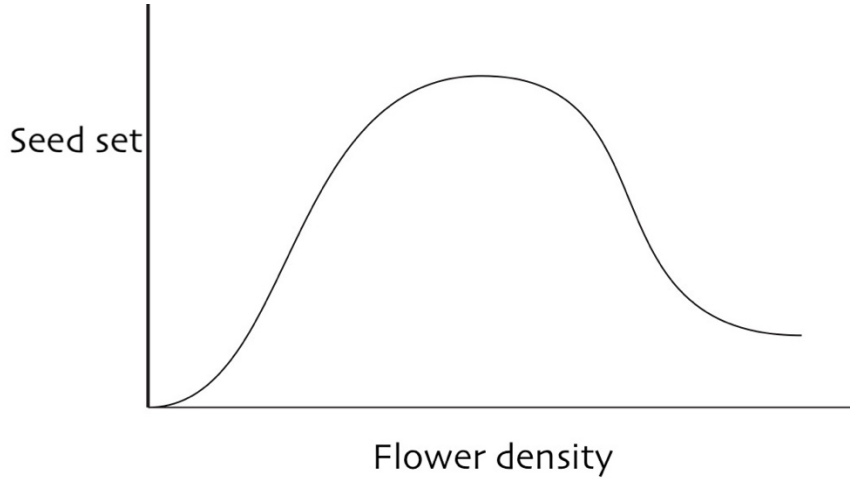

Figure 1: Schematic and hypothetical graph representing *seed set* as a function of *flower density*. Optimal flower density (ODF) marks the top point. To the left of the ODF represents concentration effect (i.e. increasing seed set due to increasing floral display) and pollinators competing for plants. To the right of the OFD represents dilution effects (i.e. reduced seed set as flower density increases, and pollinators visit proportionally fewer flowers) and plants competing for pollinators.
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Today, fragmentation of natural landscapes is considered among the most serious threats to biodiversity (Sodhi & Ehrlich, 2010). For plants, fragmentation do in general lead to smaller populations, smaller patches, increased isolation and hence an overall decline in abundance (Hobbs & Yates, 2003). Such changes might directly affect the reproductive success of the plants in question, but also indirectly the pollinators with which they interact. Conversely, a change in frequency or abundance of pollinators can indirectly affect plant reproductive success, due to e.g. reduced pollen transfer (Dauber et al., 2010; Nielsen & Ims, 2000; Sodhi & Ehrlich, 2010). Insight in how plant-pollinator interactions vary on different spatial scales (e.g. between areas, populations and patches) is crucial for future and present conservation schemes. Such insight should, ideally, be based on analysis seen from both the plant and the pollinators perspective (Nielsen et al., 2012; Nielsen & Ims, 2000), and should compare similar systems experiencing different levels fragmentation (Sodhi & Ehrlich, 2010).

Islands, offering closed or nearly closed study systems, represent excellent areas for conducting experiments (McArthur & Wilson, 1967). For Eløen and Sletter, two islands in the outer Oslo fiord, much research effort has been focused on birdlife and floristic mapping

(Kasbo, 1981; Strandli, 1990). The insect fauna and plant-pollinators interactions remain, however, largely unknown. In the present study, I use Eløen and Sletter as study systems to assess seed set and flower visitation of the sticky catchfly, *Viscaria vulgaris* (synonyms: *Lychnis viscaria*, *Silene viscaria).*

On both Islands, *V. vulgaris* is found in populations of different flower density and in patches of different size. The plant is visited by both legitimate pollinators (such as *Bombus lapidarius*, *B. pascuorum* and *B. hortorum*), primary nectar robbers (such as *B. terrestris*, *B. lucorum* and *B. hypnorum*) and secondary nectar robbers (mainly *Apis mellifera*)(Jennersten et al., 1988) . First, I assess how seed set (as a measure of plant reproductive success) and frequency of flower visitation vary between islands (area level), between populations of different flower density (population level) and between patches of different size (patch level). Further, I statistically examine the impact of legitimate pollinators and nectar robbers on *V. vulgaris* seed set, and whether the effect of pollinators vary between different spatial scales (i.e. area, population or patch level). Also, I investigate pollinator dependency and potential pollinator and resource limitation of the focal plant species by hand pollination and bagging experiments. Finally, I conduct an experiment on seed germination.

Specifically, I ask the following questions:

1. Does the frequency of different *V. vulgaris* flower visitors vary between
   a) islands, b) populations and c) patch size?
2. Is there a variation in *V. vulgaris* seed set between
   a) islands b) populations and c) patches?
3. Does the effect of pollinators on seed set vary between
   a) islands, b) populations and c) patches?

   And, finally,
4. Can any conclusion based on my findings be drawn in terms of optimal flower density for *V. vulgaris?*

2. Methods

2.1. Study system

2.1.1. The area

The field work was conducted on Eløen (synonym: Eldøya or Eløya) and Store Sletter (hereby Sletter), two volcanic islands in the outer Oslo fiord, in Moss and Råde municipalities, in Viken county, southeast Norway. The islands are one kilometre off the coast, with Eløen approximately two kilometres north of Sletter (figure 2).

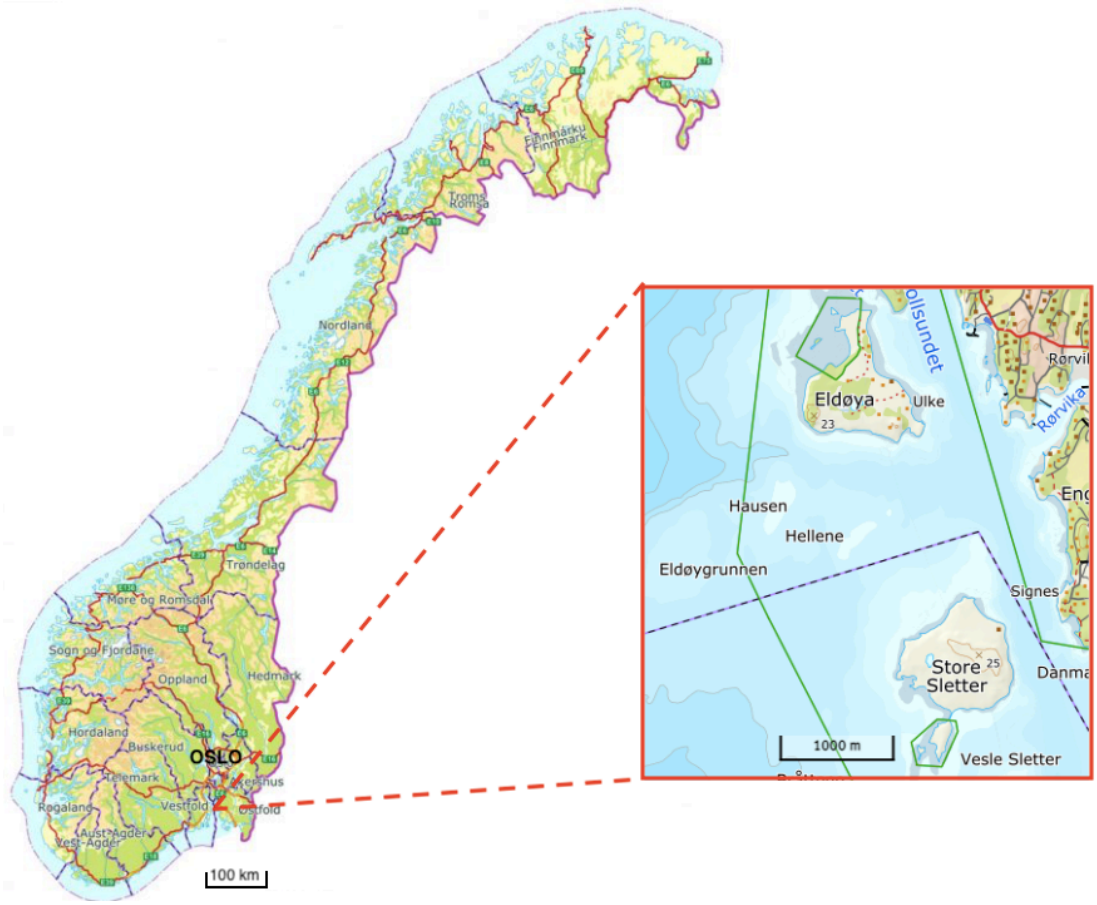

Figure 2: Eløen and Sletter are two volcanic islands in the outer Oslo fiord, in Moss and Råde municipalities, in Viken county, southeast Norway. Modified from Norgeskart.no.

Eløen and Sletter are approximately 0.7 and 0.6 km^2 in size. The islands´ climate is characterized by dry and windy conditions, and the soil is nutrient poor. A great part of both islands´ surface areas consist of grazed, dry meadows, which support an impressive diversity of herbaceous plants (Kasbo, 1981; Strandli, 1990; Strandli et al., 2002).

The two islands differ substantially in landscape complexity. First, while Sletter is practically free for trees and shrubs (appendix B: figure B.1 and B.2), Eløen is characterized by overgrowth and reforestation. This is partly due to difference in grazing pressure. While Sletter has been consistently and often intensively grazed by sheep and cattle for several hundred years, grazing was reduced on Eløen after the second world war. Today, Sletter is grazed throughout the season. As for Eløen, animals are restricted to the S part of the island until August, after which they are allowed also on the N part. Second, while people have inhabited Eløen for more than 400 years, there has never been settlements on Sletter. On Eløen, cultivation of land (including e.g. traditional mowing of meadows and cultivation of fruit trees, but never use of fertilizer) has occurred on a modest scale and in direct connection with individual houses. Today, there are eleven cabins on the island, as well as small woodlands, pine- and fruit trees. On Sletter, by contrast, there are no houses and no trees. Yet small amounts of fertilizer have been applied to the island, which probably is the reason for its somewhat poorer flora and higher number of grass species as compared to Eløen. On Eløen, the peculiar mix of grazed dry meadows, rose bushes, junipers and other shrubs as well as pines, small woodlands and fruit trees creates a landscapes that today is found few other places in Norway (Kasbo, 1981; Strandli, 1990; Strandli et al., 2002) The island can likely be considered as "semi natural grassland" (i.e. meadows and pastures that are not intensely cultivated or fertilized). Semi natural grassland typically possess remarkably high small-scale species richness (Eriksson et al., 2002; Kull & Zobel, 1991), particularly on poor soils (Wehn et al., 2020). Somewhat higher abundance and diversity of species could therefore be expected at Eløen compared to Sletter.

2.1.2. The plant

Among the flowering plants dominating Eløen and Sletter in early summer is the sticky catchfly, *V. vulgaris. V. vulgaris* is a perennial herb in the carnation family *Caryophyllaceae,* with flowering season from mid-May to June (Jennersten & Nilsson, 1993; Stenberg & Mossberg, 2018). The plant is abundant in the lowlands of north and central Europe, thriving on sandy or rocky, calcium poor soil in sunny and open habitats.

The flowers of *V. vulgaris* are pentamerous with dark pink petals and a corolla depth of approximately 12 mm. At the base of the corolla tube, *V. vulgaris* produce nectar 24 h a day (Jennersten, 1988a). It normally reaches a height of 30 to 40 cm. The individuals of *V. vulgaris* form distinct tussocks consisting of one to 150 flower stalks, hereby inflorescences. Several tussocks commonly form distinct patches, consisting of a few to several thousand individuals, and several patches make up a population (figure 3). Each inflorescence produces one to approximately 50 flowers gathered in clusters arranged in a cylindrical spike. The flowers within each cluster can be classified into orders depending on their relative position (Jennersten, 1991; Nielsen & Ims, 2000) (figure 4).

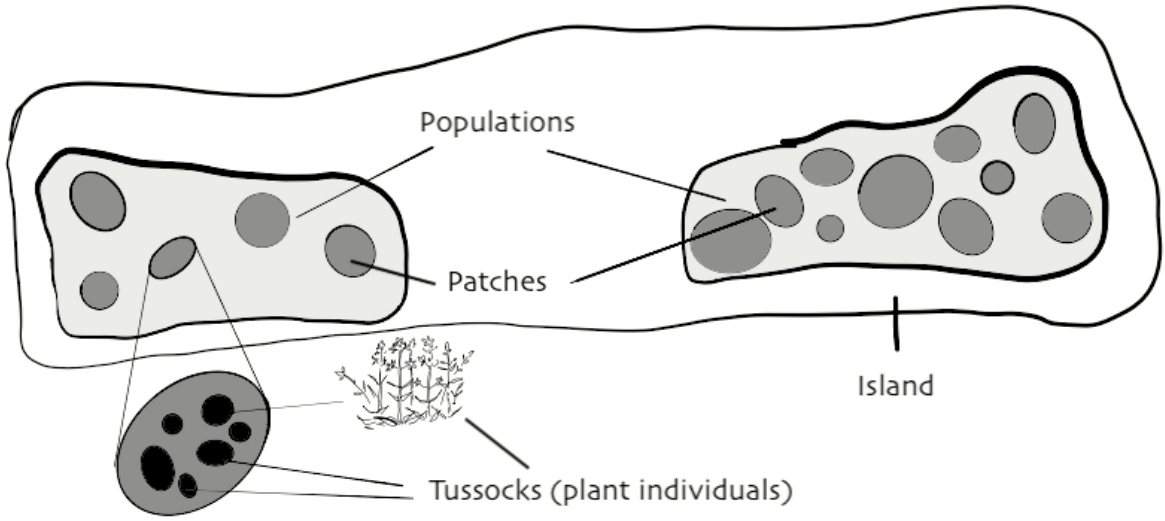

Figure 3: Schematic representation of *V. vulgaris* growing forms. Plant individuals form distinct tussocks consisting of one to 150 inflorescences. Several tussocks form distinct patches, and several patches make up a population. In the present study, one dense population (right) and one sparse population (left) was found on each island. Modified from Nielsen & Ims (2000).

V. vulgaris flowers are protandrous, i.e. the male part matures before the female part. First, ten stamens in two sequenced whorls of five will become mature (appendix B: figure B.3). The anthers release pollen for about two days, succeeded by five stigmas becoming receptive (appendix B, figure B.4) (Jennersten et al., 1988). Following fertilization, *V. vulgaris* ovaries develop into capsules with up to approx. 500 seeds, with highest seed set typically found in 1st order flowers (Jennersten, 1991). Protandry reduces but does not inhibit self-fertilization

(Jennersten, 1988) , meaning that both auto- and allogamy might result in seed development. The seed set (i.e. number of pollinated seeds per available ovules) does, however, decrease significantly by self-fertilization (Jennersten et al., 1988). At maturity, capsules of *V. vulgaris* open and release the developed seeds (Nielsen & Ims, 2000).

Figure 4: *V. vulgaris* Inflorescence *(left).* Schematic representation of *V. vulgaris* inflorescence (right). Height positions (1, 2, 3, 4) for flower clusters, and order (I, II, III) for each flower within the cluster is marked. Modified from Jennersten (1991) and Nielsen & Ims (2000).

2.1.3. The pollinators

Although *V. vulgaris* produces nectar 24 h/d, 90% of the flower visits are diurnal (Jennersten, 1988). Very broadly, the *V. vulgaris* visitors can be divided into three groups: nectar feeders, pollen feeders and seed predators (Jennersten et al., 1988).

Nectar feeders include various species of Lepidoptera and Hymenoptera and can again be divided into "*legitimate*" and "*robbing*" visitors. Legitimate visitors are insects with proboscis > 10 mm – i.e. insects that can reach the nectar at the base of the ovary. Legitimate visitors include medium- and long tongued bumblebees (such as *Bombus hortorum, B. pascuorum, B. lapidarius)* and several species of diurnal butterflies and nocturnal moths, particularly species of the Sphingidae family (Jennersten et al., 1988). Nectar robbing visitors include primary and secondary robbing insects with proboscis < 10 mm. Primary robbers (typically short tongued

bumblebees such as *B. terrestris, B. lucorum* and *B. hypnorum*) bite holes at the bottom of the corolla to access the nectar, and consequently do not touch the sexual parts of the flower. Secondary robbers (typically the honeybee *Apis mellifera*) use the holes already made to access the nectar ((Jennersten et al., 1988), *pers obs.* Henninge Torp Bie). According to Jennersten et al. (1998), nectar robbing has little or no negative effect on *V. vulgaris* seed set. Pollen feeders include honeybees and common species of bumblebees, as well as various species of the order Coleoptera and Diptera. Pollen feeding insects do in general only visit flowers in male stage. Finally, seed predators include night active, ovipositing insects whose larvae feed on *Viscaria* seeds, such as the beetle *Sabinia viscariae* and three moth species (*Hadena confusa*, *Perizoma hydrata* and *Coleophora graminicolella*).

Long tongued species of Hymenoptera and Lepidoptera are probably the most important pollinators of *V. vulgaris*, as they legitimately visit flowers in both male and female phase and hence partake in pollen transfer. Among these, long tongued bumblebees are likely the overall most efficient. They forage systematically in that they typically start at the bottom flowers and work upwards along the flowering stalks, visiting several flowers per plant, several plants in a patch, and transferring considerable amounts of conspecific pollen grain as they work. (Jennersten, 1988b; Jennersten et al., 1988; Jennersten & Nilsson, 1993). Butterflies, on the other hand, forage "sporadically": they typically transfer less pollen and visit fewer flowers in one spot than what do bumblebees (Jennersten, 1988a). Yet, while bumblebees normally forage within a few hundred metres of the nest, butterflies are drifters and typically travel longer distances. They may therefore play a valuable role in long distance cross-pollination of *V. vulgaris* (Berge et al., 1998; Jennersten, 1988; Jennersten, 1988b; Jennersten & Nilsson, 1993). Moths, finally, may participate in nocturnal pollination, but are probably more important as *V. vulgaris* pollinators in areas where summer nights are longer than in the Scandinavian countries (Jennersten, 1988b; Jennersten et al., 1988).

2.2. Study design and data collection

2.2.1. Selection and description of study system

Selection of study system was done the 18th of May 2020 and involved surveying Eløen and Sletter for *V. vulgaris*. On both islands, *V. vulgaris* was found in *dense* and *sparse* populations.

A *dense population* was operationally defined as a population with less than 10 m between distinct patches and *sparse* as a population with 25 m or more between patches (see figure 3 for schematic representation of patches and populations). On Eløen, the dense population was found in the S/SW part of the island and the sparse in the N part. On Sletter, the dense population was found in the N part of the island, and the sparse in the mid-S part.

A total of 30 "sites" were selected on the two islands combined, including 20 sites (hereby site 1 – 20) on Eløen and ten (hereby site 21 – 30) on Sletter (figure 5). Each site included a patch consisting of one or more tussocks of *V. vulgaris*. On both islands, half of the sites belonged to the dense and half to the sparse population of *V. vulgaris.* For each site, the patch was categorized as small (S), medium (M) or large (L) defined after number of flowering individuals (i.e. tussocks) not more than five meters apart. Small patches were defined as < 8 tussocks, medium patches as 8 – 15 tussocks and large patches as > 15 tussocks (appendix B, figure B.5 and B.6) (Nielsen & Ims, 2000).

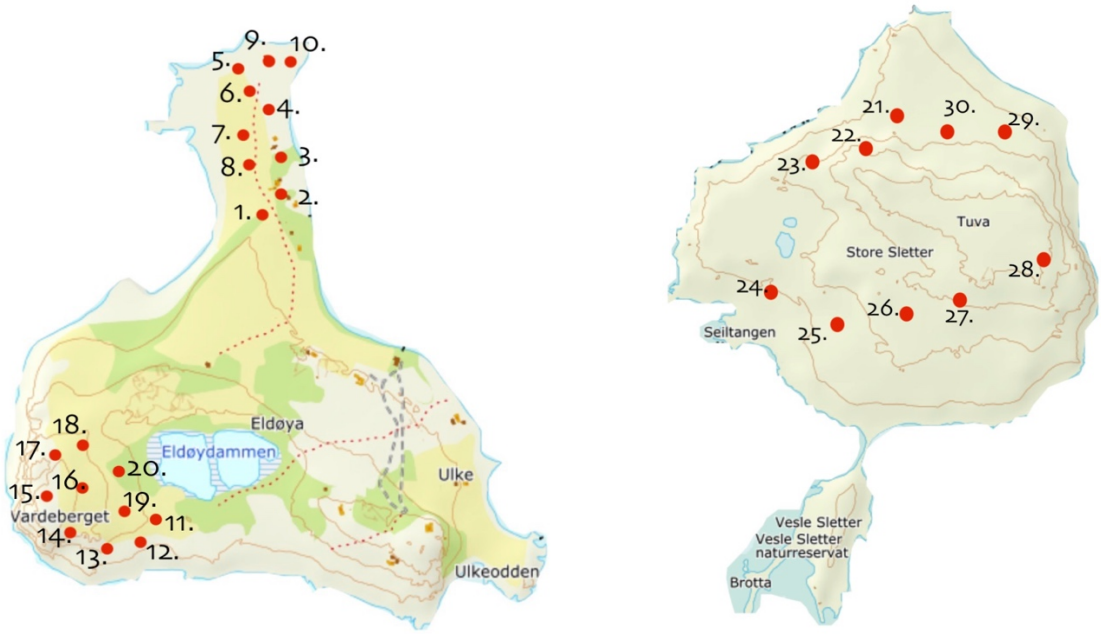

Figure 5: Approximately placement of site 1 – 20 (Eløen, left) and site 21 – 30 (Sletter, right). On Eløen, site 1 – 10 (Eløen N) are in the sparse population and site 11 – 20 (Eløen S – SW) in the dense population. On Sletter, site 21 – 23 and 29 – 30 (Sletter N) are in the sparse population and 24 – 28 (Sletter mid- S) are in the dense population. Green colour symbolizes forest, yellow shrubs and meadows, beige open areas and blue water. Modified from Norgeskart.no.

2.2.2. Functional grouping and observation of flower visitation

In order to assess spatial variation in flower visitation, *V. vulgaris* flower visitors were observed and recorded on Eløen and Sletter. The observations were done from the end of May and until *V. vulgaris* flowering was over (i.e. when most inflorescences were withered, and the majority of the flower visitors had switched to other forage plants). At Eløen (site 1-20), each site was observed nine times (28.05 – 31.05, 02. – 03.06, 06.06, 08. – 09.06) and at Sletter (site 21 – 30) each site was visited four times (03. – 04.06, 06.06, 10.06).

Before initiating an observation, number of inflorescences belonging to one tussock at the study site and number of flowers per inflorescence were counted. Number of inflorescences varied from ten to fifty, depending upon insect activity (i.e. if a high number of insects were present, a lower number of inflorescences were observed to ensure precise counting). If < 30 inflorescences were observed, all individual flowers were counted. If 30 or more inflorescences were observed, number of flowers per inflorescence was for simplicity calculated as the average of 30 inflorescences randomly picked from the tussock. Flower visitation was recorded for 10 min per site. On 10.06, however, observations were recorded for 5 min only, due to approaching bad weather. All days, a visit to one single flower was counted as one visit. Hence, the same insect could account for several visits to the same inflorescence and to other inflorescences.

Visitors were divided into three functional groups according to their forage behaviour on *V. vulgaris*: legitimate pollinators, primary nectar robbers and secondary nectar robbers. Group one, legitimate pollinators consisted of bumblebees with proboscis > 10 mm, and included *B. hortorum, B. pascuorum* and *B. lapidarius*. The latter might actually have proboscis < 10 mm (Goulson & Darvill, 2004; Sowig, 1989), but was grouped as a legitimate pollinators in agreement with Jennersten (1988), and as neither queens nor workers were observed nectar robbing in the course of the fieldwork. Group two, primary nectar robbers, consisted of bumblebees with proboscis < 10 mm and included *B. terrestris, B. lucorum, B. hypnorum.* Whether the latter actually function as a primary or as a secondary robber is unclear, as its mandibles may be too soft to penetrate flower petals (*pers. Comm*. Dave Goulson). However, due to relatively short proboscis length (Crowther et al., 2014; Jennersten et al., 1988), *B. hypnorum* was grouped together with the other short tongued bumblebees, and therefore

considered a primary robber Finally, group three, honeybees (*A. mellifera*), were assessed as secondary nectar robbers (figure 6).

Figure 6: Flower visitors were divided into three functional groups: legitimate pollinators (i.e. bumblebees with proboscis > 10 mm, right), primary nectar robbers (i.e. bumblebees with proboscis < 10 mm and robust mandibles, middle) and secondary nectar robbers (honeybees, i.e. bees with proboscis < 10 mm and soft mandibles, left).

From the observation of flower visitation, two different measures of site attractiveness were estimated: 1) number of flower visits for given site at given date, and 2) average frequency of flower visitation for the given site over all days of observation. For 1), flower visits for each functional group was used as the response variable in the statistical analysis of flower visitation models. Here, number of flowers observed (= number of individual flowers on observed inflorescences) * duration (min) of observation were included as offset variable (see part 2.3.1) (Reitan & Nielsen, 2016). For 2), average visitation frequency for one site was calculated as:

1. $$\frac{\sum_{i=1}^{n} \frac{Pol1i,\ Pol2i,\ Pol3i}{Fi}}{n}$$

where i = field day ($n \mid 1 \leq n \leq 14$), $Pol1_i$ = legitimate pollinator observations at field day i, $Pol2_i$ = primary robber observations at field day i and $Pol3_i$ = secondary robber observations at day i. F_i = number of flowers observed at day i. Separate estimates for average flower visitation were done for the three groups of pollinators.

Average flower visitation was used as a covariate representing visitation frequency of the different functional group in the seed set model. In addition to flower visitation, temperature at each observation was recorded with a handheld digital thermometer.

2.2.3. Assessment of spatial variation in seed set

To assess spatial variation in *V. vulgaris* seed set, three *V. vulgaris* flower capsules were collected from each site at Eløen and Sletter (site 1 – 30) between the 10th and 19th of June. In this time period, capsules were fully developed but not yet open. Only flower capsules resulting from 1st order flowers were collected (figure 4) (Nielsen & Ims, 2000). At each site, the collected capsules belonged to different inflorescences, but the same tussock.

Following collection, the capsules were dissected, and seeds and ovules counted. For each three capsules belonging to the same site (e.g. all capsules from site 7), number of seeds and ovules were counted collectively and classified into five different categories under a stereo microscope. The categories were as follow: type I: *pollinated* (healthy, big seeds), type II *possibly pollinated dark* (healthy, but small seeds), type III: *possibly pollinated light* (healthy, but small and bright coloured seeds), type IV: *aborted* (clearly unhealthy, crushed seeds) and finally type V: *unpollinated* (tiny, undeveloped ovules) (figure 7). The mean value of the three capsules from the same site was used for statistical analysis (Jennersten & Nilsson, 1993). The three first categories (*pollinated* and *possibly pollinated light* and -*dark*) were all considered "pollinated seeds" in the statistical analysis (see part 2.2.5., germination experiment).

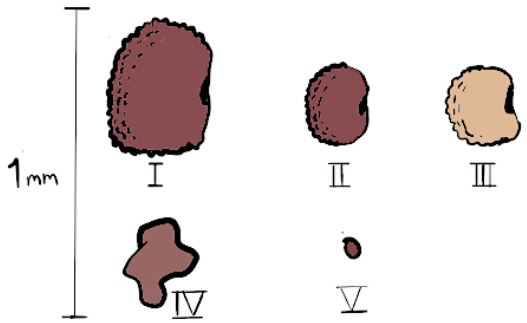

Figure 7: Schematic representation of the seed/ ovule categories (I – V) 1: *pollinated*, II: *possibly pollinated dark*, III: *possibly pollinated light*, IV: *aborted*, V: *unpollinated.* Type I – III were considered pollinated seeds in the present study.

2.2.4. Plant breeding system and flower treatments

To assess *V. vulgaris* breeding system (i.e. the relative effect of self-pollination, optimal pollination and natural pollination on seed set), four different flower treatments were conducted for each Eløen site (i.e. site 1 – 20) between 26th of May and 7th of June. The treatments were B (bagging), Pi (hand pollination and bagging), Po (hand pollination and unbagged) and F ("free", or naturally pollinated). For all flower treatments, only 1st order flowers were chosen (figure 4) (Nielsen & Ims, 2000). Developed capsules were collected between 10th and 19th of June.

To assess the effects of self-pollination, *V. vulgaris* flowers in bud stage were "bagged", i.e. excluded from flower visitation (treatment B). The bagging was done between the 26th and 27th of May, when many tussocks were still in bud stage, and by the means of sheep-proof cages. The cages were built in dimension 0.5m x 0.3m x 0.3m, with angular legs so that they could be hammered into the ground (figure 8, appendix B: figure B.7 and B.8). Each cage was completely covered in mosquito net. From each bagged tussock, three flowers from different inflorescences were marked for later recognition and thereafter left untouched.

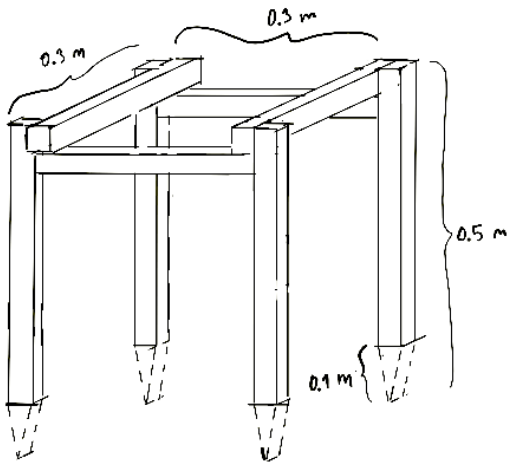

Figure 8: To assess the effects of self-pollination, cages covered in mosquito net (dim. 0.5 x 0.3 x 0.3 m) were put over *V. vulgaris* tussocks in bud stage. The lower parts of the cage legs were made angular so that they could be hammered into the ground.

To assess the effect of optimal pollination and whether the cages negatively affected seed set, three flowers were hand pollinated inside the cage (treatment Pi, pollination inside) and

three flowers outside the cage (treatment Po, pollination outside) at each site between the 28th of May and the 7th of June. Each group of three flowers were from the same tussock but different inflorescences. For each flower, the five stigmas were pollinated with pollen from the anthers of three different flowers belonging to three different tussocks growing more than five meters away (figure 9). The pollination was done by gently brushing the anthers on the stigma until the stigma became grey-blue (as the colour of the pollen). A total of 120 flowers were hand pollinated across the island.

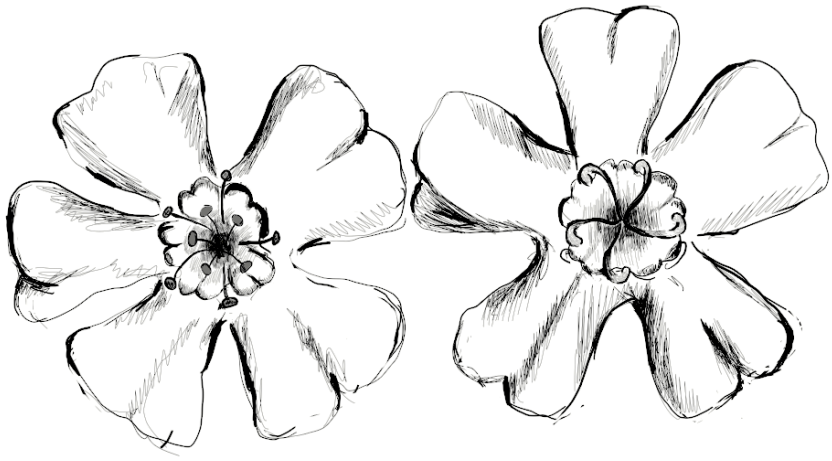

Figure 9: *V. vulgaris* in male (left) and female (right) stage. In male stage, ten stamens in two sequenced whorls of five will become mature. Two days later, the anthers are succeeded by five receptive stigmas. When hand pollinating, pollen from the anthers of three different flowers belonging to three different tussocks growing more than five meters away was gently brushed on the five stigmas.

Finally, to assess the effect of natural pollination (and hence whether the plants experienced pollinator limitation), three flowers outside the cage were marked and then left untouched (treatment F, "free") at each site. These were the same flowers as used to assess spatial variations in seed set (part 2.2.2).

When developed, capsules resulting from the different flower treatments were collected. Succeeding collection, the capsules were dissected, and seeds and ovules counted and categorized following the same procedure as described in part 2.2.3. Again, seeds and ovules from the three capsules belonging to the same treatment at the same site (e.g. all capsules resulting from B treatment at site 7), were considered collectively and the mean value was used for statistical analysis.

2.2.5. Germination experiment

To examine germination success for different categories of seeds and ovules, a germination experiment was conducted on seed type I- IV (i.e. on *pollinated*, *possibly pollinated* (both dark and bright) and *aborted* seeds) (figure 7). For each category, a total of thirty seeds were collected from three different sites (site 1, 5 and 15). Groups of ten seeds belonging to one category were placed in 90 mm petri dishes. The seeds were placed on filter paper moistened with 5 ml distilled water. The petri dishes where enclosed and kept in a growth cabinet in 4 degrees and dark conditions for 2 weeks for stratification. Following, the petri dishes where kept in a growth cabinet with 18 h illumination per day and 20 degrees. Germination was assumed when the radicle emerged (Whittington et al., 1988).

2.3. Statistical analysis

All statistical analyses were done in R version 3.6.3. via Rstudio, version 1.2.5001. for Mac OS X (RStudio Team, 2020; R Core Team, 2020).

To examine how flower visitation and seed set varied with spatial (and non-spatial) factors, a Generalized Linear Mixed Model (GLMM) approach was applied. Based on the collected data, four models were made. These included three models explaining the variation in flower visitation conducted by legitimate pollinators (M1), primary nectar robbers (M2) and secondary nectar robbers (M3), and finally, one model explaining variation in seed set (M4).

First, each model was generated by using the ´*glmer*´ function from the ´lme4´ package, assuming a Poisson distribution (Bates et al., 2015). Next, the models were tested for over-dispersion (i.e. variance greater than mean) by using the *´dispersion_glmer´* function from the ´blmeco´ package (Korner-Nievergelt et al., 2015). As the output was not between 0.75 and 1.4 for any model, overdispersion was detected in every case. Followingly, a negative binomial distribution was assumed and the ´*glmer.nb*´ function in R from the ´lme4´ package was applied (Bates et al., 2015).

To identify the models that best explained variance for the different response variables, a backward selection procedure was applied using the Akaike Information Criterion (AIC) as model selection criterion. Initially, a full model was made for each response variable, including all fixed effects. Fixed effects included in the full models for M1 – M3 were *Island,*

Population, Patch Size and *Temperature*, and for M4, *Island, Population, Patch Size*, *BumblebeeLegFrq*, *BumblebeeRobFrq* and *HoneybeeFrq* (table 1). Random effects included for M1 – M3 were *Site* and *Date*, and for M4, *Site* (table 2). For M1 – M3 and for M4, *number of individual flowers observed multiplied with min of observation* and *total amount of ovules* were included as offset variables, respectively (Reitan & Nielsen, 2016).

Table 1: Fixed effects included in GLMM analysis of variation in flower visitation (M1- M3; legitimate pollinators, primary nectar robbers and secondary nectar robbers, respectively) and seed set (M4).

	Fixed effect	**Explanation**	**Why it was included**
M1- M3: Flower visitation	Island	Eløen (E) or Sletter (S)	To examine possible difference in visitation frequency between islands
	Population	Sparse (S) or dense (D) plant population	To examine possible difference in visitation frequency between sparse and dense plant populations
	Patch Size	Large (L), medium (M) or small (S) patch size	To examine possible difference in visitation frequency between patches of different size
	Temp	Temperature	To examine the correlation between temperature and visitation frequency by functional group in question
M4: Seed set	Island	Eløen (E) or Sletter (S)	To examine possible differences in seed set between islands
	Population	Sparse (S) or dense (D) plant population	To examine possible differences in seed set between sparse and dense plant populations
	Patch Size	Large (L), medium (M) or small (S) patch size	To examine possible differences in seed set between patches of different size
	BumblebeeLegFrq	Average visits of legitimate pollinators for the site in question	To examine the correlation between seed set and visitation frequency of legitimate pollinators
	BumblebeeRobFrq	Average visits of primary robbers for the site in question	To examine the correlation between seed set and visitation frequency of primary nectar robbers
	HoneybeeFrq	Average visits of secondary robbers for the site in question	To examine the correlation between seed set and visitation frequency of secondary nectar robbers

Table 2: Random effects included in GLMM analysis of variation in flower visitation (M1- M3; legitimate pollinators, primary nectar robbers and secondary nectar robbers , respectively) and seed set (M4).		
	Random effect	**Random effect**
Seed set and *flower visitation*	Site	Site where capsules were collected, or observation of flower visitation conducted (1-30)
Flower visitation	Date	Date when observation of flower visitation was conducted (1-14, initiated at first field day)

To assess whether the effect of pollinators on seed set varied on different spatial scales (i.e. on island, population and patch level), to-way interactions and combinations of two-way interactions between spatial factors and pollinators were tested in the M4 (seed set) model selection. Only the interactions with legitimate pollinators were assesses, as nectar robbers have no important effect on *V. vulgaris* seed set (Jennersten et al., 1988). The interactions included *Island * BumblebeeLegFrq*, *Population * BumblebeeLegFrq* and *Patch Size * BumblebeeLegFrq*.

For all models, variables and interactions between variables were removed using the *´drop1´* function until no further reduction in AIC value could be obtained. See Appendix C for how model selection was carried out for the different models (C.1 – C.4 for M1 – M4, respectively).

3. Results

3.1. Functional grouping of flower visitors

As much as 98.8 % of the visits to *V. vulgaris* flowers on Eløen and Sletter was done by bumblebees and honeybees. The remaining 1.2% was done by species of the Diptera order and was excluded for further assessment due to the low number.

Of the three functional groups of flower visitors, legitimate pollinators accounted for 23.5 %, primary robbers for 44.7 % and secondary robbers (*A. mellifera*) for 30.6 % of the flower visits. Among the bee species, *B. terrestris* was the most frequent visitor and accounted for 30.7 % total flower visits (figure 10, appendix A: table A.1).

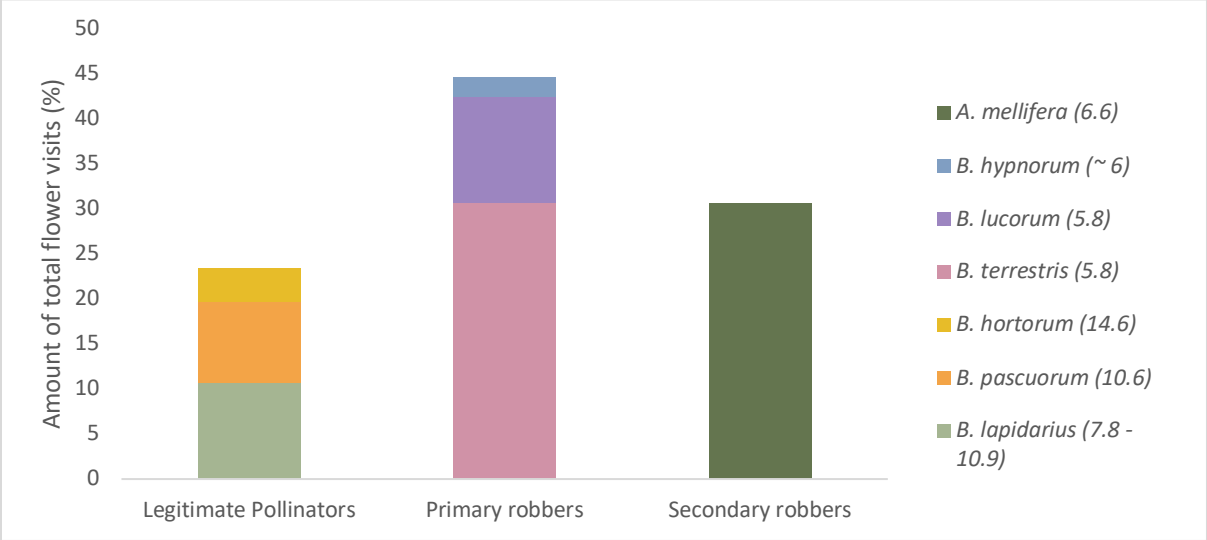

Figure 10: Percentage of flower visits to *V. vulgaris* by legitimate pollinators (left), primary nectar robbers (middle) and secondary nectar robbers (right). Average proboscis length specified in mm behind the name of each species is based on Goulson and Darwill (2004), Sowig (1989), Jennersten et. al (1988) and Crowther (2014).

3.2. Spatial variation in visitation frequency

Legitimate pollinators

The best model explaining variation in visitation frequency conducted by legitimate pollinators (M1) included *Island* and *Population* as fixed effects. Frequency of legitimate pollinators was lower on Sletter compared to Eløen, and in sparse compared to dense plant populations. The effect of patch size and temperature was not included in the best model (table 3).

Primary robbers

The best model explaining variation in visitation frequency conducted by primary robbers (M2) included *Island*, *Population* and *Patch Size* as fixed effects. As for legitimate pollinators, the frequency of primary robbers was lower on Sletter compared to Eløen and in sparse compared to dense plant populations. In contrast to M1, primary robbing frequency decreased significantly with decreasing patch size. The effect of temperature was not included in the best model (table 3).

Secondary robbers

The best model explaining variation in visitation frequency conducted by secondary robbers (M3, honeybees) included *Island*, *Population*, *Patch Size* and *Temperature* as fixed effects. For honeybees, Sletter had zero observations. Similar as for primary robbers, honeybee visitation decreased with decreasing patch size. Interestingly, and contrary to the bumblebee findings, honeybee visitation frequency increased in sparse plant populations. Finally, visitation frequency was positively correlated with temperature (table 3).

Table 3: Summary of the estimates in the best models explaining variation in flower visitation conducted legitimate pollinators, primary nectar robbers and secondary nectar robbers (M1 – M3). Island S = Sletter. Population S = sparse. Patch size S = small, M = medium.

	Fixed effects	Estimate	SE
M1: legitimate pollinators	Intercept	-3.747	0.244
	Island S	-1.768	0.459
	Population S	-1.399	0.371
M2: Primary robbers	Intercept	-2.997	0.262
	Island S	-0.783	0.436
	Population S	-0.628	0.371
	Patch Size M	-0.576	0.323
	Patch Size S	-1.535	0.462
M3: Secondary robbers	Intercept	-8.251	1.450
	Island S	-25.594	85.334

	Population S	0.908	0.533
	Patch Size M	-0.727	0.512
	Patch size S	-2.662	0.7556
	Temp	0.171	0.058

3.3. Spatial variation in seed set

A total of 90 flower capsules subject to natural pollination were collected, whereof 30 capsules were from Sletter and 60 from Eløen (i.e. three capsules per site, 1 – 30). Seed potential (i.e. number of available ovules) ranged from 107 to 418 and did not differ significantly between sparse and dense plant populations (mean = 207.4 and 197.5, respectively, Welch two sample t test, p-value =0.41) (table 4).

Table 4: Mean number of available ovules in sparse and dense plant populations.

Population	Mean number of ovules	Range
Sparse	207.4	107 - 418
Dense	197.5	113 - 350

The best model explaining variation in seed set (M4) included *Patch Size* as fixed effect, as well as the interaction between *Population* and *BumblebeeLegFrq* (visitation frequency conducted by legitimate pollinators) (table 5). Neither the effect of Island nor of visitation frequency conducted by primary or secondary robbers were included. Seed set was in general lower in sparse compared to dense populations and was highest in small and lowest in medium sized patches with large patches in between. Seed set was significantly and positively correlated with visitation frequency from legitimate pollinators – but only in sparse populations. At a visitation frequency of 0.2 (bees per flower per min) and higher, seed set in sparse plant populations was predicted to surpass that in dense populations (which basically stayed the same irrespective of the visitation frequency) (figure 11).

Table 5: Summary of the estimates in the best model explaining variation in seed set (M4). Population S = sparse. Patch size S = small, M = medium. BumblebeeLegFrq = frequency of legitimate pollinators.

		Estimate	SE
Fixed effects	Intercept	-0.207	0.170
	Population S	-0.998	0.211
	BumblebeeLeg Frq.	0.167	0.830
	Patch Size M	-0.347	0.143
	Patch Size S	0.102	0.198
Interactions	Population P: BumblebeeLeg Frq.	5.099	1.790

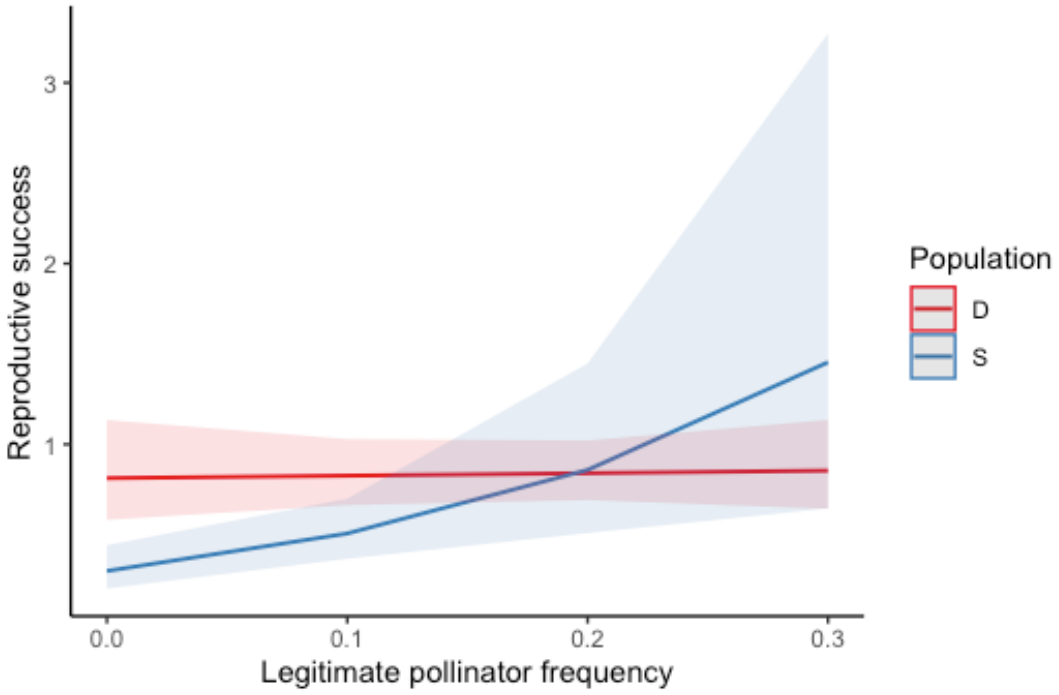

Figure 11: Predicted plant reproductive success as a function of legitimate pollinator frequency in sparse (S) and dense (D) population. Plant reproductive success (y-axis) is calculated as number of pollinated seeds per seed potential (i.e. seed:ovule ratio). Legitimate pollinator frequency (x-axis) is calculated as bees per flower per min (formula 1, part 2.2.2.). The shaded area represents the 95 % confidence interval of the fitted effects. Maximum visitation frequency was ~ 0.30 in the dense population and ~ 0.17 in the sparse population, meaning that the rightmost part of the sparse population graph is based on extrapolated values. Seed set in sparse populations is predicted to surpass that in dense populations for visitation frequencies > 0.2.

3.4. *V. vulgaris* breeding system

A total of 240 flower capsules (three per treatment per site) were supposed to be collected at Eløen (site 1 – 20). However, in the dense plant population, 25/30 of the flowers that were hand pollinated outside the cage (treatment Po) were eaten or otherwise destroyed by grazing cattle and sheep. Hence, only 215 flower capsules were collected.

In both the dense and the sparse plant population, seed set was lowest for bagged flowers (treatment B) (figure 12, appendix A: table A.2). However, only in the dense population was bagged flower seed set significantly lower than the flower treatment that achieved highest seed set (table 6, left). No significant effect of the cage (i.e. difference in seed set between hand pollinated flowers inside and outside the cage, treatment Pi and treatment Po) was detected, neither within populations, nor considering both populations together (table 6, middle). Possible effects of the cage could hence be considered negligible.

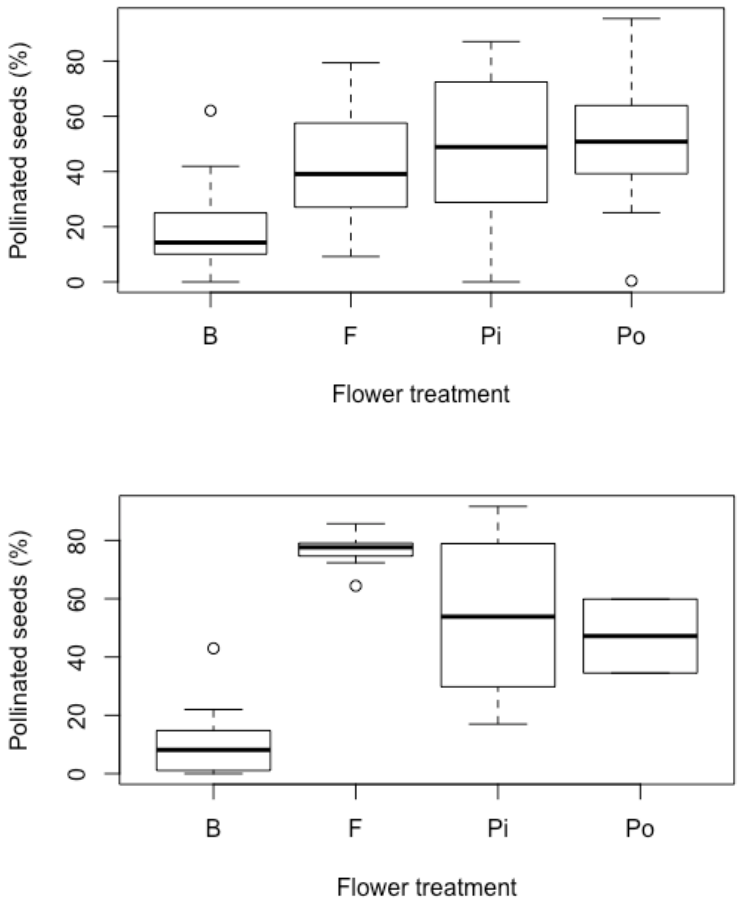

Figure 12: Percentage pollinated seed of available ovules in sparse plant population (top) and dense plant population (bottom). B = bagged, F = naturally pollinated, Pi = hand pollinated inside cage and Po = hand pollinated outside cage.

Seed set was highest for naturally pollinated flowers (treatment F) In the dense population (avg. 71.99 % pollinated seeds) and for hand pollinated flowers outside the cage (treatment Po) in the sparse population (avg. 49.47 % pollinated seeds) (figure 12, appendix A.2). In the sparse population, avg. seed set was 40.71 % for F flowers, meaning that hand pollination increased seed set with 21.5 % (treatment F vs treatment Po: 40.71 % vs. 49.47 %, respectively). Further, F flower avg. seed set was almost 80 % higher in the dense compared to the sparse population (71.99 % vs. 40.71 %), representing thus the only treatment for which seed set differed significantly between populations (p-value <0.001, table 6, right).

Table 6: P-values from Welch two sample t-test comparing highest and lowest seed set in dense and sparse populations (left), seed set following hand pollination inside and outside cage (middle), and seed set in between flower treatments in sparse and dense plant populations (right). For lowered text, s = sparse population, d = dense population and tot = both populations. B = bagged, F = naturally pollinated, Pi = hand pollinated inside cage and Po = hand pollinated outside cage. Statistically significant results (i.e. p-value < 0.001) are marked in bold font.

Highest / Lowest	p-value	Po / Pi	p-value	Dense / Sparse	p-value
Po_s / B_s	0.0107	Po_s / Pi_s	0.8997	B_d / B_s	0.2384
F_d / B_d	**< 0.001**	Po_d / Pi_d	0.6903	F_s / F_d	**<0.001**
		Po_{tot} / Pi_{tot}	0.5768	Pi_s / Pi_d	0.6277
				Po_s / Po_d	0.8942

3.5. Germination experiment

After seven days, 96.67 % (29/30) of the seeds in the category *pollinated* (type I, big, healthy seeds) and 96.67% (29/30) of the seeds in the category *possibly pollinated dark* (type II, healthy, but small seeds) had germinated (table 7). For the category *possibly pollinated bright* (type III, healthy, but small, bright seeds) only 0.07 % (2/30) germinated, and for the aborted (type IV, crunched, unhealthy seeds) germination percentage was zero. Another week in the growth cabinet did not increase germination for any of the categories, and I declared the experiment for over. Although type III had poor germination success, it clearly differed from unpollinated ovules in size and form (figure 7). I therefore included seeds type I – III (*pollinated*, *possibly pollinated dark* and *possibly pollinated light* as pollinated seeds in the analysis.

Table 7: Germination success for seeds type I – IV: *pollinated, possibly pollinated (light and dark)* and *aborted,* respectively, measured in percentage germinated seeds after 1 and 2 weeks in the growth cabinet. Collection site and flower treatment is listed. Seeds type I, II and IV germinated as expected.

Type		Site	Treatment	1 week	2 weeks
I	*Pollinated*	1, 5, 15	F, F, F	96.67 %	96.67 %
II	*Possibly pollinated dark*	1, 5, 15	F, F, F	96.67%	96.67%
III	*Possibly pollinated light*	1, 5, 15	B, B, B	0.07%	0.07%
IV	*Aborted*	1, 5, 15	Pi, B, Pi	0.00%	0.00%

4. Discussion

As posed in the introduction, the first three research questions in this study sought to examine spatial variation in 1) *V. vulgaris* flower visitation 2) *V. vulgaris* seed set and 3) effect of pollinators on *V. vulgaris* seed set. The fourth question aimed to evaluate whether any conclusions could be drawn regarding the plant's optimal flower density. In the following section, I will, in the light of my results, answer the question in respective order.

4.1. Spatial variation in flower visitation – question 1

Variation in flower visitation between Eløen and Sletter

Visitation frequency of all bee groups was lower on Sletter than on Eløen, in spite of similar *V. vulgaris* density on the two islands. The difference was particularly pronounced for honeybees, for which no *V. vulgaris* visits were observed Sletter. Why so? As neither island have beehives (*pers. comm.* Christian Sibbern), the honeybees must have travelled from the mainland. In this context, Sletter is both somewhat smaller and more isolated than Eløen (Strandli et al., 2002) and might therefore be harder to reach for mainland bees (McArthur & Wilson, 1967). However, this theory does not explain the difference in flower visitations by bumblebees. Could my findings reflect overall lower insect abundance on Sletter? As mentioned in the introduction, the islands differ in complexity. While Sletter mainly consists of dry meadows, Eløen can probably be considered semi-natural grassland. This would make the latter a likely spot for high insect abundance and diversity (Eriksson et al., 2002; Kull & Zobel, 1991) and explain the high observed bee visitation frequency compared to Sletter.

Variation in flower visitation between populations of different density

While lower visitation frequency was recorded for both bumblebee groups (i.e. legitimate pollinators and primary robbers) in sparse populations, the opposite was true for honeybees. As previously stated, an accumulation of studies has reported lower visitation frequencies in less dense flower areas (e.g. Aguilar et al., 2006; ; Dauber et al., 2010; Jennersten, 1988; ; Jennersten & Nilsson, 1993; Nielsen et al., 2012; Rathcke & Jules, 1993) and the findings were therefore as expected for the bumblebees. Why then, was honeybee visitation higher in the sparse population? A possibility is that my results reflect *Apis-Bombus* exclusive competition (see e.g. Goulson & Sparrow, 2008; Nielsen et al., 2012; Thomson, 2004). However, as

honeybees need primary robbers (i.e. short tongued bumblebees) to access *V. vulgaris* nectar, the *Apis-Bombus* relationship in the present study is evidently more complex than one of mere competition. A more plausible explanation may lay in honeybee forage behaviour. Honeybees optimize forage by exploiting specific hotspots to which they recruit nestmates (Nielsen et al., 2012). In the present study, site 5 and site 9 (both belonging to the sparse population on Eløen) were almost consistently honeybee dominated (appendix A: figure A.1). While site 5 was the largest, site 5 and 9 were the most flower dense, and therefore the presumably most attractive patches in the sparse population. These patches might represent honeybee "hotspots", which again could explain the high honeybee visitation in a relatively flower sparse population. If so, curiously, my findings may imply that honeybees actually are more affected by variations in patch size than by variation in population density.

Variation in flower visitation in relation to patch size

As previously mentioned, larger patches are often assumed to attract more pollinators due to larger floral display (Dauber et al., 2010; Nielsen & Ims, 2000; Ramsey & Vaughyon, 2000; Sih, 1987). In agreement with this, visitation frequency of both primary and secondary robbers (i.e. short tongued bumblebees and honeybees) decreased with decreasing patch size, of which the effect was strongest for honeybees (table 4). By contrast, no correlation was found between legitimate pollinators (i.e. long tongued bumblebees) and patch size. While it is tempting to conclude this section with "variations in patch size has little or no effect on long tongued bumblebees", absence of evidence is not necessarily evidence of absence. It is worth to look closer at my functional grouping of bumblebees, and the concept of resource portioning. Resource portioning, i.e. how different species co-exist in the same landscape by e.g. exploiting resources in different locations (Westphal et al., 2006), is the main topic in a study on the influence of patch size on bumblebees by Sowig (1989). In the study, Sowig found that short tongued bumblebees and honeybees dominated large flower patches, whereas long tongued bumblebees frequented patches of smaller size. Do long tongued bees "prefer" sub- optimal patches? According to Sowig, bees have different forage strategies, and what an "optimal" patch size is will vary between species (i.e. resource portioning by preference). Yet, if long tongued bees prefer smaller patches, why was no such effect observed in my study? As described in part 2.2.2., I grouped *B. lapidarius* as a legitimate

pollinator and hence with long tongued bees. In the study of Sowig, however, *B. lapidarius* is considered short tongued. Interestingly, by grouping *B. lapidarius* with the primary robbers (i.e. short tongued bumblebees) in the present study, legitimate pollinators (i.e. long tongued bumblebees, here *B. pascuorum* and *B. hortorum*) are significantly and negatively correlated with patch size (appendix D: D.1 and D.2). Do long tongued bumblebees really prefer small patches, or could the variation in patch size preference between bee groups be due to competition? Granted that large patches are the optimal choice for all groups, one could imagine long tongued bumblebees to be restricted to small patches when numbers of short tongued bumblebees or honeybees are high. Yet, although interspecific competition between two species of bumblebees (Inouye, 1978) and between bumblebees and honeybees (Goulson & Sparrow, 2008) have been suggested in other studies, competitive interactions are hard if not impossible to verify in cases were species are grouped together (as in the present study). My reflections can therefore serve as no more than mere speculations. Summing up, it seems likely that different bee species frequent patches of different size. This is probably a result of resource portioning, either due to competitive exclusion or different forage strategies. However, as my result show, such effects might be obscured when several species are grouped together.

4.2 Spatial variation in seed set – question 2

Flower treatment: *V. vulgaris* benefits form animal pollination

In agreement with other studies on *V. vulgaris* (e.g. Jennersten, 1988a; Nielsen & Ims, 2000), my results confirm that *V. vulgaris* reproductive success largely benefits from animal pollination. First, in the flower treatment experiment, bagged flowers (treatment B, i.e. flowers that did not receive insect visitation) achieved the lowest seed set in both plant populations (figure 12). Second, naturally pollinated flowers (treatment F) received increased pollinator visitation and had significantly higher seed set (see part 4.1) in the dense compared to the sparse population (table 6). As no significant difference in seed set was detected for flower treatments other than F (table 6), variations in F flower seed set was more likely due to difference in pollinator service than to difference in resource limitation between populations. Finally, in the germination experiment, type III seeds (figure 7) had poor germination success (table 7). Naturally, this could have been a result of underdevelopment due to too early collection. It is, however, interesting that all type III seeds were collected from bagged flowers

and thus a result of self-pollination (table 7). Did I by coincidence collect only bagged flowers too early? Or can the poor germination rather be linked to self-pollination? While this clearly asks for more research, one would in either case expect spatial variations in *V. vulgaris* seed set to at least partly correlate with legitimate pollinator visitation.

Variation in seed set between islands and populations

No variation in seed set was observed between Islands in spite of lower visitation frequency of legitimate pollinators on Sletter (part 4.1.). Why so? A possibility is that honeybee visits (of which none were recorded on Sletter) have a negative effect on seed set that was not detected in this study, which somehow equalized the positive effect of visitation frequency by legitimate pollinators on Eløen. Another feasibility is that Eløen plants had reached a "saturation point"- i.e. a point where increased pollinator visitation did not result in higher seed set. The saturation point could be caused by e.g. resource limitation (see e.g. Zhao et al., 2014) or high levels of geitonogamy (Finer & Morgan, 2003). However, which of the two that is more important in this context cannot be deduced based on the present study. Turning now to variation in *V. vulgaris* seed set on population level, it was in general lower in sparse compared to dense populations (but see part 4.3.). This is in accordance with the general assumption that plant reproductive success decreases in more fragmented areas (e.g. Aguilar et al., 2006; Jennersten, 1988; Rathcke & Jules, 1993), and, as also visitation frequency of legitimate pollinators was lower, indicates pollinator limitation for *V. vulgaris* in sparse populations. Uniting the findings from island and populations, my results indicate that *V. vulgaris* may experience pollinator limitation on population level (in sparse populations), and limitations in resources or pollen quality on island level (on Eløen).

Variation in seed set in relation to patch size

The relationship between seed set and patch size was somewhat complex, with highest seed set in small patches, and lowest in patches of medium size (figure 13). This is unexpected of many reasons. First, legitimate pollinator visitation was not correlated with patch size (section 4.1). Second, the observed relationship between seed set and patch size is reverse to the previously mentioned "optimal patch size theory" (Rathcke, 1983). Third, and specifically for *V. vulgaris*, both Nielsen & Ims (2000) and Jennersten (1993) have reported an increase in *V.*

vulgaris seed set with increasing patch size (although the definition of patch size in Jennersten´s study differs from mine to such a degree that comparisons between the two hardly can be considered helpful). How can the presents study's relationship between seed set and patch size be explained? For the high observed seed set in small patches, and as discussed in part 4.1., some species of legitimate pollinators (*B. pascuorum* and *B. hortorum*) were actually positively correlated with small patches (and therefore likely to influence seed set) (appendix D: D.1). Further, Goulson (2000) suggests that searching for unvisited inflorescences is easier in small patches, and that bumblebees therefore visit a higher proportion of the total number of flowers. Hence, even if allover *frequency* of legitimate pollinators did not increase in small patches, their *efficiency* might have. The observed lower seed set in medium compared to large patches is harder to explain. It is possible that information was lost in this study´s classification of patch size, and that other cut-off points might be more relevant for pollinators. Although this is a likely explanation, my findings may also indicate an interesting trade-off between elevated pollination efficiency (as seen in small patches (Goulson, 2000)) and high number of pollinators due to large flower display (as seen in large patches (e.g. Nielsen & Ims, 2000)). As medium patches have neither, they might experience lower seed set. If so, Rathcke´s model could, at least for some plant species in some areas, be exchanged for a trade-off model where big and small patches are more optimal than those of medium size. I have found no results similar to mine, so this hypothesis clearly asks for further investigation.

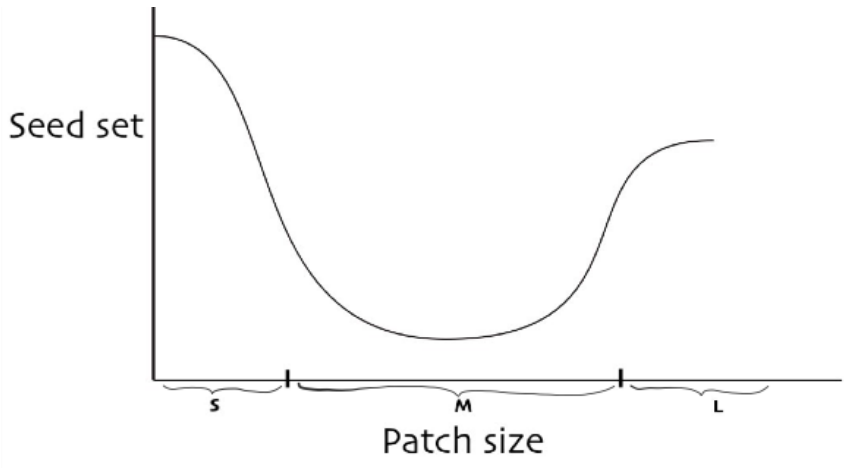

Figure 13: Schematic graph representing the present study´s relationship between seed set and patch size (i.e. S> L > M). Seemingly reverse to the theory about optimal patch size (Rathcke 1983) and figure 1 in the introduction.

4.3. Variation in effect of pollinators between plant populations – question 3

Seed set was not correlated to visitation frequency of primary or secondary robbers, indicating that nectar robbing has little or no detrimental effect on *V. vulgaris* reproductive success (but see discussion on honeybees, part 4.2.). This is in agreement with findings from Jennersten et al. (1988). As anticipated, seed set was positively correlated with legitimate pollinators – but the effect was, interestingly, only seen in sparse populations. Seed set in dense populations was in general higher than in sparse populations (part 4.2.). However, it did not change with increasing visitation frequencies. In sparse populations, on the other hand, seed set increased with visitation frequency and was at 0.2 (bees per flower per min) predicted to surpass that in dense populations (figure 11). Why so? A possible explanation is pollinator forage behavior. In this study, long tongued bumblebees were the only observed pollinators of *V. vulgaris*. While bumblebees are efficient in terms of pollen transfer, they normally visit several flowers on each inflorescence, and several inflorescences of the same individual. Therefore, and particularly for *V. vulgaris* where one individual often includes hundreds of flowers, bumblebees may in fact facilitate selfing by geitonogamy and not actual cross pollination. As proposed by Finer and Morgan (2003), pollinator-mediated geitonogamy will be especially likely in populations where plants grow close together. In sparse populations, by contrast, bumblebees may be forced to fly longer distances between patches or flower individuals, and hence in a greater degree partake in cross pollination. This is in agreement with a study on *Cynoglossum officinale* conducted by Klinkhamer et al. (1989). In the study, the authors argue that isolated plants receive pollen from more distant and less related plants, which likely reduces inbreeding. Such increased pollinator movement and consequent cross pollination is probably more likely when visitation frequency reaches a certain number and competition between pollinators for plants starts. Hence, the predicted 0.2 threshold in the present study might mark the point where bumblebees in sparse populations start to compete for flowers rather than flowers for bees.

4.4. Optimal flower density and saturation point – question 4

My results suggest that optimal flower density for *V. vulgaris* depends upon legitimate pollinator visitation. For low visitation frequencies, the plants seed set was highest in dense populations. For increased frequencies, however, seed set was predicted to be highest in

sparse populations. As seed set in dense populations did not increase with increasing visitation frequency, the plants reproductive success had seemingly reached a "saturation point" at a seed:ovule ratio of ~ 0.8 (figure 11). In a study on *Trifolium pratense,* Hegland (2014) reports a "saturation effect" above which higher insect visitation did not result in increased fruit set. He suggests it to reflect reduced pollen quality and increased inbreeding. Similarly, in my study, resource limitation was unlikely (see part 4.2.) and the observed saturation point may therefore indicate high levels of pollinator mediated geitonogamy in the dense populations. Turning to the sparse populations, visitation frequencies > 0.17 are based on extrapolated values (corresponding to a seed:ovule ratio of ~ 0.76). As the graph continues to rise after 0.17 (instead of flattening out, which would be the case if a saturation point had been reached), it is clear that my results do not suggest a saturation point for sparse populations. However, as seed:ovule ratio cannot surpass 1 (i.e. the graph can in reality not rise indeterminately), and as a further increase in legitimate pollinator visitation is unlikely to affect seed set negatively (i.e. the graph is unlikely to fall significantly for visitation frequencies > 0.17) it is probable that a saturation point for sparse populations would be found for visitation frequencies somewhere > 0.17, corresponding to seed:ovule ratio between 0.8 and 1 (figure 14). For exactly what visitation frequency sparse plant populations would reach a saturation point, could be an interesting focus for future studies.

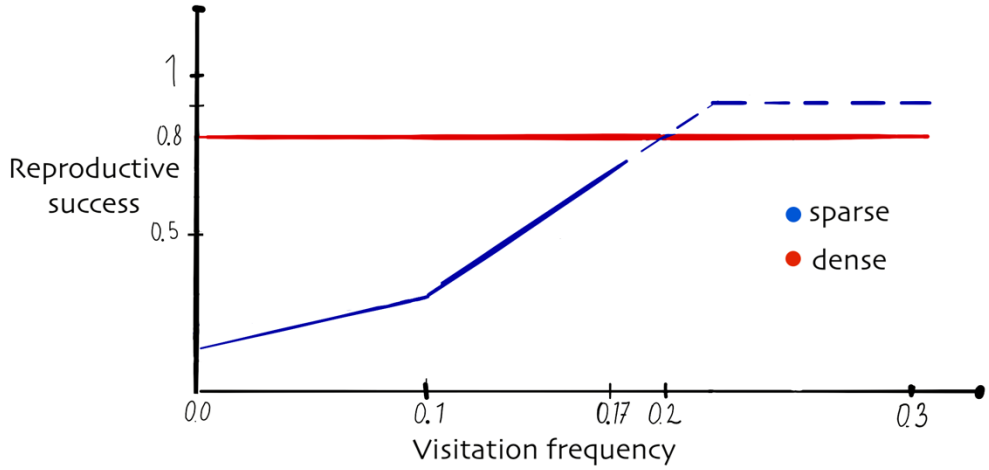

Figure 14: Schematic graph representing plant reproductive success as a function of legitimate pollinator frequency in sparse (blue) and dense (red) populations. Observed data in solid lines and predicted and hypothesized scenario (saturation point of sparse populations at visitation frequencies > 0.17) in dotted lines. Reproductive success (y-axis) is measured as seed:ovule ratio and visitation frequency (x-axis) in bees per flower per min. Modified from figure 11.

To sum up, my results suggest that *V. vulgaris´* saturation point – and therefore also reproductive success – have the potential to be *higher* in sparse than in dense populations, probably due to lower levels of geitonogamy in less dense flower areas. Can this be extrapolated to other plants species? My findings largely build upon *V. vulgaris* growing forms (i.e. numerous flowers per plant individual and hence higher probability of geitonogamy) and main pollinator forage behavior (i.e. bumblebees' systematic visitation of several flowers per flowering stalk), meaning that generalizations must be coupled with caution. Nevertheless, that similar proportions of pollinators to flowers can have a more positive effect in sparse than in dense populations is likely true for many plant species. This emphasizes the importance of maintaining viable pollinator populations in areas where plant species targeted for conservation are sparse or patchily distributed, e.g. by ensuring high diversity of other nectar and pollen sources throughout the season (Nielsen & Ims, 2000).

4.5. Notes on abiotic, biotic and spatial factors

In the present study, temperature had a positive and linear effect on honeybees only. Somewhat contrasting to my findings, a study by Nielsen et al (2017) reports a "honeybee-peak" at ~ 25 °C and a linear relationship between bumblebees and temperature. However, results from both the named study and my own imply that honeybees in a greater degree than bumblebees are affected by temperature. Variation in observed bee-temperature relationship might be due to factors such as wind, weather conditions, competition from other species, host plant or nectar concentration. Clearly, biotic and abiotic factors other than temperature and spatial variables might be well as important when analysing variation in flower visitation (Hobbs & Yates, 2003).

5. Conclusive remarks

The main purpose of this study was to examine spatial variation in flower visitation and seed set of *V. vulgaris*, and, based on the result, evaluate whether any conclusions could be drawn regarding the plant´s optimal flower density. The major finding was the relationship between seed set, legitimate pollinators and plant population density. In agreement with the common assumption that plant reproductive success decreases in less dense flower areas, I found that *V. vulgaris* seed set was higher in dense than in sparse populations. However, seed set in dense populations did not change with increasing visitation frequency, indicating a saturation point for plant reproductive success. For sparse populations, on the other hand, pollinator visitation had a significant, positive effect, and seed set was predicted to surpass that in dense populations for frequencies > 0.2 (bees per flower per min). These results may have important implications.

First, they emphasize the significance of analyzing interactions between effects. Second, they underline the importance of distinguishing between efficient pollinators and other flower visitors when assessing plant-pollinator interactions. Different groups or species may respond differently to spatial variations and may have contrasting effect on seed set in the host plant. Last, but not least, my results show that *V. vulgaris* optimal flower density depends upon pollinator visitation frequency, and that, *given enough efficient pollinators,* reproductive success of animal pollinated plants has potential to be *higher* in sparse than in dense populations. This might be due to elevated levels of geitonogamy in dense flower aggregations. My results highlight the importance of maintaining viable pollinator communities in areas where conservation targeted plant species are sparse or patchily distributed. However, as my findings build largely upon *V. vulgaris* growing form (i.e. numerous flowers per plant individual) and pollinator forage behavior (i.e. bumblebees systematic forage strategy), caution should be applied when extrapolating the results to other plant-pollinator systems.

What would I do different if I were to redo the study? An obvious and important limitation of my research is the limited number of observations and therefore reliance upon extrapolated data. This study would clearly benefit from a higher sample size over several seasons. A second constraint is the subjective definition of spatial variables. Categories such as “large”, “medium” and “small” as well as “dense” and “sparse” might serve for comparison within a

singular study, but clearly complicate meta-analysis. For future research (including my own), I would highly recommend consistency in definitions, e.g. by use of countable (continuous) measurements or standardized categories. Notwithstanding these limitations, my study offers additional insight into plant – pollinator interactions. It offers another brick in the wall concerning our understanding of pollination: perhaps the best-studied and certainly among the most beautiful interactions in the world - and inevitably driven by self-interest.

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Appendices

Appendix A

Table A.1: Percentage of total visits to *V. vulgaris* done by different, functional groups. Proboscis length of species is based on studies by Goulson and Darwill (2004), Sowig (1989), Jennersten et. al (1988) and Crowther (2014)

Functional group	Species	Proboscis length, mm	Percentage visits by species	Percentage visits by functional group
Legitimate pollinators	*B. lapidarius*	7.8 - 10.9	10.7	**23.5**
	B. pascuorum	10.6	9.0	
	B. hortorum	14.6	3.7	
Primary nectar robbers	*B. terrestris*	5.8	30.7	**44.7**
	B. lucorum	5.8	11.8	
	B. hypnorum	~ 6	2.2	
Secondary nectar robbers	*A. mellifera*	6.6	30.6	**30.6**
	TOTAL			**98.8**

Table A.2: Average percentage seed set for the different flower treatments in the sparse and dense plant population. Standard error is calculated manually as $\frac{\sigma_x}{\sqrt{n}}$

Sparse plant population			
Treatment	**Mean**	**SE**	**Range**
B	20.37 %	5.85	0.00 – 62.04 %
F	40.71 %	7.00	9.21 – 79.38 %
Pi	47.83 %	9.91	0.00 – 87.06 %
Po	49.47 %	8.23	0.38 – 95.41 %
Dense plant population			
Treatment	**Mean**	**SE**	**Range**
B	11.58 %	4.16	0.00 – 42.96 %
F	71.99 %	1.94	64.41 – 85.73 %
Pi	54.16 %	8.15	17.00 – 91.67 %
Po	47.20 %	12.68	34.51 – 59.88 %

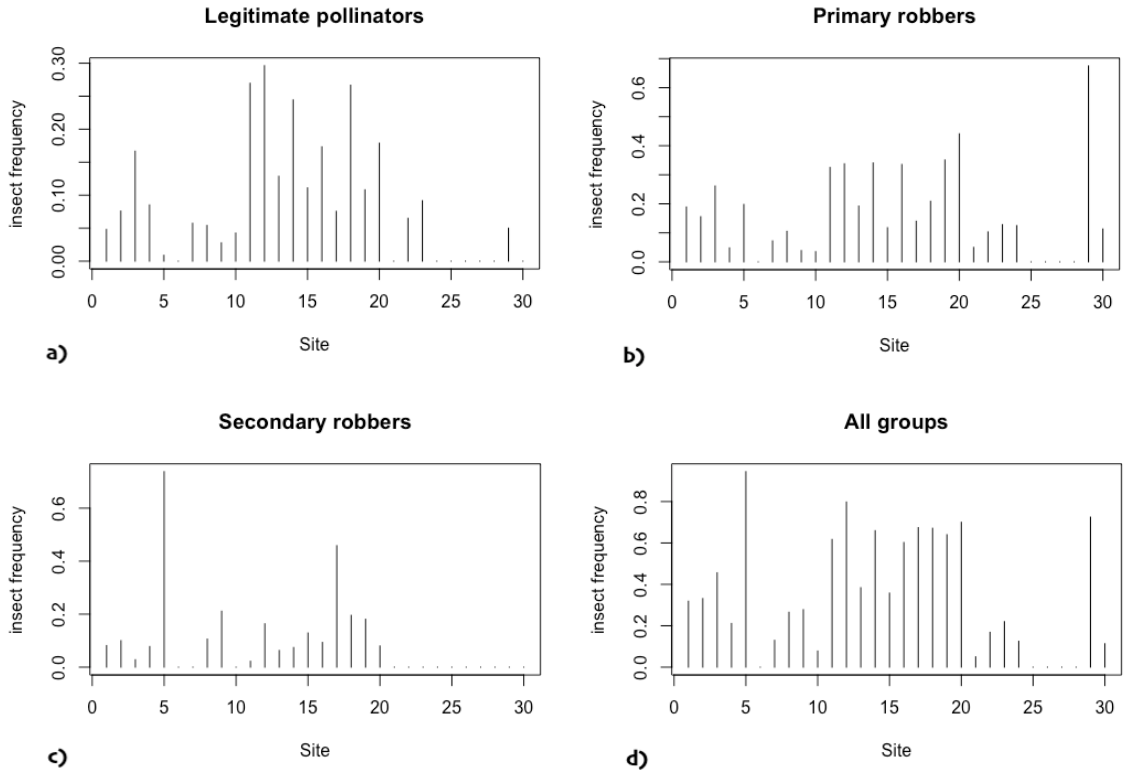

Figure A.1: Average flower visitation at site 1 – 30 for a) legitimate pollinators, b) primary nectar robbers, c) secondary nectar robbers and d) all groups. Visitation frequency is measured as bee per flower per min.

Appendix B

Figure B.1: Sletter is practically free for trees and shrubs. Here, view from mid Sletter towards the north. Photo: Henninge Torp Bie

Figure B.2: Sletter is practically free for trees and shrubs. Here, S Sletter. Photo: Henninge Torp Bie

Figure B.3: Male stage in *V. vulgaris*. Ten stamens in two sequenced whorls of five become mature first. First sequence (left) and second sequence (right). Photo: André Navarro

Figure B.4: Female stage in *V. vulgaris*. Following the stamens, five stigmas become receptive. Stigmas appearing following stamens (left) and receptive stigmas (right). Photo: André Navarro

Figure B.5: Small patches were defined as < 8 tussocks. Here, small patch, site 28, SE Sletter. Photo: Henninge Torp Bie.

Figure B.6: Medium patches were defined as 8 – 15 tussocks and large patches as > 15 tussocks. Here, large patch, site 19, Eløen S. Photo: Henninge Torp Bie.

Figure B.7: To bag flowers in bud stage, sheep proof cages were built in dimension 0.5m x 0.3m x 0.3m. Photo: André Navarro.

Figure B.8: The cages were built with angular legs so that they could be hammered into the ground (left). Each cage was completely covered in mosquito net (right). Photo: André Navarro.

Appendix C

C.1: Flower visitation conducted by legitimate pollinators (long tongued bumblebees, M1)

Making a full model. All fixed effects included

```
modelBBLeg = glmer.nb(formula= BumblebeeLeg ~ Island + Population + PatchSize +
Temp + (1 | Site) + (1 | Date), offset = log(FlowerOffset), data= dataInsect )
summary(modelBBLeg)

## Generalized linear mixed model fit by maximum likelihood (Laplace
##   Approximation) [glmerMod]
##  Family: Negative Binomial(0.3816)  ( log )
## Formula: BumblebeeLeg ~ Island + Population + PatchSize + Temp + (1 |
##     Site) + (1 | Date)
##    Data: dataInsect
##  Offset: log(FlowerOffset)
##
##      AIC      BIC   logLik deviance df.resid
##    898.3    928.9   -440.1    880.3      212
##
## Scaled residuals:
##     Min      1Q  Median      3Q     Max
## -0.6062 -0.5366 -0.3947  0.2012  4.7171
##
## Random effects:
##  Groups Name        Variance Std.Dev.
##  Site   (Intercept) 0.01533  0.1238
##  Date   (Intercept) 0.14141  0.3760
## Number of obs: 221, groups:  Site, 30; Date, 11
##
## Fixed effects:
##             Estimate Std. Error z value Pr(>|z|)
## (Intercept) -2.83904    1.15696  -2.454 0.014132 *
## IslandS     -1.79116    0.49812  -3.596 0.000323 ***
## PopulationP -1.26579    0.41946  -3.018 0.002547 **
## PatchSizeM  -0.13650    0.35539  -0.384 0.700915
## PatchSizeS  -0.25591    0.46288  -0.553 0.580360
## Temp        -0.03397    0.04437  -0.766 0.443866
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##             (Intr) IslndS PpltnP PtchSM PtchSS
## IslandS     -0.286
## PopulationP -0.140  0.353
## PatchSizeM  -0.186 -0.330 -0.375
## PatchSizeS  -0.074 -0.279 -0.698  0.509
## Temp        -0.974  0.266  0.139  0.077  0.015
```

Starting drop1()

```
modelBBLeg = glmer.nb(formula= BumblebeeLeg ~ Island + Population + PatchSize + Tem
p + (1 | Site) + (1 | Date), offset = log(FlowerOffset), data= dataInsect )

drop1(modelBBLeg)

## Warning in checkConv(attr(opt, "derivs"), opt$par, ctrl = control$checkConv, :
## Model failed to converge with max|grad| = 0.0108804 (tol = 0.002, component 1)

## Single term deletions
##
## Model:
## BumblebeeLeg ~ Island + Population + PatchSize + Temp + (1 |
##     Site) + (1 | Date)
##            npar    AIC
## <none>          898.29
## Island        1 909.01
## Population    1 907.63
## PatchSize     2 894.59
## Temp          1 896.85
```

Dropping Patch Size

```
modelBBLeg = glmer.nb(formula= BumblebeeLeg ~ Island + Population + Temp + (1 | Sit
e) + (1 | Date), offset = log(FlowerOffset), data= dataInsect )

drop1(modelBBLeg)

## Warning in checkConv(attr(opt, "derivs"), opt$par, ctrl = control$checkConv, :
## Model failed to converge with max|grad| = 0.0147176 (tol = 0.002, component 1)

## Single term deletions
##
## Model:
## BumblebeeLeg ~ Island + Population + Temp + (1 | Site) + (1 |
##     Date)
##            npar    AIC
## <none>          894.57
## Island        1 906.63
## Population    1 912.72
## Temp          1 893.07
```

Dropping Temperature

```
modelBBLeg = glmer.nb(formula= BumblebeeLeg ~ Island + Population + (1 | Site) + (1
| Date), offset = log(FlowerOffset), data= dataInsect )

drop1(modelBBLeg)

## Warning in checkConv(attr(opt, "derivs"), opt$par, ctrl = control$checkConv, :
## Model failed to converge with max|grad| = 0.0150553 (tol = 0.002, component 1)

## Single term deletions
##
## Model:
## BumblebeeLeg ~ Island + Population + (1 | Site) + (1 | Date)
##            npar    AIC
## <none>          893.06
## Island        1 904.68
## Population    1 910.71
```

No further decrease in AIC available. Final model as follows:

```
modelBBLeg = glmer.nb(formula= BumblebeeLeg ~ Island + Population + (1 | Site) + (1
| Date), offset = log(FlowerOffset), data= dataInsect )

summary(modelBBLeg)

## Generalized linear mixed model fit by maximum likelihood (Laplace
##   Approximation) [glmerMod]
##  Family: Negative Binomial(0.3986)  ( log )
## Formula: BumblebeeLeg ~ Island + Population + (1 | Site) + (1 | Date)
##    Data: dataInsect
##  Offset: log(FlowerOffset)
##
##      AIC      BIC   logLik deviance df.resid
##    893.1    913.4   -440.5    881.1      215
##
## Scaled residuals:
##     Min      1Q  Median      3Q     Max
## -0.6188 -0.5419 -0.3700  0.2152  4.6150
##
## Random effects:
##  Groups Name        Variance Std.Dev.
##  Site   (Intercept) 0.08548  0.2924
##  Date   (Intercept) 0.18165  0.4262
## Number of obs: 221, groups:  Site, 30; Date, 11
##
## Fixed effects:
##             Estimate Std. Error z value Pr(>|z|)
## (Intercept)  -3.7473     0.2441 -15.352  < 2e-16 ***
## IslandS      -1.7684     0.4592  -3.851 0.000118 ***
## PopulationP  -1.3993     0.3085  -4.535 5.75e-06 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##             (Intr) IslndS
## IslandS     -0.364
## PopulationP -0.435  0.207
```

C.2: Flower visitation conducted by primary robbers (short tongued bumblebees, M2)

Making a full model. All fixed effects included

```
modelBBRob = glmer.nb(formula= BumblebeeRob ~ Island + Population + PatchSize + Tem
p + (1 | Site) + (1 | Date), offset = log(FlowerOffset), data= dataInsect )

summary(modelBBRob)

## Generalized linear mixed model fit by maximum likelihood (Laplace
##   Approximation) [glmerMod]
##  Family: Negative Binomial(0.3419)  ( log )
## Formula: BumblebeeRob ~ Island + Population + PatchSize + Temp + (1 |
##     Site) + (1 | Date)
##    Data: dataInsect
##  Offset: log(FlowerOffset)
##
##      AIC      BIC   logLik deviance df.resid
##   1100.4   1131.0   -541.2   1082.4      212
##
## Scaled residuals:
##     Min      1Q  Median      3Q     Max
## -0.5802 -0.5402 -0.4055  0.1990  4.9887
##
## Random effects:
##  Groups Name        Variance Std.Dev.
##  Site   (Intercept) 0.04851  0.2203
##  Date   (Intercept) 0.05439  0.2332
## Number of obs: 221, groups:  Site, 30; Date, 11
##
## Fixed effects:
##             Estimate Std. Error z value Pr(>|z|)
## (Intercept) -1.59032    1.02222  -1.556  0.11977
## IslandS     -0.98234    0.45845  -2.143  0.03213 *
## PopulationP -0.76634    0.37935  -2.020  0.04337 *
## PatchSizeM  -0.56578    0.31712  -1.784  0.07441 .
## PatchSizeS  -1.43669    0.46197  -3.110  0.00187 **
## Temp        -0.05597    0.03973  -1.409  0.15882
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##             (Intr) IslndS PpltnP PtchSM PtchSS
## IslandS     -0.350
## PopulationP -0.374  0.364
## PatchSizeM  -0.093 -0.154 -0.186
## PatchSizeS   0.104 -0.133 -0.562  0.367
## Temp        -0.968  0.262  0.291  0.001 -0.114
```

Starting drop1()

```
modelBBRob = glmer.nb(formula= BumblebeeRob ~ Island + Population + PatchSize + Tem
p + (1 | Site) + (1 | Date), offset = log(FlowerOffset), data= dataInsect )


drop1(modelBBRob)
```

```
## Warning in checkConv(attr(opt, "derivs"), opt$par, ctrl = control$checkConv, :
## Model failed to converge with max|grad| = 0.00801746 (tol = 0.002, component 1)

## Warning in checkConv(attr(opt, "derivs"), opt$par, ctrl = control$checkConv, :
## unable to evaluate scaled gradient

## Warning in checkConv(attr(opt, "derivs"), opt$par, ctrl = control$checkConv, :
## Model failed to converge: degenerate Hessian with 1 negative eigenvalues

## Warning in checkConv(attr(opt, "derivs"), opt$par, ctrl = control$checkConv, :
## Model failed to converge with max|grad| = 0.00403377 (tol = 0.002, component 1)

## Single term deletions
##
## Model:
## BumblebeeRob ~ Island + Population + PatchSize + Temp + (1 |
##     Site) + (1 | Date)
##            npar    AIC
## <none>          1100.4
## Island        1 1102.7
## Population    1 1102.3
## PatchSize     2 1106.0
## Temp          1 1100.2
```

Dropping Temperature

```
modelBBRob = glmer.nb(formula= BumblebeeRob ~ Island + Population + PatchSize + (1
| Site) + (1 | Date), offset = log(FlowerOffset), data= dataInsect )

## Warning in checkConv(attr(opt, "derivs"), opt$par, ctrl = control$checkConv, :
## Model failed to converge with max|grad| = 0.010667 (tol = 0.002, component 1)

drop1(modelBBRob)

## Single term deletions
##
## Model:
## BumblebeeRob ~ Island + Population + PatchSize + (1 | Site) +
##     (1 | Date)
##            npar    AIC
## <none>          1100.2
## Island        1 1101.4
## Population    1 1101.0
## PatchSize     2 1106.4
```

No further decrease in AIC available. Final model as follows:

```
modelBBRob = glmer.nb(formula= BumblebeeRob ~ Island + Population + PatchSize + (1
| Site) + (1 | Date), offset = log(FlowerOffset), data= dataInsect )

## Warning in checkConv(attr(opt, "derivs"), opt$par, ctrl = control$checkConv, :
## Model failed to converge with max|grad| = 0.010667 (tol = 0.002, component 1)

summary(modelBBRob)

## Generalized linear mixed model fit by maximum likelihood (Laplace
##   Approximation) [glmerMod]
##  Family: Negative Binomial(0.3441)  ( log )
## Formula: BumblebeeRob ~ Island + Population + PatchSize + (1 | Site) +
##     (1 | Date)
##    Data: dataInsect
##  Offset: log(FlowerOffset)
##
##      AIC      BIC   logLik deviance df.resid
##   1100.2   1127.4   -542.1   1084.2      213
##
## Scaled residuals:
##     Min      1Q  Median      3Q     Max
## -0.5810 -0.5402 -0.4152  0.2041  4.3737
##
## Random effects:
##  Groups Name        Variance Std.Dev.
##  Site   (Intercept) 0.06432  0.2536
##  Date   (Intercept) 0.09691  0.3113
## Number of obs: 221, groups:  Site, 30; Date, 11
##
## Fixed effects:
##             Estimate Std. Error z value Pr(>|z|)
## (Intercept)  -2.9979     0.2616 -11.459  < 2e-16 ***
## IslandS      -0.7827     0.4357  -1.797 0.072407 .
## PopulationP  -0.6282     0.3709  -1.694 0.090350 .
## PatchSizeM   -0.5757     0.3231  -1.782 0.074770 .
## PatchSizeS   -1.5350     0.4617  -3.325 0.000885 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##             (Intr) IslndS PpltnP PtchSM
## IslandS     -0.339
## PopulationP -0.347  0.294
## PatchSizeM  -0.389 -0.162 -0.183
## PatchSizeS  -0.061 -0.075 -0.548  0.351
```

C.3: Flower visitation conducted by secondary robbers (honeybees, M3)

Making a full model. All fixed effects included.

```
modelHoney = glmer.nb(formula= Honeybee ~ Island + Population + PatchSize + Temp + (
1 | Site) + (1 | Date), offset = log(FlowerOffset), data= dataInsect )

summary(modelHoney)

## Generalized linear mixed model fit by maximum likelihood (Laplace
##   Approximation) [glmerMod]
##  Family: Negative Binomial(0.3278)  ( log )
## Formula: Honeybee ~ Island + Population + PatchSize + Temp + (1 | Site) +
##     (1 | Date)
##    Data: dataInsect
##  Offset: log(FlowerOffset)
##
##      AIC      BIC   logLik deviance df.resid
##    817.4    848.0   -399.7    799.4      212
##
## Scaled residuals:
##     Min      1Q  Median      3Q     Max
## -0.5669 -0.4859 -0.2236  0.0000  3.6358
##
## Random effects:
##  Groups Name        Variance Std.Dev.
##  Site   (Intercept) 0.4370   0.6611
##  Date   (Intercept) 0.1331   0.3648
## Number of obs: 221, groups:  Site, 30; Date, 11
##
## Fixed effects:
##              Estimate Std. Error z value Pr(>|z|)
## (Intercept)  -8.25105    1.44960  -5.692 1.26e-08 ***
## IslandS     -25.59406   85.33351  -0.300 0.764231
## PopulationP   0.90833    0.53273   1.705 0.088188 .
## PatchSizeM   -0.72683    0.51155  -1.421 0.155366
## PatchSizeS   -2.66180    0.75557  -3.523 0.000427 ***
## Temp          0.17102    0.05751   2.974 0.002941 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##             (Intr) IslndS PpltnP PtchSM PtchSS
## IslandS      0.000
## PopulationP -0.255  0.000
## PatchSizeM  -0.081  0.000 -0.255
## PatchSizeS   0.030  0.000 -0.517  0.362
## Temp        -0.969  0.000  0.172 -0.029 -0.050
## optimizer (Nelder_Mead) convergence code: 0 (OK)
## Model is nearly unidentifiable: large eigenvalue ratio
##  - Rescale variables?
```

Starting drop1()

```
modelHoney = glmer.nb(formula= Honeybee ~ Island + Population + PatchSize + Temp + (
1 | Site) + (1 | Date), offset = log(FlowerOffset), data= dataInsect )

drop1(modelHoney)

## Single term deletions
##
## Model:
## Honeybee ~ Island + Population + PatchSize + Temp + (1 | Site) +
##     (1 | Date)
##            npar    AIC
## <none>          817.43
## Island        1 840.93
## Population    1 818.02
## PatchSize     2 825.35
## Temp          1 822.22
```

No further decrease in AIC available. Model stays as initial.

C.4: Variation in seed set (M4)

Making a full model. All fixed effects included

```
modelSeedSet = glmer.nb(formula= Seeds ~ Island + Population + PatchSize + Bumblebe
eLegFrq + BumblebeeRobFrq + HoneybeeFrq + (1 | Site), offset = log(SeedPotentialPer
Capsule), data= dataSeed )
```

```
## Warning in theta.ml(Y, mu, weights = object@resp$weights, limit = limit, :
## iteration limit reached
```

```
summary(modelSeedSet)
```

```
## Generalized linear mixed model fit by maximum likelihood (Laplace
##   Approximation) [glmerMod]
##  Family: Negative Binomial(48664.36)  ( log )
## Formula:
## Seeds ~ Island + Population + PatchSize + BumblebeeLegFrq + BumblebeeRobFrq +
##     HoneybeeFrq + (1 | Site)
##    Data: dataSeed
##  Offset: log(SeedPotentialPerCapsule)
##
##      AIC      BIC   logLik deviance df.resid
##    318.6    332.6   -149.3    298.6       20
##
## Scaled residuals:
##      Min       1Q   Median       3Q      Max
## -1.36799 -0.11246 -0.01021  0.09719  0.57409
##
## Random effects:
##  Groups Name        Variance Std.Dev.
##  Site   (Intercept) 0.09664  0.3109
## Number of obs: 30, groups:  Site, 30
##
## Fixed effects:
##                  Estimate Std. Error z value Pr(>|z|)
## (Intercept)     -0.401201   0.399746  -1.004  0.31555
## IslandS         -0.006095   0.195049  -0.031  0.97507
## PopulationP     -0.629848   0.211404  -2.979  0.00289 **
## PatchSizeM      -0.290399   0.232997  -1.246  0.21263
## PatchSizeS       0.032122   0.331759   0.097  0.92287
## BumblebeeLegFrq  1.024336   1.198128   0.855  0.39258
## BumblebeeRobFrq  0.145658   0.646678   0.225  0.82179
## HoneybeeFrq      0.146428   0.586709   0.250  0.80292
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##             (Intr) IslndS PpltnP PtchSM PtchSS BmblLF BmblRF
## IslandS     -0.572
## PopulationP -0.379  0.421
## PatchSizeM  -0.785  0.166  0.042
## PatchSizeS  -0.650  0.044 -0.354  0.762
## BumblbLgFrq -0.681  0.658  0.426  0.358  0.244
## BumblbRbFrq -0.653  0.075  0.112  0.617  0.592  0.012
## HoneybeeFrq -0.691  0.438 -0.032  0.573  0.619  0.415  0.360
```

Testing interactions between spatial variables and legitimate pollinators

Testing: Island * BumblebeeLegFrq

```
modelSeedSet = glmer.nb(formula= Seeds ~ Island * BumblebeeLegFrq + Population + Pa
tchSize + BumblebeeRobFrq + HoneybeeFrq + (1 | Site), offset = log(SeedPotentialPer
Capsule), data= dataSeed )

## Warning in theta.ml(Y, mu, weights = object@resp$weights, limit = limit, :
## iteration limit reached

summary(modelSeedSet)

## Generalized linear mixed model fit by maximum likelihood (Laplace
##   Approximation) [glmerMod]
##  Family: Negative Binomial(48689.91)  ( log )
## Formula:
## Seeds ~ Island * BumblebeeLegFrq + Population + PatchSize + BumblebeeRobFrq +
##     HoneybeeFrq + (1 | Site)
##    Data: dataSeed
##  Offset: log(SeedPotentialPerCapsule)
##
##      AIC      BIC   logLik deviance df.resid
##    320.6    336.0   -149.3    298.6       19
##
## Scaled residuals:
##      Min       1Q   Median       3Q      Max
## -1.36725 -0.11282 -0.01012  0.09801  0.57409
##
## Random effects:
##  Groups Name        Variance Std.Dev.
##  Site   (Intercept) 0.09663  0.3109
## Number of obs: 30, groups:  Site, 30
##
## Fixed effects:
##                            Estimate Std. Error z value Pr(>|z|)
## (Intercept)               -0.399239   0.406528  -0.982  0.32607
## IslandS                   -0.009488   0.215573  -0.044  0.96489
## BumblebeeLegFrq            1.021214   1.223285   0.835  0.40382
## PopulationP               -0.629530   0.212291  -2.965  0.00302 **
## PatchSizeM                -0.292173   0.238194  -1.227  0.21997
## PatchSizeS                 0.031861   0.333460   0.096  0.92388
## BumblebeeRobFrq            0.140673   0.661535   0.213  0.83160
## HoneybeeFrq                0.145346   0.594886   0.244  0.80698
## IslandS:BumblebeeLegFrq    0.131638   3.472678   0.038  0.96976
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##             (Intr) IslndS BmblLF PpltnP PtchSM PtchSS BmblRF HnybFr
## IslandS     -0.569
## BumblbLgFrq -0.685  0.630
## PopulationP -0.374  0.369  0.427
## PatchSizeM  -0.788  0.230  0.365  0.036
## PatchSizeS  -0.649  0.049  0.246 -0.349  0.753
## BumblbRbFrq -0.652  0.138  0.016  0.099  0.629  0.584
## HoneybeeFrq -0.694  0.419  0.421 -0.030  0.574  0.623  0.359
## IslndS:BmLF  0.107 -0.407 -0.055  0.046 -0.181 -0.006 -0.181 -0.035
```

Testing: Population * BumblebeeLegFrq

```
modelSeedSet = glmer.nb(formula= Seeds ~ Island + Population * BumblebeeLegFrq + Pa
tchSize + BumblebeeRobFrq + HoneybeeFrq + (1 | Site), offset = log(SeedPotentialPer
Capsule), data= dataSeed )

## Warning in checkConv(attr(opt, "derivs"), opt$par, ctrl = control$checkConv, :
## Model failed to converge with max|grad| = 0.00335093 (tol = 0.002, component 1)

## Warning in theta.ml(Y, mu, weights = object@resp$weights, limit = limit, :
## iteration limit reached

summary(modelSeedSet)

## Generalized linear mixed model fit by maximum likelihood (Laplace
##   Approximation) [glmerMod]
##  Family: Negative Binomial(36949.54)  ( log )
## Formula:
## Seeds ~ Island + Population * BumblebeeLegFrq + PatchSize + BumblebeeRobFrq +
##     HoneybeeFrq + (1 | Site)
##    Data: dataSeed
##  Offset: log(SeedPotentialPerCapsule)
##
##      AIC      BIC   logLik deviance df.resid
##    312.2    327.6   -145.1    290.2       19
##
## Scaled residuals:
##      Min       1Q   Median       3Q      Max
## -1.54124 -0.12714  0.02837  0.12060  1.08804
##
## Random effects:
##  Groups Name        Variance Std.Dev.
##  Site   (Intercept) 0.06846  0.2616
## Number of obs: 30, groups:  Site, 30
##
## Fixed effects:
##                             Estimate Std. Error z value Pr(>|z|)
## (Intercept)                  -0.6054     0.3546  -1.708  0.08771 .
## IslandS                       0.1680     0.1771   0.948  0.34291
## PopulationP                  -1.1010     0.2340  -4.705 2.54e-06 ***
## BumblebeeLegFrq               0.8742     1.0323   0.847  0.39707
## PatchSizeM                   -0.1578     0.2060  -0.766  0.44374
## PatchSizeS                    0.4329     0.3127   1.384  0.16622
## BumblebeeRobFrq               0.2661     0.5659   0.470  0.63826
## HoneybeeFrq                   0.9107     0.5644   1.614  0.10659
## PopulationP:BumblebeeLegFrq   6.5810     2.0200   3.258  0.00112 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##             (Intr) IslndS PpltnP BmblLF PtchSM PtchSS BmblRF HnybFr
## IslandS     -0.593
## PopulationP -0.181  0.122
## BumblbLgFrq -0.663  0.619  0.359
## PatchSizeM  -0.797  0.222 -0.082  0.349
## PatchSizeS  -0.663  0.166 -0.493  0.213  0.766
## BumblbRbFrq -0.661  0.096  0.052  0.018  0.627  0.576
## HoneybeeFrq -0.699  0.515 -0.277  0.369  0.599  0.685  0.363
## PpltnP:BmLF -0.172  0.300 -0.631 -0.048  0.192  0.391  0.062  0.416
```

Testing: Patch Size * BumblebeeLegFrq

```
modelSeedSet = glmer.nb(formula= Seeds ~ Island + Population + PatchSize * BumblebeeLegFrq + BumblebeeRobFrq + HoneybeeFrq + (1 | Site), offset = log(SeedPotentialPerCapsule), data= dataSeed )

## Warning in checkConv(attr(opt, "derivs"), opt$par, ctrl = control$checkConv, :
## Model failed to converge with max|grad| = 0.00251547 (tol = 0.002, component 1)

summary(modelSeedSet)

## Generalized linear mixed model fit by maximum likelihood (Laplace
##   Approximation) [glmerMod]
##  Family: Negative Binomial(40900.75)  ( log )
## Formula:
## Seeds ~ Island + Population + PatchSize * BumblebeeLegFrq + BumblebeeRobFrq +
##     HoneybeeFrq + (1 | Site)
##    Data: dataSeed
##  Offset: log(SeedPotentialPerCapsule)
##
##      AIC      BIC   logLik deviance df.resid
##    316.2    333.0   -146.1    292.2       18
##
## Scaled residuals:
##      Min       1Q   Median       3Q      Max
## -1.45130 -0.10626 -0.02304  0.19501  0.88935
##
## Random effects:
##  Groups Name        Variance Std.Dev.
##  Site   (Intercept) 0.07492  0.2737
## Number of obs: 30, groups:  Site, 30
##
## Fixed effects:
##                              Estimate Std. Error z value Pr(>|z|)
## (Intercept)                    0.3098     0.5043   0.614  0.53896
## IslandS                        0.2174     0.1960   1.109  0.26748
## PopulationP                   -0.5021     0.1945  -2.581  0.00985 **
## PatchSizeM                    -1.1074     0.4495  -2.464  0.01375 *
## PatchSizeS                    -1.0931     0.5293  -2.065  0.03893 *
## BumblebeeLegFrq               -0.3540     1.3492  -0.262  0.79303
## BumblebeeRobFrq               -1.0376     0.7686  -1.350  0.17703
## HoneybeeFrq                   -0.6266     0.6491  -0.965  0.33436
## PatchSizeM:BumblebeeLegFrq     3.8609     2.2549   1.712  0.08686 .
## PatchSizeS:BumblebeeLegFrq    10.5268     3.7475   2.809  0.00497 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##             (Intr) IslndS PpltnP PtchSM PtchSS BmblLF BmblRF HnybFr PSM:BL
## IslandS     -0.248
## PopulationP -0.129  0.457
## PatchSizeM  -0.878 -0.107 -0.158
## PatchSizeS  -0.806 -0.225 -0.379  0.901
## BumblbLgFrq -0.808  0.402  0.226  0.657  0.555
## BumblbRbFrq -0.804 -0.107 -0.057  0.795  0.786  0.387
## HoneybeeFrq -0.809  0.223 -0.131  0.725  0.735  0.619  0.596
## PtchSzM:BLF  0.692  0.152  0.188 -0.877 -0.752 -0.594 -0.635 -0.565
## PtchSzS:BLF  0.386  0.433  0.217 -0.527 -0.664 -0.267 -0.449 -0.326  0.483
```

Testing combinations of interactions

Testing: Island * BumblebeeLegFrq + Population * BumblebeeLegFrq

```
modelSeedSet = glmer.nb(formula= Seeds ~ Island * BumblebeeLegFrq + Population * Bu
mblebeeLegFrq + PatchSize + BumblebeeRobFrq + HoneybeeFrq + (1 | Site), offset = lo
g(SeedPotentialPerCapsule), data= dataSeed )

## Warning in theta.ml(Y, mu, weights = object@resp$weights, limit = limit, :
## iteration limit reached

summary(modelSeedSet)

## Generalized linear mixed model fit by maximum likelihood (Laplace
##   Approximation) [glmerMod]
##  Family: Negative Binomial(35755.97)  ( log )
## Formula: Seeds ~ Island * BumblebeeLegFrq + Population * BumblebeeLegFrq +
##     PatchSize + BumblebeeRobFrq + HoneybeeFrq + (1 | Site)
##    Data: dataSeed
##  Offset: log(SeedPotentialPerCapsule)
##
##      AIC      BIC   logLik deviance df.resid
##    313.7    330.5   -144.9    289.7       18
##
## Scaled residuals:
##      Min       1Q   Median       3Q      Max
## -1.57237 -0.11899  0.03862  0.10721  1.13000
##
## Random effects:
##  Groups Name        Variance Std.Dev.
##  Site   (Intercept) 0.06676  0.2584
## Number of obs: 30, groups:  Site, 30
##
## Fixed effects:
##                             Estimate Std. Error z value Pr(>|z|)
## (Intercept)                  -0.6507     0.3555  -1.830 0.067198 .
## IslandS                       0.2354     0.1987   1.184 0.236296
## BumblebeeLegFrq               0.9220     1.0212   0.903 0.366586
## PopulationP                  -1.1316     0.2355  -4.805 1.55e-06 ***
## PatchSizeM                   -0.1206     0.2098  -0.575 0.565420
## PatchSizeS                    0.4593     0.3116   1.474 0.140516
## BumblebeeRobFrq               0.3568     0.5726   0.623 0.533198
## HoneybeeFrq                   0.9717     0.5665   1.715 0.086314 .
## IslandS:BumblebeeLegFrq      -2.2031     3.0860  -0.714 0.475284
## BumblebeeLegFrq:PopulationP   6.9415     2.0673   3.358 0.000786 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##             (Intr) IslndS BmblLF PpltnP PtchSM PtchSS BmblRF HnybFr IS:BLF
## IslandS     -0.597
## BumblbLgFrq -0.661  0.576
## PopulationP -0.139  0.018  0.336
## PatchSizeM  -0.804  0.304  0.352 -0.125
## PatchSizeS  -0.669  0.199  0.218 -0.506  0.765
## BumblbRbFrq -0.672  0.183  0.030  0.011  0.645  0.581
## HoneybeeFrq -0.707  0.518  0.371 -0.302  0.610  0.691  0.380
## IslndS:BmLF  0.171 -0.470 -0.066  0.174 -0.240 -0.108 -0.213 -0.143
## BmblbLgF:PP -0.212  0.371 -0.024 -0.646  0.241  0.409  0.108  0.442 -0.228
```

Testing: Island * BumblebeeLegFrq + PatchSize * BumblebeeLegFrq

```
modelSeedSet = glmer.nb(formula= Seeds ~ Island * BumblebeeLegFrq + Population + Pa
tchSize * BumblebeeLegFrq + BumblebeeRobFrq + HoneybeeFrq + (1 | Site), offset = lo
g(SeedPotentialPerCapsule), data= dataSeed )

## Warning in theta.ml(Y, mu, weights = object@resp$weights, limit = limit, :
## iteration limit reached

summary(modelSeedSet)

## Generalized linear mixed model fit by maximum likelihood (Laplace
##   Approximation) [glmerMod]
##  Family: Negative Binomial(37184.75)  ( log )
## Formula:
## Seeds ~ Island * BumblebeeLegFrq + Population + PatchSize * BumblebeeLegFrq +
##     BumblebeeRobFrq + HoneybeeFrq + (1 | Site)
##    Data: dataSeed
##  Offset: log(SeedPotentialPerCapsule)
##
##      AIC      BIC   logLik deviance df.resid
##    316.5    334.7   -145.2    290.5       17
##
## Scaled residuals:
##      Min       1Q   Median       3Q      Max
## -1.51204 -0.11821 -0.02329  0.13159  1.00136
##
## Random effects:
##  Groups Name        Variance Std.Dev.
##  Site   (Intercept) 0.0693   0.2632
## Number of obs: 30, groups:  Site, 30
##
## Fixed effects:
##                             Estimate Std. Error z value Pr(>|z|)
## (Intercept)                   0.4282     0.4629   0.925 0.354947
## IslandS                       0.3869     0.2148   1.801 0.071665 .
## BumblebeeLegFrq              -0.6142     1.2156  -0.505 0.613374
## PopulationP                  -0.4807     0.1869  -2.571 0.010127 *
## PatchSizeM                   -1.2608     0.4032  -3.127 0.001767 **
## PatchSizeS                   -1.3672     0.4934  -2.771 0.005588 **
## BumblebeeRobFrq              -1.1706     0.7265  -1.611 0.107133
## HoneybeeFrq                  -0.7907     0.6099  -1.296 0.194884
## IslandS:BumblebeeLegFrq      -4.4885     3.0259  -1.483 0.137985
## BumblebeeLegFrq:PatchSizeM    4.9039     2.0046   2.446 0.014432 *
## BumblebeeLegFrq:PatchSizeS   13.0037     3.5214   3.693 0.000222 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##              (Intr) IslndS BmblLF PpltnP PtchSM PtchSS BmblRF HnybFr IS:BLF
## IslandS      -0.205
## BumblbLgFrq -0.782  0.349
## PopulationP -0.148  0.409  0.246
## PatchSizeM  -0.867 -0.141  0.617 -0.148
## PatchSizeS  -0.779 -0.301  0.503 -0.380  0.885
## BumblbRbFrq -0.798 -0.090  0.341 -0.037  0.778  0.754
## HoneybeeFrq -0.794  0.163  0.582 -0.129  0.706  0.715  0.574
## IslndS:BmLF -0.071 -0.506  0.051 -0.052  0.137  0.256  0.038  0.099
## BmblbLF:PSM  0.648  0.228 -0.545  0.180 -0.852 -0.729 -0.588 -0.526 -0.232
## BmblbLF:PSS  0.335  0.515 -0.213  0.205 -0.488 -0.651 -0.388 -0.294 -0.352
##             BLF:PSM
```

```
## IslandS
## BumblbLgFrq
## PopulationP
## PatchSizeM
## PatchSizeS
## BumblbRbFrq
## HoneybeeFrq
## IslndS:BmLF
## BmblbLF:PSM
## BmblbLF:PSS  0.468
```

Testing: Population * BumblebeeLegFrq + PatchSize * BumblebeeLegFrq

```
modelSeedSet = glmer.nb(formula= Seeds ~ Island + Population * BumblebeeLegFrq + Pa
tchSize * BumblebeeLegFrq + BumblebeeRobFrq + HoneybeeFrq + (1 | Site), offset = lo
g(SeedPotentialPerCapsule), data= dataSeed )

## Warning in theta.ml(Y, mu, weights = object@resp$weights, limit = limit, :
## iteration limit reached

summary(modelSeedSet)

## Generalized linear mixed model fit by maximum likelihood (Laplace
##   Approximation) [glmerMod]
##  Family: Negative Binomial(36655.82)  ( log )
## Formula:
## Seeds ~ Island + Population * BumblebeeLegFrq + PatchSize * BumblebeeLegFrq +
##     BumblebeeRobFrq + HoneybeeFrq + (1 | Site)
##    Data: dataSeed
##  Offset: log(SeedPotentialPerCapsule)
##
##      AIC      BIC   logLik deviance df.resid
##    316.1    334.3   -145.0    290.1       17
##
## Scaled residuals:
##      Min       1Q   Median       3Q      Max
## -1.52328 -0.11334  0.01819  0.12226  1.11649
##
## Random effects:
##  Groups Name        Variance Std.Dev.
##  Site   (Intercept) 0.06808  0.2609
## Number of obs: 30, groups:  Site, 30
##
## Fixed effects:
##                              Estimate Std. Error z value Pr(>|z|)
## (Intercept)                 -0.44272    0.79067  -0.560   0.5755
## IslandS                      0.16974    0.21512   0.789   0.4301
## PopulationP                 -1.06049    0.50609  -2.095   0.0361 *
## BumblebeeLegFrq              0.53870    1.46840   0.367   0.7137
## PatchSizeM                  -0.33054    0.80642  -0.410   0.6819
## PatchSizeS                   0.24003    1.29270   0.186   0.8527
## BumblebeeRobFrq              0.04751    1.22338   0.039   0.9690
## HoneybeeFrq                  0.70907    1.25500   0.565   0.5721
## PopulationP:BumblebeeLegFrq  6.21914    4.95427   1.255   0.2094
## BumblebeeLegFrq:PatchSizeM   0.91833    3.38409   0.271   0.7861
## BumblebeeLegFrq:PatchSizeS   0.79423   10.36407   0.077   0.9389
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
## Correlation of Fixed Effects:
##             (Intr) IslndS PpltnP BmblLF PtchSM PtchSS BmblRF HnybFr PP:BLF
## IslandS      0.226
## PopulationP  0.701  0.549
## BumblbLgFrq -0.809  0.091 -0.363
## PatchSizeM  -0.956 -0.436 -0.811  0.709
## PatchSizeS  -0.921 -0.495 -0.904  0.628  0.967
## BumblbRbFrq -0.927 -0.424 -0.743  0.582  0.930  0.918
## HoneybeeFrq -0.931 -0.287 -0.830  0.680  0.925  0.940  0.866
## PpltnP:BmLF -0.781 -0.420 -0.928  0.467  0.834  0.907  0.778  0.865
## BmblbLF:PSM  0.882  0.441  0.760 -0.698 -0.952 -0.902 -0.861 -0.850 -0.765
## BmblbLF:PSS  0.823  0.566  0.886 -0.529 -0.892 -0.952 -0.840 -0.865 -0.914
##             BLF:PSM
## IslandS
## PopulationP
## BumblbLgFrq
## PatchSizeM
## PatchSizeS
## BumblbRbFrq
## HoneybeeFrq
## PpltnP:BmLF
## BmblbLF:PSM
## BmblbLF:PSS  0.838
```

Testing combination of all interactions: Island * BumblebeeLegFrq + Population * BumblebeeLegFrq + PatchSize * BumblebeeLegFrq

```
modelSeedSet = glmer.nb(formula= Seeds ~ Island * BumblebeeLegFrq + Population * Bum
blebeeLegFrq + PatchSize * BumblebeeLegFrq + BumblebeeRobFrq + HoneybeeFrq + (1 | Si
te), offset = log(SeedPotentialPerCapsule), data= dataSeed )

## Warning in theta.ml(Y, mu, weights = object@resp$weights, limit = limit, :
## iteration limit reached

summary(modelSeedSet)

## Generalized linear mixed model fit by maximum likelihood (Laplace
##   Approximation) [glmerMod]
##  Family: Negative Binomial(35009.49)  ( log )
## Formula: Seeds ~ Island * BumblebeeLegFrq + Population * BumblebeeLegFrq +
##     PatchSize * BumblebeeLegFrq + BumblebeeRobFrq + HoneybeeFrq +
##     (1 | Site)
##    Data: dataSeed
##  Offset: log(SeedPotentialPerCapsule)
##
##      AIC      BIC   logLik deviance df.resid
##    317.2    336.9   -144.6    289.2       16
##
## Scaled residuals:
##     Min      1Q  Median      3Q     Max
## -1.5558 -0.1071  0.0193  0.1158  1.1615
##
## Random effects:
##  Groups Name        Variance Std.Dev.
##  Site   (Intercept) 0.06543  0.2558
## Number of obs: 30, groups:  Site, 30
##
```

```
## Fixed effects:
##                              Estimate Std. Error z value Pr(>|z|)
## (Intercept)                   -0.2011     0.7100  -0.283   0.7770
## IslandS                        0.3021     0.2246   1.345   0.1786
## BumblebeeLegFrq                0.1663     1.4721   0.113   0.9101
## PopulationP                   -0.9288     0.4159  -2.233   0.0255 *
## PatchSizeM                    -0.6028     0.6934  -0.869   0.3847
## PatchSizeS                    -0.2349     1.0511  -0.223   0.8232
## BumblebeeRobFrq               -0.2738     1.0350  -0.265   0.7913
## HoneybeeFrq                    0.3130     1.1000   0.285   0.7760
## IslandS:BumblebeeLegFrq       -3.2360     3.0730  -1.053   0.2923
## BumblebeeLegFrq:PopulationP    4.9246     4.1235   1.194   0.2324
## BumblebeeLegFrq:PatchSizeM     2.2826     3.1253   0.730   0.4652
## BumblebeeLegFrq:PatchSizeS     4.6077     7.5951   0.607   0.5441
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##             (Intr) IslndS BmblLF PpltnP PtchSM PtchSS BmblRF HnybFr IS:BLF
## IslandS      0.072
## BumblbLgFrq -0.824  0.153
## PopulationP  0.591  0.399 -0.279
## PatchSizeM  -0.945 -0.298  0.720 -0.719
## PatchSizeS  -0.901 -0.373  0.628 -0.849  0.954
## BumblbRbFrq -0.904 -0.261  0.565 -0.628  0.902  0.881
## HoneybeeFrq -0.913 -0.130  0.678 -0.761  0.899  0.922  0.819
## IslndS:BmLF -0.267 -0.543  0.201 -0.252  0.317  0.369  0.238  0.287
## BmblbLgF:PP -0.698 -0.237  0.406 -0.896  0.748  0.850  0.676  0.810  0.247
## BmblbLF:PSM  0.850  0.348 -0.699  0.657 -0.939 -0.875 -0.813 -0.803 -0.370
## BmblbLF:PSS  0.755  0.477 -0.494  0.819 -0.837 -0.922 -0.758 -0.808 -0.411
##             BLF:PP BLF:PSM
## IslandS
## BumblbLgFrq
## PopulationP
## PatchSizeM
## PatchSizeS
## BumblbRbFrq
## HoneybeeFrq
## IslndS:BmLF
## BmblbLgF:PP
## BmblbLF:PSM -0.659
## BmblbLF:PSS -0.855  0.782
```

Best interaction/ combination of interactions: Population * BumblebeeLegFrq

Starting drop1()

```
modelSeedSet = glmer.nb(formula= Seeds ~ Island + Population * BumblebeeLegFrq + Pa
tchSize + BumblebeeRobFrq + HoneybeeFrq + (1 | Site), offset = log(SeedPotentialPer
Capsule), data= dataSeed )

## Warning in checkConv(attr(opt, "derivs"), opt$par, ctrl = control$checkConv, :
## Model failed to converge with max|grad| = 0.00335093 (tol = 0.002, component 1)

## Warning in theta.ml(Y, mu, weights = object@resp$weights, limit = limit, :
## iteration limit reached

drop1(modelSeedSet)
```

```
## Single term deletions
##
## Model:
## Seeds ~ Island + Population * BumblebeeLegFrq + PatchSize + BumblebeeRobFrq +
##     HoneybeeFrq + (1 | Site)
##                            npar    AIC
## <none>                          312.22
## Island                        1 311.07
## PatchSize                     2 316.73
## BumblebeeRobFrq               1 310.44
## HoneybeeFrq                   1 312.62
## Population:BumblebeeLegFrq    1 318.57
```

Dropping Primary Nectar Robbers (short tongued bumblebees)

```
modelSeedSet = glmer.nb(formula= Seeds ~ Island + Population * BumblebeeLegFrq + Pa
tchSize + HoneybeeFrq + (1 | Site), offset = log(SeedPotentialPerCapsule), data= da
taSeed )

## Warning in checkConv(attr(opt, "derivs"), opt$par, ctrl = control$checkConv, :
## Model failed to converge with max|grad| = 0.00211001 (tol = 0.002, component 1)

drop1(modelSeedSet)

## Single term deletions
##
## Model:
## Seeds ~ Island + Population * BumblebeeLegFrq + PatchSize + HoneybeeFrq +
##     (1 | Site)
##                            npar    AIC
## <none>                          310.44
## Island                        1 309.21
## PatchSize                     2 315.01
## HoneybeeFrq                   1 310.63
## Population:BumblebeeLegFrq    1 316.62
```

Dropping Island

```
modelSeedSet = glmer.nb(formula= Seeds ~ Population * BumblebeeLegFrq + PatchSize +
HoneybeeFrq + (1 | Site), offset = log(SeedPotentialPerCapsule), data= dataSeed )

## Warning in theta.ml(Y, mu, weights = object@resp$weights, limit = limit, :
## iteration limit reached

drop1(modelSeedSet)
```

```
## Single term deletions
##
## Model:
## Seeds ~ Population * BumblebeeLegFrq + PatchSize + HoneybeeFrq +
##     (1 | Site)
##                             npar    AIC
## <none>                           309.21
## PatchSize                      2 314.06
## HoneybeeFrq                    1 308.64
## Population:BumblebeeLegFrq     1 314.62
```

Dropping Secondary Nectar Robbers (honeybees)

```
modelSeedSet = glmer.nb(formula= Seeds ~ Population * BumblebeeLegFrq + PatchSize +
(1 | Site), offset = log(SeedPotentialPerCapsule), data= dataSeed )

## Warning in theta.ml(Y, mu, weights = object@resp$weights, limit = limit, :
## iteration limit reached

drop1(modelSeedSet)

## Single term deletions
##
## Model:
## Seeds ~ Population * BumblebeeLegFrq + PatchSize + (1 | Site)
##                             npar    AIC
## <none>                           308.64
## PatchSize                      2 312.71
## Population:BumblebeeLegFrq     1 312.67
```

No further decrease in AIC available. Final model as follows:

```
modelSeedSet = glmer.nb(formula= Seeds ~ Population * BumblebeeLegFrq + PatchSize +
(1 | Site), offset = log(SeedPotentialPerCapsule), data= dataSeed )

## Warning in theta.ml(Y, mu, weights = object@resp$weights, limit = limit, :
## iteration limit reached

summary(modelSeedSet)

## Generalized linear mixed model fit by maximum likelihood (Laplace
##   Approximation) [glmerMod]
##  Family: Negative Binomial(38261.88)  ( log )
## Formula: Seeds ~ Population * BumblebeeLegFrq + PatchSize + (1 | Site)
##    Data: dataSeed
##  Offset: log(SeedPotentialPerCapsule)
##
##      AIC      BIC   logLik deviance df.resid
##    308.6    319.9   -146.3    292.6       22
##
```

```
## Scaled residuals:
##      Min       1Q   Median       3Q      Max
## -1.55323 -0.08443 -0.01381  0.16976  0.93219
##
## Random effects:
##  Groups Name        Variance Std.Dev.
##  Site   (Intercept) 0.07516  0.2742
## Number of obs: 30, groups:  Site, 30
##
## Fixed effects:
##                            Estimate Std. Error z value Pr(>|z|)
## (Intercept)                 -0.2069     0.1702  -1.216  0.22406
## PopulationP                 -0.9984     0.2110  -4.731 2.23e-06 ***
## BumblebeeLegFrq              0.1667     0.8302   0.201  0.84082
## PatchSizeM                  -0.3471     0.1431  -2.426  0.01528 *
## PatchSizeS                   0.1024     0.1982   0.517  0.60541
## PopulationP:BumblebeeLegFrq  5.0985     1.7897   2.849  0.00439 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##             (Intr) PpltnP BmblLF PtchSM PtchSS
## PopulationP -0.477
## BumblbLgFrq -0.827  0.455
## PatchSizeM  -0.656  0.049  0.396
## PatchSizeS  -0.319 -0.549  0.200  0.472
## PpltnP:BmLF  0.250 -0.642 -0.360 -0.021  0.264
```

Appendix D

D.1: Flower visitation conducted by legitimate bumblebees, excluding *B. lapidarius*

Making a full model. All fixed effects included.

```
modelBBLegNew
= glmer.nb(formula= BumblebeeLeg ~ Island + Population + PatchSize + Temp + (1 | Sit
e) + (1 | Date), offset = log(FlowerOffset),data= dataHumleNew )

## boundary (singular) fit: see ?isSingular

summary(modelBBLegNew)

## Generalized linear mixed model fit by maximum likelihood (Laplace
##   Approximation) [glmerMod]
##  Family: Negative Binomial(0.3142)  ( log )
## Formula: BumblebeeLeg ~ Island + Population + PatchSize + Temp + (1 |
##     Site) + (1 | Date)
##    Data: dataHumleNew
##  Offset: log(FlowerOffset)
##
##      AIC      BIC   logLik deviance df.resid
##    724.6    755.2   -353.3    706.6      212
##
## Scaled residuals:
##     Min      1Q  Median      3Q     Max
## -0.5519 -0.4932 -0.4245  0.1202  4.9523
##
## Random effects:
##  Groups Name        Variance  Std.Dev.
##  Site   (Intercept) 1.550e-11 3.937e-06
##  Date   (Intercept) 1.047e-10 1.023e-05
## Number of obs: 221, groups:  Site, 30; Date, 11
##
## Fixed effects:
##             Estimate Std. Error z value Pr(>|z|)
## (Intercept) -3.50741    0.94404  -3.715 0.000203 ***
## IslandS     -1.59686    0.42827  -3.729 0.000193 ***
## PopulationP -1.66321    0.41028  -4.054 5.04e-05 ***
## PatchSizeM   0.80631    0.34078   2.366 0.017978 *
## PatchSizeS   1.04499    0.47946   2.180 0.029294 *
## Temp        -0.04243    0.03612  -1.175 0.240034
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##             (Intr) IslndS PpltnP PtchSM PtchSS
## IslandS     -0.402
## PopulationP -0.318  0.348
## PatchSizeM  -0.140 -0.202 -0.301
## PatchSizeS   0.004 -0.201 -0.695  0.489
## Temp        -0.971  0.350  0.253  0.037 -0.049
## optimizer (Nelder_Mead) convergence code: 0 (OK)
## boundary (singular) fit: see ?isSingular
```

Starting drop1()

```
drop1(modelBBLegNew)

## Single term deletions
##
## Model:
## BumblebeeLeg ~ Island + Population + PatchSize + Temp + (1 |
##     Site) + (1 | Date)
##            npar    AIC
## <none>          724.57
## Island        1 734.72
## Population    1 737.97
## PatchSize     2 727.63
## Temp          1 723.89
```

Dropping Temperature

```
modelBBLegNew
= glmer.nb(formula= BumblebeeLeg ~ Island + Population + PatchSize + (1 |Site) + (1 |
 Date), offset = log(FlowerOffset),data= dataHumleNew )

## boundary (singular) fit: see ?isSingular

drop1(modelBBLegNew)
## boundary (singular) fit: see ?isSingular
## boundary (singular) fit: see ?isSingular
## boundary (singular) fit: see ?isSingular
## Single term deletions
##
## Model:
## BumblebeeLeg ~ Island + Population + PatchSize + (1 | Site) +
##     (1 | Date)
##            npar    AIC
## <none>          723.89
## Island        1 732.76
## Population    1 736.17
## PatchSize     2 727.03
```

No further decrease in AIC available. Final model as follows:

```
summary(modelBBLegNew)

## Generalized linear mixed model fit by maximum likelihood (Laplace
##   Approximation) [glmerMod]
##  Family: Negative Binomial(0.3154)  ( log )
## Formula: BumblebeeLeg ~ Island + Population + PatchSize + (1 | Site) +
##     (1 | Date)
##    Data: dataHumleNew
##  Offset: log(FlowerOffset)
##
##      AIC      BIC   logLik deviance df.resid
##    723.9    751.1   -353.9    707.9      213
##
## Scaled residuals:
##     Min      1Q  Median      3Q     Max
## -0.5508 -0.4943 -0.4244  0.1167  5.8901
```

```
## Random effects:
##  Groups Name        Variance  Std.Dev.
##  Site   (Intercept) 3.529e-12 1.879e-06
##  Date   (Intercept) 2.470e-02 1.572e-01
## Number of obs: 221, groups:  Site, 30; Date, 11
##
## Fixed effects:
##             Estimate Std. Error z value Pr(>|z|)
## (Intercept)  -4.5845     0.2330 -19.675  < 2e-16 ***
## IslandS      -1.4366     0.4130  -3.478 0.000504 ***
## PopulationP  -1.5693     0.4059  -3.866 0.000111 ***
## PatchSizeM    0.8174     0.3405   2.401 0.016368 *
## PatchSizeS    1.0314     0.4797   2.150 0.031562 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##             (Intr) IslndS PpltnP PtchSM
## IslandS     -0.259
## PopulationP -0.275  0.310
## PatchSizeM  -0.418 -0.221 -0.295
## PatchSizeS  -0.185 -0.213 -0.706  0.481
## optimizer (Nelder_Mead) convergence code: 0 (OK)
## boundary (singular) fit: see ?isSingular
```

D.2: Flower visitation conducted by primary robbers, including *B. lapidarius*

Making a full model. All fixed effects included.

```
modelBBRobNew
= glmer.nb(formula= BumblebeeRob ~ Island + Population + PatchSize + Temp + (1 | Site) +
(1 | Date), offset = log(FlowerOffset),data= dataHumleNew )

summary(modelBBRobNew)

## Generalized linear mixed model fit by maximum likelihood (Laplace
##   Approximation) [glmerMod]
##  Family: Negative Binomial(0.3201)  ( log )
## Formula: BumblebeeRob ~ Island + Population + PatchSize + Temp + (1 |
##     Site) + (1 | Date)
##    Data: dataHumleNew
##  Offset: log(FlowerOffset)
##
##      AIC      BIC   logLik deviance df.resid
##   1139.1   1169.7   -560.5   1121.1      212
##
## Scaled residuals:
##     Min      1Q  Median      3Q     Max
## -0.5625 -0.5223 -0.4087  0.1369  4.6732
##
## Random effects:
##  Groups Name        Variance Std.Dev.
##  Site   (Intercept) 0.01610  0.1269
##  Date   (Intercept) 0.07577  0.2753
## Number of obs: 221, groups:  Site, 30; Date, 11
##
## Fixed effects:
##             Estimate Std. Error z value Pr(>|z|)
## (Intercept) -1.60696    1.08353  -1.483 0.138052
## IslandS     -1.09306    0.46927  -2.329 0.019843 *
## PopulationP -0.78101    0.37514  -2.082 0.037348 *
## PatchSizeM  -0.72584    0.31243  -2.323 0.020170 *
## PatchSizeS  -1.58795    0.46054  -3.448 0.000565 ***
## Temp        -0.04432    0.04217  -1.051 0.293289
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##             (Intr) IslndS PpltnP PtchSM PtchSS
## IslandS     -0.352
## PopulationP -0.372  0.358
## PatchSizeM  -0.101 -0.122 -0.186
## PatchSizeS   0.109 -0.147 -0.554  0.365
## Temp        -0.970  0.262  0.297  0.014 -0.110
```

Starting drop1()

```
drop1(modelBBRobNew)
## Warning in checkConv(attr(opt, "derivs"), opt$par, ctrl = control$checkConv, :
## Model failed to converge with max|grad| = 0.0076595 (tol = 0.002, component 1)
## Single term deletions
##
## Model:
## BumblebeeRob ~ Island + Population + PatchSize + Temp + (1 |
##     Site) + (1 | Date)
##            npar    AIC
## <none>          1139.1
## Island        1 1142.1
## Population    1 1141.2
## PatchSize     2 1146.7
## Temp          1 1138.2
```

Dropping Temperature

```
modelBBRobNew
= glmer.nb(formula= BumblebeeRob ~ Island + Population + PatchSize + (1 |Site) + (1 | Date
), offset = log(FlowerOffset),data= dataHumleNew )

drop1(modelBBRobNew)

## Warning in checkConv(attr(opt, "derivs"), opt$par, ctrl = control$checkConv, :
## Model failed to converge with max|grad| = 0.00799943 (tol = 0.002, component 1)
## Single term deletions
##
## Model:
## BumblebeeRob ~ Island + Population + PatchSize + (1 | Site) +
##     (1 | Date)
##            npar    AIC
## <none>          1138.2
## Island        1 1140.3
## Population    1 1139.4
## PatchSize     2 1146.0
```

No further decrease in AIC available. Final model as follows:

```
summary(modelBBRobNew)

## Generalized linear mixed model fit by maximum likelihood (Laplace
##   Approximation) [glmerMod]
##  Family: Negative Binomial(0.322)  ( log )
## Formula: BumblebeeRob ~ Island + Population + PatchSize + (1 | Site) +
##     (1 | Date)
##    Data: dataHumleNew
##  Offset: log(FlowerOffset)
##
##      AIC      BIC   logLik deviance df.resid
##   1138.2   1165.3   -561.1   1122.2      213
##
## Scaled residuals:
##     Min      1Q  Median      3Q     Max
## -0.5638 -0.5226 -0.4035  0.1982  4.2297
```

```
## Random effects:
##  Groups Name        Variance Std.Dev.
##  Site   (Intercept) 0.02763  0.1662
##  Date   (Intercept) 0.10517  0.3243
## Number of obs: 221, groups:  Site, 30; Date, 11
##
## Fixed effects:
##             Estimate Std. Error z value Pr(>|z|)
## (Intercept)  -2.7243     0.2648 -10.289  < 2e-16 ***
## IslandS      -0.9351     0.4391  -2.130 0.033203 *
## PopulationP  -0.6726     0.3635  -1.850 0.064277 .
## PatchSizeM   -0.7260     0.3167  -2.292 0.021904 *
## PatchSizeS   -1.6617     0.4563  -3.642 0.000271 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##             (Intr) IslndS PpltnP PtchSM
## IslandS     -0.361
## PopulationP -0.331  0.283
## PatchSizeM  -0.378 -0.136 -0.189
## PatchSizeS  -0.031 -0.081 -0.539  0.358
```