

Reproduction and pollination in bilberry (*Vaccinium myrtillus*) along
two elevational gradients in western Norway



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Master of Science in Biology

Biodiversity, Evolution and Ecology



Department of Biology
University of Bergen
March 2022

Front page:

Bumblebee (*B. pascuorum*) on bilberry in Sogn, Norway, 2021. Photo: Hedda V. B. Ørbæk

Acknowledgements

I would like to thank my supervisors *Dr. Aud H. Halbritter*, *Asoc. Prof. Bjørn Arild Hatteland* and *Prof. Stein Joar Hegland* for the possibility to conduct this study. To my main supervisor *Aud* for always finding time and patiently guiding me through all parts of this process. To *Bjørn Arild* for brilliant ideas and encouragement, especially on the buzzing side of things. To *Stein Joar* for valuable knowledge and insights in this field and during fieldwork. I would also like to thank *Mark A. Gillesipe* for help and guidance with data and fieldwork. To my field-companion *Maren* for coming to Kaupanger helping me with fieldwork. To *Louise* and *Kenneth* for helping with barcoding of *Bombus* individuals. To the *EECRG writing group* and *Jon* for help with constructive feedback on my drafts and to *Frida* for brilliant map skills. To the *Rclub* for help with statistical analysis, and a special thanks to *Joe* for statistical help. To *the Natural History Collections* in Bergen for lending material. And finally, a special thanks to everyone in *verdens beste lesesal* for much needed encouragement. This study was partly funded by *Myrdyrkningsfondet*.

Abstract

Climate change affects species distributions and abundances, as well as driving changes in species phenological events. Plant-pollinator mutualisms may be vulnerable to such changes, as disruption of the overlap in temporal timing or spatial distribution can alter interaction opportunities between them. Possible effects of climate change on plants and pollinators are numerous and will vary among species and regions, and it is still unclear whether these relationships are resilient under novel climatic conditions. Bilberry (*Vaccinium myrtillus*) is a functional important species in boreal ecosystems, both outcrossed by insects and self-pollinated, however the overall importance of pollination on bilberry reproduction is not fully understood. This study used both observational and experimental approaches to investigate bilberry phenology and reproduction in relation to temperature, snowmelt and pollination, over two growing seasons (2020 and 2021), along two elevational gradients from the sub-montane zone to the subalpine zone in western Norway. Bilberry phenology advanced with increasing temperature and snowmelt along both gradients, and bilberry flowering was in synchrony with pollinator activity across all sites. Interestingly, while the number of flowers, fruits ovules and seed weight decreased with elevation, fruit set was higher at subalpine sites. Seed set varied between sites, with contrasting patterns between the gradients. These results show that the optimum elevation for bilberry vegetative performance might reflect the optimum for bilberry yields, but not for fruit and seed set. *Bombus* community composition and overall pollinator activity varied between sites, and fruit production was higher along the gradient with the highest pollinator abundance, however no clear sign of pollen limitation was found in hand cross-pollinated flowers compared to open-pollinated flowers across elevations. This study contributes to the knowledge of elevational effects, and consequently impact of climate variables such as temperature and snowmelt patterns, on bilberry phenology, pollination, and pollinator communities, as well as several variables on bilberry reproductive traits, within a boreal forest biome.

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1 Introduction

Global warming influence ecosystems across the world, by altering the timing of phenological events, such as flowering and pollinator activity, as well as abundance and species distributions (Walther *et al.* 2002; Parmesan & Yohe 2003; Parmesan 2006). Alterations on the species level exert varying impacts on the relationships among species and can even reshape trophic interactions (Tylianakis *et al.* 2008; Pyke *et al.* 2016; Mathiasson & Rehan 2020). In Norway, future climate scenarios project increases in both annual temperatures and rainfall, as well as their extremes (Hanssen-Bauer *et al.* 2009; IPCC 2021). It is therefore important to understand how species interactions will respond to a changing climate in order to predict how ecological communities will change in the future, especially functional important species which play important roles in maintaining diverse and dynamic ecosystems (Lurgi *et al.* 2012; Millon *et al.* 2014).

Plant-pollinator interactions represent an integral part of the functioning of terrestrial ecosystems, contributing to its complexity and interconnection by allowing for co-existence of multiple species (Costanza *et al.* 1997; Kearns *et al.* 1998; IPBES 2019). Pollination also serve humanity directly through the production, yield, and quality of food crops (Klein *et al.* 2007; IPBES 2016). Flowering phenology, quality and abundance directly influence resource availability for pollinators at a community scale (Memmott *et al.* 2007; Hegland *et al.* 2009; Moquet *et al.* 2017a). At the same time, wild pollinators contribute to essential mechanisms in the reproduction of a majority of flowering plants, ensuring successful seed production, as well as maintaining genetic and trait diversity (Burd 1994; Dodd *et al.* 1999; Ollerton *et al.* 2011). Moreover, many flowering species are considered pollen limited, when reduced reproductive success are caused by insufficient pollination (Burd 1994; Larson & Barrett 2000; Ashman *et al.* 2004).

Plant-pollinator mutualisms may be vulnerable to climate change, as disruption of the overlap in temporal timing or spatial distribution can alter interaction opportunities between them. For example, cumulative evidence show that climate warming accelerates both flowering initiation and insect emergence (Fitter & Fitter 2002; Bartomeus *et al.* 2011). Some studies demonstrates linear relationships between temperature and plant-pollinator mutualisms (Bartomeus *et al.* 2011; Rafferty & Ives 2011), however other alternative patterns are also described (Forrest & Thomson 2011; Kudo & Ida 2013; Pyke *et al.* 2016; Weaver & Mallinger 2022). In addition, other cues may covary with temperature for flower initiation and pollinator emergence (Sparks

et al. 2000; Parmesan 2006), making quantification of future responses to climate change complex. Because abiotic changes alter species niches, we typically observe northward or, in mountain regions, uphill shifts of species distributions, caused by increasing temperatures (Hickling *et al.* 2006; Kelly & Goulден 2008; Grytnes *et al.* 2014). For example, plant species richness has increased in Europe's mountains (Steinbauer *et al.* 2018), and Fourcade *et al.* (2018) showed that bumblebee species richness have increased in higher elevations and decreased in low elevation communities, in some parts of western and inland Norway in recent time. Temporal and spatial asynchrony between plant and pollinators may affect plant reproductive success, potentially altering resource availability for the next generation of pollinators as well as other dependent species. Especially critical is cascading effects which may follow mismatches between bumblebee queens and their main food plants, influencing pollination services later in the season (Waser & Real 1979; Hegland *et al.* 2009). Possible effects of climate change on plants and pollinators are manifold and will vary among species and regions, and it is still unclear whether these relationships are resilient under novel climatic conditions (Hegland *et al.* 2009; Kerr *et al.* 2015; Schenk *et al.* 2017; Martinet *et al.* 2021).

Bilberry (*Vaccinium myrtillus* L.) is a functional important species in boreal ecosystems and among the first insect-pollinated species to flower in spring. The plant provides important food resources for both vertebrate and invertebrate herbivores (Jacquemart 1993; Selås 2001; Hjältén *et al.* 2004; Stenset *et al.* 2016). The species also influence main components in the boreal forest ecosystem dynamics, such as soil nutrient and carbon cycles, as well as seedling regeneration (Nilsson & Wardle 2005; Kolari *et al.* 2006). The plant dominates field layers of forest communities and also occurs in heathlands and alpine areas (Ritchie 1956). Bilberry is both outcrossed by insects, but also able to self-pollinate, permitting reproduction in habitats where pollinators are scarce (Jacquemart & Thompson 1996). However, experimental studies have shown reduced reproductive success with self-pollination in comparison with cross-pollination (Jacquemart & Thompson 1996; Jacquemart 1997; Nuortila *et al.* 2002), yet the overall importance of pollination on bilberry reproduction is not fully understood.

Bumblebees, solitary bees and syrphid flies have been identified as the main pollinators of *Vaccinium* species (Jacquemart 1993), of which the former is regarded to be among the most efficient pollinators (Moquet *et al.* 2017b). Because bilberry is among the early flowering species, the plant is an especially important food source early in the year, providing essential nectar and pollen resources for bumblebees (*Bombus* ssp.) and solitary bees, including the

Bilberry Mining Bee, *Andrena lappicona* (Kevan *et al.* 1993; Carvell *et al.* 2017; Eckerter *et al.* 2021). Whereas solitary bees are considered a major pollinator at low elevations, bumblebees dominate the pollinator fauna at higher elevations, which may be explained by thermoregulator abilities in bumblebees that poses fewer weather constrains (McCall & Primack 1992). Bumblebee species are also among the dominating visitors of flowering plants in northern countries (Lázaro *et al.* 2008; Totland *et al.* 2013), however there is no comprehensive studies on their importance in boreal ecosystems.

Climate change is expected to affect bilberry phenology and reproduction in several ways, as integral parts of its life cycle are linked to climatic conditions, such as temperature and the duration of the snow cover. Temperature is expected to influence both bud formation and flower initiation (Selås 2000; Pato & Obeso 2012; Selas *et al.* 2015), and snow cover as well as snowmelt patterns are important at local and regional scales, as it determines local thermal conditions. For example, a stable insulating snow cover during winter is important for bilberry, protecting against frost damage (Inouye 2008; Wipf *et al.* 2009; Kreyling *et al.* 2012; Gerdol *et al.* 2013). In addition, pollination services may vary, as the abundance of bees and other pollinators may be highly variable between different areas (Hodkinson 2005). Local weather conditions also determine pollinator activity (Corbet 1990; Vicens & Bosch 2000; Peat & Goulson 2005), and may indirectly affect fruit and seed quantity and quality. Because of climatic variability across elevations, latitudes and regions, and the fact that species responses to a changing climate are highly site-specific under different climatic contexts (Hegland *et al.* 2009; Delnevo *et al.* 2018), additional studies in different regions will contribute to our understanding of bilberry phenology in relation to future climate change scenarios. Moreover, recent studies assessing berry production in boreal plants have focused on rodents, climatic factors, and plant community composition, but only occasionally on the importance of biotic interactions with pollinators on fruit and seed production (but see Boulanger-Lapointe *et al.* 2017; Eckerter *et al.* 2019). Information about the importance of insect pollination on bilberry reproduction and how climate change will influence this interaction is still limited and subject for further exploration, especially because pollination services are likely to change in the future (Biesmeijer *et al.* 2006; IPBES 2016). Understanding how phenology and reproduction in bilberry responds to direct effects of environmental conditions (temperature and snowmelt), as well as indirect effects of environmental variation (pollinator abundance and activity), can provide a better understanding of how these communities will change in the future.

Investigating how climate change affects biodiversity generally requires long-term monitoring. However, natural elevational gradients allow the study of ecosystem responses to longer term climate trends, over short-term monitoring (Walther *et al.* 2002; Hegland *et al.* 2009; Blois *et al.* 2013). Because abiotic factors, including air temperatures, length of vegetation period and nutrient availability decrease with altitude, whereas precipitation and frequency of frost tends to increase (Ziska *et al.* 1992; Körner & Körner 1999; Heegaard 2002), the environmental gradient (in space) serves as a proxy for environmental change (in time), allowing for research on possible temporal or spatial changes that may occur in a changing climate. Temperature and timing of snowmelt are determinants of plant and insect performance, as well as plant phenology (Sparks *et al.* 2000; Wipf *et al.* 2009) and most likely linked to insect emergence (Alford 1969; Roy & Sparks 2000; Forister & Shapiro 2003; Kudo & Ida 2013). Provided that results are interpreted with some caution, elevational gradients can therefore be a useful tool when studying plant-pollinator interactions in relation to future climate scenarios.

In this study, we investigate bilberry phenology and reproduction in relation to temperature, snowmelt and pollination, over two seasons (2020 and 2021), along two natural elevational gradients from the sub-montane zone to the subalpine zone in western Norway (Figure 1). Only a few studies in Norway have focused on bilberry phenology and reproduction along natural elevational gradients (but see; Nielsen *et al.* 2007; Berge 2018; Benevenuto *et al.* 2020a; Benevenuto *et al.* 2020b), and literature is limited on bilberry reproduction in relation to pollination services here (but see; Olsen *et al.* 2017). By using both observational and experimental approaches, and recordings of *Bombus* diversity and abundance, flower visitation rates and plant phenology and reproduction, we aim to answer the following questions:

1. Is there a difference in phenology of bilberry along the elevational gradients?
2. Is there a difference in flower visitation and abundance and/or richness of bumblebees during the flowering period of bilberry along the elevational gradients?
3. Is there a mismatch between flowering of bilberry and activity of pollinators along the elevational gradients?
4. Is there a difference in bilberry reproductive traits along the elevational gradients?
5. Is there an indication of pollen limitation in bilberry along the elevational gradients?

Based on previous studies, we can make several predictions on bilberry phenology and reproduction in response to temperature, snowmelt, and availability of pollinators. Because plants are sensitive to temperature, as well as timing of snowmelt, we can expect an overall shift of plant phenology towards earlier occurrences and a longer flowering and pollinator duration with decreasing elevation. At forest sites, we typically observe higher abundance of pollinators compared to high elevations, as the availability of pollinators have been found to decrease with elevation (Totland 1993; Blionis & Vokou 2001). We therefore expect that pollinator activity and abundance are higher at lower elevations compared to high elevations. In Norway, lowland to highland habitats differ substantially in the availability of pollinators, and as more cold adapted species are found at higher elevation, species richness might increase from the sub-montane to the subalpine sites. Because cross-pollination have been found to increase bilberry fruit and seed set, we expect bilberry fruit and seed set to increase with increased pollinator activity and abundance. Moreover, because climatic conditions in subalpine habitats, including low temperatures, rapid temperature fluctuations, low amount of nutrients and high amounts of precipitation, create unfavourable conditions for plant performance in comparison with lower elevations, we expect plant performance to decrease with elevation.

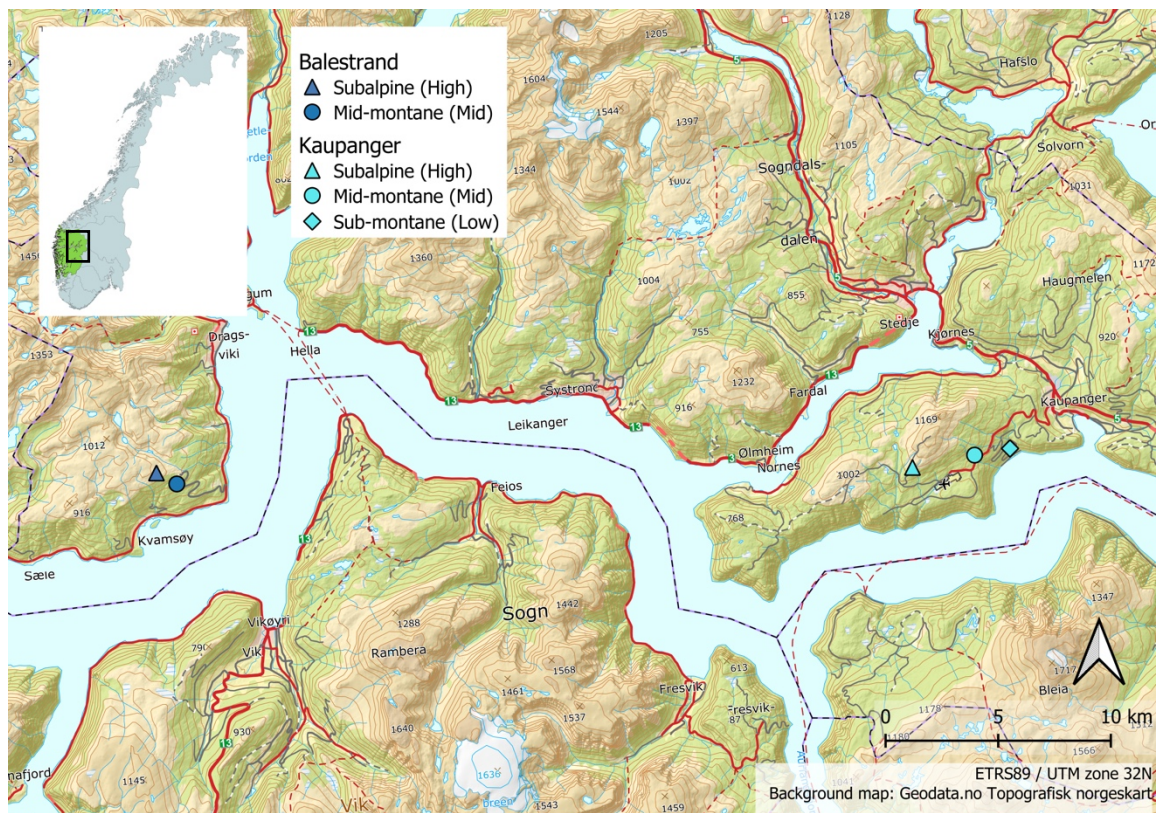


Figure 1. Map of sampling areas and gradient locations included in this study. The gradients are located in Balestrand and Kaupanger with elevations located in the submontane (low), midmontane (mid) and subalpine (high) zones. Mapsource topografisk norgeskart, GeoNorge

2 Material and Methods

2.1 Site description

The study was conducted in Sogn, western Norway, during the growing season of 2020 (May-September) and *V. myrtillus* flowering peak of 2021 (May-June). During the spring of 2020, two elevational gradients were established along the Sognefjord, which stretch from drier continental climate in the inner branches to wetter more oceanic climate closer to the coastline (Figure 1). The Sognefjord is characterized by fjords and mountains ranging from 0 - 2,400 m.a.s.l, with marked topographical variation. The two elevational gradients therefore represent variations in environmental conditions, over short horizontal distances. Because temperature gradually decreases and precipitation increases with elevation, there is a gradual decrease in temperature and a gradual increase in precipitation, as well as delayed snowmelt from the lower to the upper parts of the gradients (Moen & Lillethun 1999). One gradient was located at the southeast slope of Storehaugfjellet, Kaupanger, located in the inner branches of the fjord. The other gradient was placed at Saurdalseggi, Balestrand located closer to the coastline. Table 1 gives the geographic locations and general climate statistics for the sites, illustrating the slightly drier climate conditions at the sites along the gradient in Kaupanger, and slightly more oceanic characteristics at the sites along the gradient in Balestrand.

Both gradients consist of two sites at different elevations (Figure 1, Table 1); one at ~500 m.a.s.l. in the mid-montane zone (mid) and one located at the tree line in the subalpine zone between 770-900 m.a.s.l. (high). Because the treeline declines towards the coast (Moen 1998), the treeline in Balestrand is approximately 100 m lower compared to Kaupanger. In 2021, data was only collected at the gradient in Kaupanger, and a low elevation site at 100 m.a.s.l in the submontane zone was added (low; Table 1). The sites along the gradient in Kaupanger were chosen based on already established locations with similar characteristics (Benevenuto *et al.* 2020b), and comparable locations based on temperature, snowmelt, light availability and vegetation structure were selected in Balestrand. All sites are located within a pine bilberry forest ecosystem, where dwarf shrubs dominate the understory of both gradients, while Scot's pine (*Pinus sylvestris* L.) and birch (*Betula pendula* Roth and *B. pubescens* Ehrh.) are most abundant in the tree layer. In Kaupanger, the submontane and mid-montane sites consist of >15-year-old clear cuts and had therefore only a few numbers of trees producing shadow effects. Here, the understory is dominated by bilberry, lingonberry (*Vaccinium vitis-idaea* L.), and crowberry (*Empetrum nigrum* L.). In Balestrand, the mid-montane site consisted of a

naturally open forest site, although slightly more trees produced shadow effects here, and the understory was mostly dominated by bilberry and heath (*Calluna vulgaris*). The subalpine site at both gradients was naturally open subalpine areas, just below the treeline. The topographic nature in Kaupanger makes the slope of the gradient steeper than the slope of the gradient in Balestrand, and the treeline is therefore somewhat more defined at the former location.

Table 1. Established locations with specific coordinates (decimal degree, DD), elevation, mean annual precipitation, mean temperatures during summer months (June-Sept 2020) and snowfree dates in Julian days

| Site | Coordinates (DD) | | Elevation <i>m.a.s.l</i> | Precipitation (mm) | | Temperature °C | | Snowfree dates | |
|-------------------------------------|------------------|------------------|-----------------------------|--------------------|---------|-----------------------|------------|-------------------|------|
| | <i>Latitude</i> | <i>Longitude</i> | | <i>Mean annual</i> | | <i>Mean June-Sept</i> | | <i>Julian day</i> | |
| | | | | 2020 | 2021 | 2020 | 2021 | 2020 | 2021 |
| <u>Balestrand, Saurdalseggi</u> | | | | | | | | | |
| Balestrand - Brannstasjon | | | 14 | 2 074.2* | - | - | - | - | - |
| Mid | 61.15 | 6.50 | 497 | - | - | - | - | 113* | - |
| High | 61.15 | 6.48 | 774 | - | - | 11.3 ± 5.59 | - | 166* | - |
| <u>Kaupanger, Storehaugsfjellet</u> | | | | | | | | | |
| Sogndal - Skardsbøfjellet | | | 713 | 1509.4* | 1032.8* | - | - | - | - |
| Low | 61.17 | 7.19 | 126 | - | - | - | 17.4 ± 7.1 | - | 54* |
| Mid | 61.17 | 7.16 | 499 | - | - | 13.9 ± 6.6 | 15. ± 6.4 | 127 | 78 |
| High | 61.16 | 7.11 | 879 | - | - | 11.8 ± 7.1 | 13.9 ± 7.8 | 159 | 125 |

*Data on precipitation obtained from MET, Norway (Note varying m.a.s.l) and data on snowfree dates from SeNorge.no

- missing values

To measure air temperatures in the field layer during the entire study, two temperature loggers (Tinytag Talk 2, TK-4014-MED; Gemini data loggers) were placed approximately 0.5 meters above ground, attached to similar sized trees facing north, at each site along the gradient in Balestrand (Table 1). Unfortunately, some loggers did not work properly, and only data from the subalpine site in Balestrand were used. Temperatures and snowmelt dates at the gradient in Kaupanger was provided by Hegland and Gillesipe from the Western Norway University of Applied Sciences, while data on snowmelt dates along the gradient in Balestrand was obtained from interpolated data from SeNorge.no. Regional annual precipitation from Balestrand and Kaupanger was obtained from The Norwegian Meteorological Institute (Met.no), with weather stations placed ~6.7 km away from the gradient in Balestrand and ~9.1 km away from the gradient in Kaupanger. Monthly air temperatures from June to September was 2-6 °C warmer

at mid-montane sites compared to subalpine sites in 2020 (Table 1). It was 1 - 2 °C warmer at the supalpine elevation in Kaupanger compared to Balestrand. Unfortunately, temperature loggers at the mid-montane site in Balestrand did not work properly. A comparison in temperature between gradients at mid elevations could therefore unfortunately not be done. In 2019-2020, annual precipitation in Balestrand and Kaupanger were 22-23 % higher compared to the normal between 1990-2020, according to the Met.no climatic statistics, and a bigger snowpack was observed this year (pers. obs). Kaupanger has less precipitation compared to Balestrand, located closer to the coastline. Snowmelt was 24-53 days earlier at lower sites compared to higher sites along both gradients.

2.2 Study Species

The study species, *Vaccinium myrtillus* (Ericaceae), is a deciduous, clonal dwarf shrub, 10 - 60 cm high with evergreen stems and with a circumpolar distribution (Flower-Ellis 1971). The plant dominates field layers of forest communities and grows in patches at high elevations (Ritchie 1956). The berries and vegetative parts of the plant are of ecological importance as an essential food source for vertebrate and invertebrate species, and the plant is therefore considered a key species in boreal ecosystems (Jacquemart 1993; Selås 2001; Hjältén *et al.* 2004; Stenset *et al.* 2016). We do not have specific information about its clone size for the study area, but have based our experiments on the assumption of the largest clone to be up to 10 m wide (Flower-Ellis 1971). Flowering are sensitive to snowmelt patterns and increased temperatures (Selås *et al.* 2015), and blooms in early spring (May-July in the study sites). Flowers are nectareous, attracting a wide range of pollinating insects, but are also able to self-pollinate, permitting reproduction in habitats where pollinators are scarce (Jacquemart & Thompson 1996). The main pollinators of bilberry are bumblebees, solitary bees and syrphid flies (Jacquemart 1993), of which the former are regarded to be among the most effective (Moquet *et al.* 2017b). Fruit production has year-to-year variation affected by factors such as environmental variables, grazing and pollinator abundance (Jacquemart 1993; Selås 2000; Hegland *et al.* 2005).

2.3 Sampling procedure

To assess how plant phenology and reproduction, as well as pollinator activity, diversity and abundance varies with elevation, both observational and experimental approaches were conducted. Observations on bilberry phenology and pollinator activity were conducted in year 2020 at mid-montane and subalpine elevations along each gradient. In year 2021, pollinator activity observations and a hand pollination experiment during the flowering peak were conducted at the submontane, mid-montane and subalpine site along the gradient in Kaupanger. In order to study bilberry phenology, pollinator activity and experimental hand-pollination trials, three 50 m long transects were placed and permanently marked at each site in areas with relatively high cover of bilberry along each gradient. At mid-montane and subalpine sites, two 0.5 x 0.5m plots were permanently marked along each transect for repeated bilberry phenology observations. Due to our assumption of the species clone size (up to 10m), the transects and plots were installed at least 15 m apart from each other.

2.3.1 *Bilberry phenology and reproductive success*

Different stages of bilberry phenology across the growing season (bud, flower, withered, ripe) and reproductive success was measured in each plot and site in 2020. All ramets in each plot were checked and the number of buds, flowers and withered flowers were counted and noted every five days, and ripe berries every five to seven days until all fruits were matured (May-September 2020). All viable fruits from each plot were collected as they matured and stored in a freezer prior to measurements. The number of seeds per fruit in the one median sized fruit per plot ($n = 24$) were counted and categorized as developed, partly developed and undeveloped, as described in Jacquemart and Thompson (1996). The remaining median sized fruits of 6-10 fruits per plot ($n = 217$) were weighed fresh, then dried at 65°C for approximately three days and then weighed again for dry mass.

2.3.2 Flower visitor observations and *Bombus* diversity

Because bumblebees, solitary bees and syrphid flies have been identified to be the main pollinators of bilberry (Jacquemart 1993), flower visitation as a measure of pollinator activity was limited to species within these groups. As *Bombus* species is considered one of the most effective pollinators of bilberry (Moquet *et al.* 2017b), and are also abundant from lowland to high elevations in Norway (Totland *et al.* 2013), this study focus on *Bombus* diversity related to bilberry. Note that in this thesis, although it is well known that different pollinators contribute to plant reproduction to variable degrees, the term pollinator and flower visitor is used interchangeably (e.g. Schemske & Horvitz 1984).

To assess how the pollinator community associated with bilberry varies with elevation, as well as how their relation to bilberry fruit production varies, floral visitor observations were performed, which is commonly used to assess animal contribution to plant reproduction (chapter 4.13: Halbritter *et al.* 2020). In 2020, pollinator visits were recorded twice every five days during the flowering period of bilberry (May-July) at all transects in each site. In 2021, flower visits were recorded at least three times on two consecutive days during the peak flowering season (May-June) at each site along the gradient in Kaupanger.

Observations were conducted between 09:00 and 18:00 on days without heavy rain and/or strong wind. In 2020, only one observer collected the data, while in 2021 two observers collected data. The observer walked along each transect for 30 minutes at a consistent walking pace until a pollinator visitor entered the transect (a visual estimation of 1 m at each side of the transect), after which the observer followed the pollinators foraging behaviour until it left the transect. Flower visits were recorded if a pollinator landed on a bilberry flower. Bumblebees were identified to family or genus, whereas other species to common names (Appendix C, Table IX).

Because flower visitor observations are highly dependent on local weather conditions (Corbet 1990; McCall & Primack 1992), we used vane traps (VTs) as a supplement and passive sampling technique to assess the bee diversity and relative abundances during the flowering period of bilberry in 2020. Blue vane traps (BVTs) and yellow vane traps (YVTs), with UV reflecting colors, have been found to be effective sampling methods for bees, especially *Bombus* ssp. (Stephen & Rao 2005; Hall 2018). Two BVTs and two YVTs were placed at the upper and lower edge at each site, hung from a tree branch approximately 0.5 m above the

ground. Traps were checked and emptied every five days. Specimens were washed, dried, and stored in a freezer before identification. Bumblebee individuals were identified to species or genus, whereas solitary bees to common names (Appendix C, Table X).

Species determination methods of Løken (1973) and Staverløkk *et al.* (2012) were used to identify *Bombus* individuals to species, and *Bombus* specimens from The Natural History Collections, University of Bergen were borrowed for comparison (Appendix D, Table XI). Morphological characterization of *B. monticola* and *B. lapponicus* as well as the subgenus *B. sensu stricto* in Norway; *B. cryptarum*, *B. lucorum*, *B. magnus*, *B. terrestris* and *B. sporadicus*, are difficult to differentiate. *B. sensu stricto* individuals were therefore identified to subgenus, whereas the few individuals of *B. monticola* and *B. lapponicus* were identified using DNA barcoding (n = 12; Appendix E, Figure II). Collected bee specimens will be archived the Natural History Collection, University of Bergen.

Genetic analysis was performed on 12 individuals of *B. monticola* and *B. lapponicus* species. DNA was extracted on the corbicula using the protocol from the Qiagen DNeasy Blood and Tissue kit (Qiagen), and stored in elution buffer at 4°C. Fragments of the mitochondrial cytochrome c oxidase I (COI) was chosen as DNA barcode, and amplified using Takara Ex Taq HS, using the forward primer LCO1490 and the reverse primer HCO2198 (Folmer *et al.* 1994). Each PCR was conducted in 25µL reaction containing 0.15 µL Taq polymerase, 2 µL dNTPs and 2.5 µL buffer, 1µL template DNA, 17.35 µL distilled H₂O and 1µL of each primer. Amplifications were carried out in a thermal cycler (Bio-Rad C1000™), with cycling conditions with an initial step of 94°C for 5 min followed by 5 cycles of 94°C for 45 sec, 45°C for 30 sec and 72°C for 1 min, then 30 cycles of 94°C for 45 sec, 50°C for 30 sec and 72°C for 1 min, and a final elongation step at 72°C for 10 min. PCR products were visualized on 1% agarose gels following standard procedures, before purification with ExoSap-IT. Sanger sequencing reactions was conducted using 3.1 Big Dye Terminator chemistry reactions. The bioinformatic desktop software Geneious (11.1.5) was used to read, edit, combine, and analyse sequencing results, combine forward and reverse reads, trimming poor quality begin- and end sequences, and edit base calls when necessary. Each sequence was assigned a taxon using the most similar published sequences on NCBI GenBank nucleotide database (acc. nb. BMB229 and MZH_GP.65740). A phylogenetic neighbor-joining tree with a distantly related and published *Bombus* sequence, *Bombus pratorum*, was chosen (acc. nb. MBF00070; Genbank) for reference guided assembly (Appendix E, figure II).

2.3.3 Hand-pollination experiment

To examine the possibility of pollen limitation in *V. myrtillus*, and whether it varies with elevation, a hand-pollination experiment was conducted during the flowering peak of 2021 (May-June) at each site along the gradient in Kaupanger (submontane, mid-montane and subalpine). Pollination trials were partly done following the protocol in (Jacquemart 1997). A total of 7-10 flowers on different ramets along each transect were hand cross-pollinated. The hand-pollinated flowers had their stigmas saturated with pollen collected from different bilberry clones to assure cross-pollination and to test pollen transfer limitation. Hand-pollinated flowers were not bagged and could therefore also receive ambient pollination in the field. Every hand-pollinated flower had two naturally open-pollinated control flowers: (1) one external control on a different ramet, (2) one internal control on the same ramet to monitor and quantify resource allocation within the ramet relative to (1) (Zimmerman & Pyke 1988). To minimize variations in bilberry performance, ramets were chosen after the following criteria: (1) placed at least 5 m away from other experimental ramets, (2) only newly open flowers on ramet, (3) at least two flowers on the ramet, (4) ± 2 flowers on the control ramets compared to the hand pollinated flower, (4) hand-pollinated flowers placed at the top branch, (5) 15cm tall (± 2 cm), (6) pollen supplementations collected from two pollen parents placed at least 15m away from the hand-pollinated flowers, over two executive days. Each experimental flower was tagged with colored sewing threads and every ramet stem marked with thin zip ties. Fruits were collected, counted, and noted between 59 and 73 days after pollination.

2.4 Statistical analyses

All statistical analysis were performed using R version 4.0.2 (RStudio Team 2020), and visualized using the ggplot2-package.

2.4.1 Flowering phenology and flower visitation

The first, peak and duration of flowering and flower visitation in Julian days for each transect at each site was calculated in order to be used as phenological estimators of flowering phenology and pollinator activity (Appendix A, Table I). Peak flowering and peak pollinator activity was calculated using the weighted mean day of occurrence (WMD; Kudo 2014). WMD is the arithmetic mean of all the dates where observations were made, weighted by the abundance of flowers or pollinators on that date.

To test for the effect of elevation and gradient on flowering phenology, separate linear models were used with first, peak and duration as response variable and elevation, gradient, and their interaction as predictors were used (package: lmer). To test for differences between elevation and gradient on flower visitation, the same model was used using the number of flower visits per transect per observation and the overall period of flower visitation, as well as flower visitation rate (flower visits per total amount of flowers per transect area) as response variables, with the predictors mentioned above. Only elevation was included as predictor variable for flower visitation in 2021. A log-link function was added to normalize residuals where needed.

To evaluate phenological synchrony between bilberry flowering and its pollinators, the WMD of peak flowering was regressed against the WMD of peak pollinator activity at each transect (package: lmer). To quantify the differences in phenological synchrony between bilberry flowering and activity of pollinators, the difference between the WMD of peak flowering and the WMD of peak pollinator activity was calculated at each site. A close match between peaks would indicate synchrony, a negative value would indicate that peak flowering occurred before peak pollinator activity and a positive value the opposite. The effects of elevation and gradient on differences in peaks at each site were tested in a linear model, with the difference in peak as response variable and elevation, gradient, and their interaction as predictor variables.

Because VT efficiency are highly determined by color, individuals captured in BVTs and YVTs per location (top bottom at each site) were added together before further analysis. Measures of *Bombus* diversity was then calculated using abundance, richness, and evenness per sampling day per trap location at each site. The effect of elevation and gradient on fauna measures were tested using separate generalized linear models, with abundance, richness and evenness as response variables and elevation, gradient and their interaction as predictors (The R stats package). A quasi-poisson distribution were added to capture over-dispersion. To visualize dissimilarities of *Bombus* communities along the elevational gradients, a non-metric multidimensional scaling ordination based on Bray-Curtis dissimilarities was used (NMDS, package: vegan). To reduce the relative influence of the most frequent species in the NMDS, *Bombus* species abundances were square-root transformed. The nonparametric multivariate analysis of variance (package: vegan), based on the Bray-Curtis matrices with 999 permutations, was used to evaluate dissimilarity measures in *Bombus* communities between elevations and sites. The rate of capture based on sampling effort was also assessed using species accumulation curves (package: vegan).

2.4.2 Reproductive traits

Abundance of flowers and fruits, as well as fruit set (number of flowers developing into a berry) in each plot was measured by using numbers from the day which had the highest amount of flowers and the highest amount of fruits within each plots. To test for the effect of elevation and gradient on total abundance of flowers and fruits, generalized linear models with the maximum numbers of flowers and fruits as response variables, and elevation, gradient and their interaction as predictors (The R stats package). A log-link function was added to normalize residuals where needed.

The effect of elevation and gradient on fruit set was tested using a generalized linear model with binomial error and a log link function with elevation, gradient, and their interaction as predictors (The R stats package). To quantify the effect of pollination on fruit set in the hand pollination experiment, the same model was used with pollination treatment (hand-pollination, open pollinated external control), elevation and their interaction as predictors. In the hand-pollination experiment, the internal open-pollinated control did not show any significant difference to the external open-pollinated control, and the former was therefore removed from

the analysis. Some experimental ramets were lost in the field, and all experimental flowers linked to lost ramets were thus excluded from the dataset.

To estimate the effect of elevation and gradient on seed set (number of developed seeds/ number of ovules), a multinomial logistic regression analysis was performed, and the Wald statistic was used to evaluate and compare (package: nnet). Because seed types in *V. myrtillus* includes three categories (developed, partly developed and undeveloped), number of developed seeds was estimated as proportions. Predictor variables includes elevation, gradient, and their interaction. Finally, to estimate the effect of elevation and gradients on the number of ovules per fruit and mean fruit weight per plot, a generalized linear model with Poisson distribution, including the predictors above (The R stats package).

2.5 Data availability

Data will be made openly available in the data repository Open Science Framework and the scripts for data curation and analysis is available at GitHub: <https://github.com/heddavic/Bilberry>

3 Results

3.1 Bilberry phenology and flower visitation

Overall, the analysed data clearly show that elevation delayed both the onset of flowering and peak flowering, and shortened flowering duration in bilberry along both gradients (Figure 2A, 2B). The onset of flowering was 19-23 days earlier at mid-montane elevations compared to subalpine elevations ($t_8 = 9.6$, $P < 0.001$; Table II, Appendix B). The peak flowering dates were 12-17 days delayed at higher elevations compared to mid-montane elevations ($t_8 = 7.6$, $P < 0.001$). Flowering duration was 1-17 days shorter at subalpine elevations compared to mid-montane elevations ($t_8 = -2.4$, $P < 0.05$). There was however no significant effect of gradient or the interaction between gradient and elevation, on onset of flowering, peak flowering, and flowering duration.

A total of 12.3 – 15. hours of observations per site (elevation mid and high, gradient Balestrand and Kaupanger) was performed and a total of 615 number of flower visits were recorded over the course of the flowering season of bilberry in 2020. In 2021, a total of nine hours of observations per site along the gradient in Kaupanger (elevation submontane “low”, mid-montane “mid”, subalpine “high”) was performed during the flowering peak, and 523 flower visits were recorded. The majority of flower visitors on *V. myrtillus* were *Bombus* species (2020: 87.5%, 2021: 63.7% of in which 6.8% were *Psithyrus* species), followed by solitary bees (2020: 10.2%, 2021: 22.9%), wasps (2020: 2.1%, 2021: 9.2%) and hoverflies (2020: 0.2%, 2021: 4.2%). The number of flower visits ranged from 0-62 visits per observation and was significantly larger at mid-montane elevations compared to subalpine elevations in 2020 ($t_{53} = -2.5$, $P < 0.05$; Table II, Appendix B). Flower visitation rate decreased with elevation ($t_8 = -3.1$, $P > 0.05$) and was higher along the gradient in Balestrand compared to Kaupanger ($t_8 = -2.9$, $P > 0.05$), however a strong negative effect of gradient was indicated ($t_8 = -2.9$, $P > 0.05$). Contrary, there was no significant differences in the number of visits with elevation during the flowering peak of 2021. While mid-montane and subalpine elevations had no major differences in flower visitation in 2021, a higher number of visits was recorded at the submontane elevation compared to higher elevations ($t_{42} = -1.9$, $P = 0.056$). Flower visitation duration, from the first observed flower visit to the last flower visit observation, had no substantial differences between elevations, but was longer along the gradient in Kaupanger compared to Balestrand ($t_8 = 2.4$,

$P < 0.05$). No significant interactions between gradient and elevation were found on total flower visits and flowering visitation duration in both years.

Peak flowering was largely concordant with peak pollinator activity across elevations and gradients ($t_{10} = 12.5$, $P < 0.001$; Figure 2A, 2B), and the synchrony between peak flowering and peak pollinator activity did not vary significantly between elevations nor gradients.

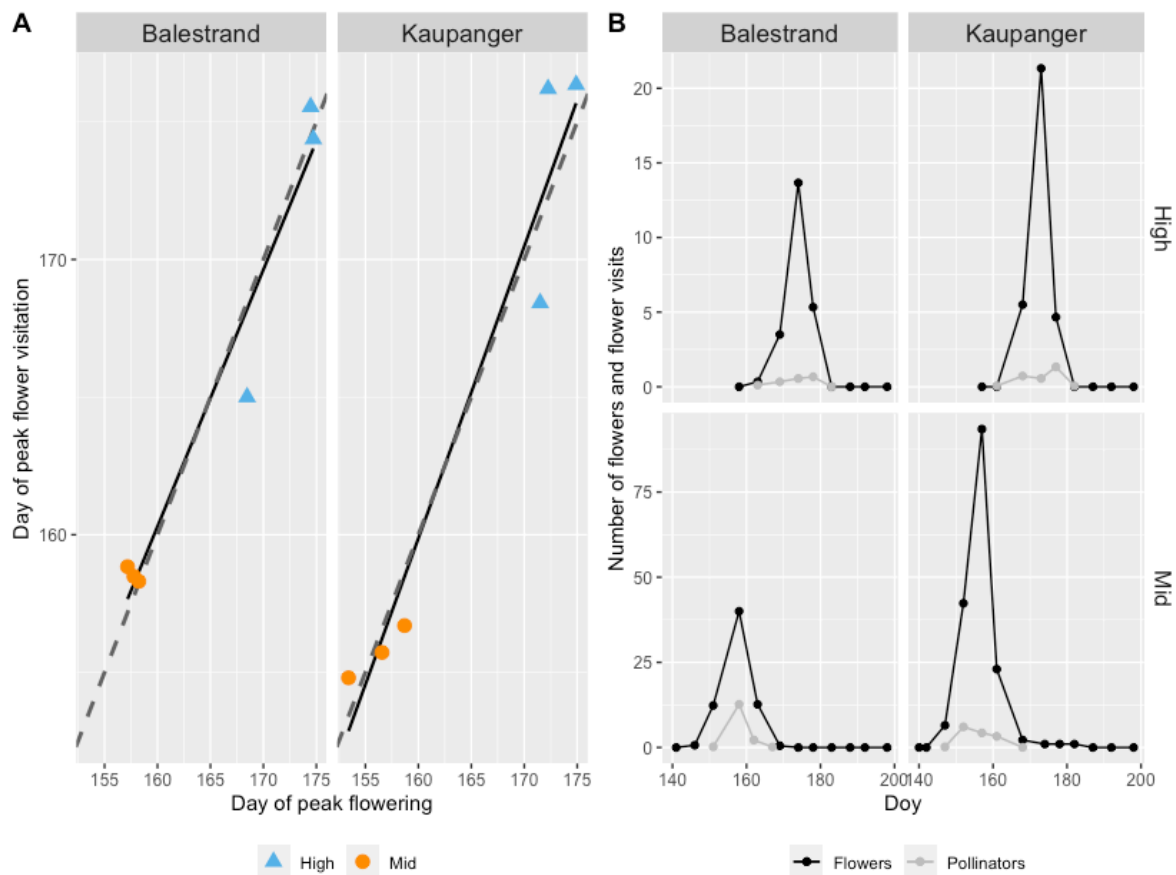


Figure 2. Flowering of bilberry and flower visits midmontane (mid) and subalpine (high) sites along two elevational gradients (Balestrand and Kaupanger) in western Norway, 2020. (A) Day of peak flowering (y-axis) and day of peak flower visitation (x-axis) at each transect and elevation. Shown are the 1:1 line (dashed line) and regression line (solid line). Symbols illustrate the elevations: mid (asterisks) and high (triangles). (B) Mean number of flowers during the flowering period of *V. myrtillus*. Note the different scale of the y axis between the high and mid elevation in (B)

3.2 *Bombus* diversity

A total of 6983 individual pollinators were caught during the flowering period of bilberry at all sites along the two gradients. Of these, 6011 individuals were flies, 659 bumblebees, 23 solitary bees (several families), 8 wasps and 282 in other groups not identified. There were substantial differences in sampling effectiveness between the two different vane trap colors, where 98%

of all individuals of bees were caught in the blue vane traps, and only 1% of bees were caught in the yellow vane traps.

Bombus individuals in traps comprised ~11 species, dominated by *B. soroeensis* (36.4%), *B. pratorum* (28.4%), *B. pascuorum* (19.1%) and *B. sensu stricto* (subgenus; 10.8%; Figure 3A, 3B). Other species relative abundances include *B. consobrinus* (1.2%), *B. monticola* (1.1%), *B. hortorum* (1.1%), *B. hypnorum* (0.8%), *B. lapponicus* (0.8%), *B. balteatus* (0.3%) and *B. jonellus* (0.2%). In total, a higher number of *Bombus* species were found at the subalpine site (n = 11) in Balestrand which included a higher number of species adapted to colder environments were (*B. monticola*, *B. lapponicus* and *B. balteatus*). The two gradients also differed in individual *Bombus* species associated with specific plant species. *B. hortorum* was more abundant in Balestrand, concordant with observations of *Digitalis purpurea* in the area (personal observation). In Kaupanger, the specialist species *B. consobrinus* was found, indicating that the plant *Aconitum septentrionale* koelle is growing in this area.

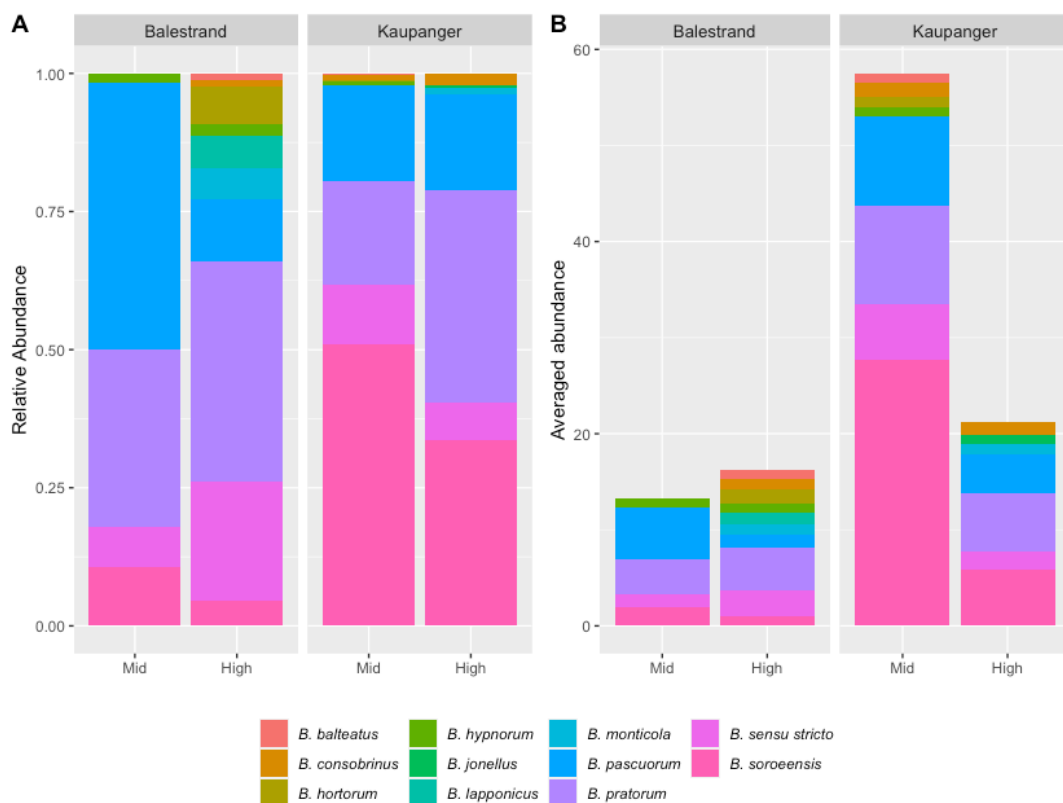


Figure 3. Relative (A) and averaged (B) abundances of *Bombus* species captured in blue and yellow VTs during the flowering period of *V. myrtilus* in 2020 at midmontane (mid) and subalpine (high) elevation along two gradients (Balestrand and Kaupanger) in western, Norway (n=659)

The NMDS ordination visualizes the distribution of bumblebee species at the four sites (Figure 4A). Four clusters show that the *Bombus* communities differ among the sites with some

overlap. *Bombus* communities were significantly different across elevations ($F_{1,28} = 7.5$, $P = 0.001$; Table III, Appendix B) and gradients ($F_{1,28} = 7.1$, $P = 0.001$) as well as their interaction ($F_{1,28} = 3.8$, $P = 0.01$). Abundance of bumblebees per trap location varied between 10-54 individuals and species richness per trap location varied between 3-5 species. Abundance decreased with elevation, although not significant (Table IV, Appendix B), and was higher along the gradient in Kaupanger compared to Balestrand ($t_{27} = 4.9$, $P < 0.001$). The interaction between elevation and gradient implies a strong positive effect on abundance in response to elevation in Kaupanger ($t_{27} = -2.8$, $P < 0.01$). Species richness increased with elevation along the gradients ($t_{27} = 2.7$, $P < 0.05$). However, the significant interaction between elevation and gradient implies that the effect of elevation on species richness depends on location, with a strong negative effect in Kaupanger ($t_{27} = -3.3$, $P < 0.01$). *Bombus* community evenness was highest at the mid-montane site in Balestrand and lowest at the mid-montane site in Kaupanger, and overall higher along the gradient in Balestrand compared to Kaupanger ($t_{27} = -2.5$, $P < 0.05$). A significant interaction between elevation and gradient was found, and the effect of elevation on evenness also depends on location, with a positive effect in Kaupanger ($t_{27} = 2.3$, $P < 0.05$). The number of observed species as a function of sampling shown in Figure 4B shows that the sampling effort during the flowering period did not fully describe *Bombus* species richness at each site.

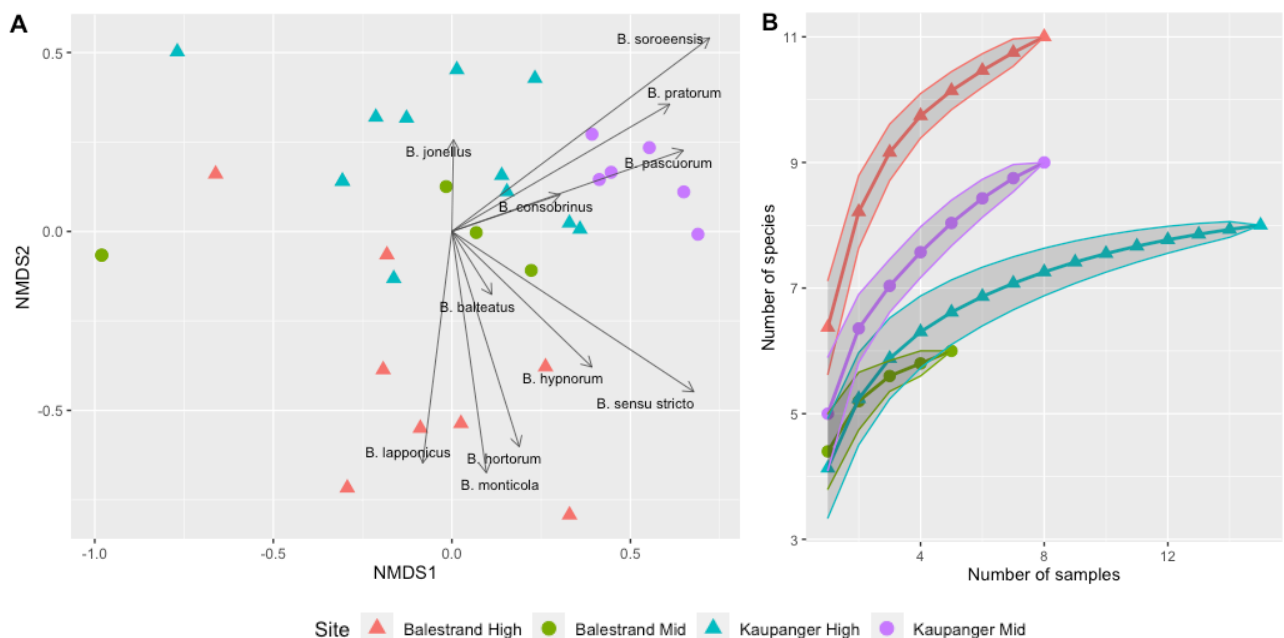


Figure 4. *Bombus* diversity represented by individuals captured in vane traps ($n=659$). (A) Non-metric multi-dimensional scaling (NMDS) plot illustrating dissimilarities in *Bombus* community composition during the flowering period of *V. myrtillus* in 2020 at midmontane “mid” and subalpine “high” elevation along two elevational gradients (Balestrand and Kaupanger) in western, Norway, 2020. The Bray-Curtis dissimilarity index was used for determination of dissimilarities among bumblebee community compositions. Stress value = 0.15, indicating a reasonable fit for the model (B) Sample based species accumulation curve for all bumblebee individuals sampled by vane traps along two elevations gradients. Curves were plotted based on data grouped across sites. The solid lines show predictions based on interpolation. 95% confidence intervals are shown as shaded areas.

3.3 Bilberry reproductive traits

The maximum number of flowers and fruits per plot ranged between 4-163 and 2-30 respectively, and mean fruit weight per plot varied between 0.016 – 0.052 mg. The number of ovules ranged from 42-112 per fruit and of the 1590 counted ovules, 359 (22.6%) fully developed into mature seeds, 378 (23.8%) partly developed seeds and 853 (53.6%) undeveloped seeds. The number of flowers and fruits was higher along the gradient in Kaupanger compared to the gradient in Balestrand (flowers: $t_{20} = 2.5$, $P > 0.05$, fruits: $t_{20} = 0.2$, $P < 0.05$), but did not differ between elevations (Figure 5A, 5B; Table V, Appendix B). However, when only looking at the gradient in Kaupanger, the number of flowers and fruits decreased with elevation ($P < 0.05$). The number of ovules per fruit and mean fruit weight per plot decreased with elevation (Figure 5C, 5D; ovules: $t_{20} = -2.1$, weight: $P < 0.05$; $t_{20} = -2.6$, $P < 0.05$; Table V, Appendix B). Mean fruit weight was overall higher along the gradient in Balestrand compared to the gradient in Kaupanger ($t_{20} = -3.1$, $P < 0.01$).

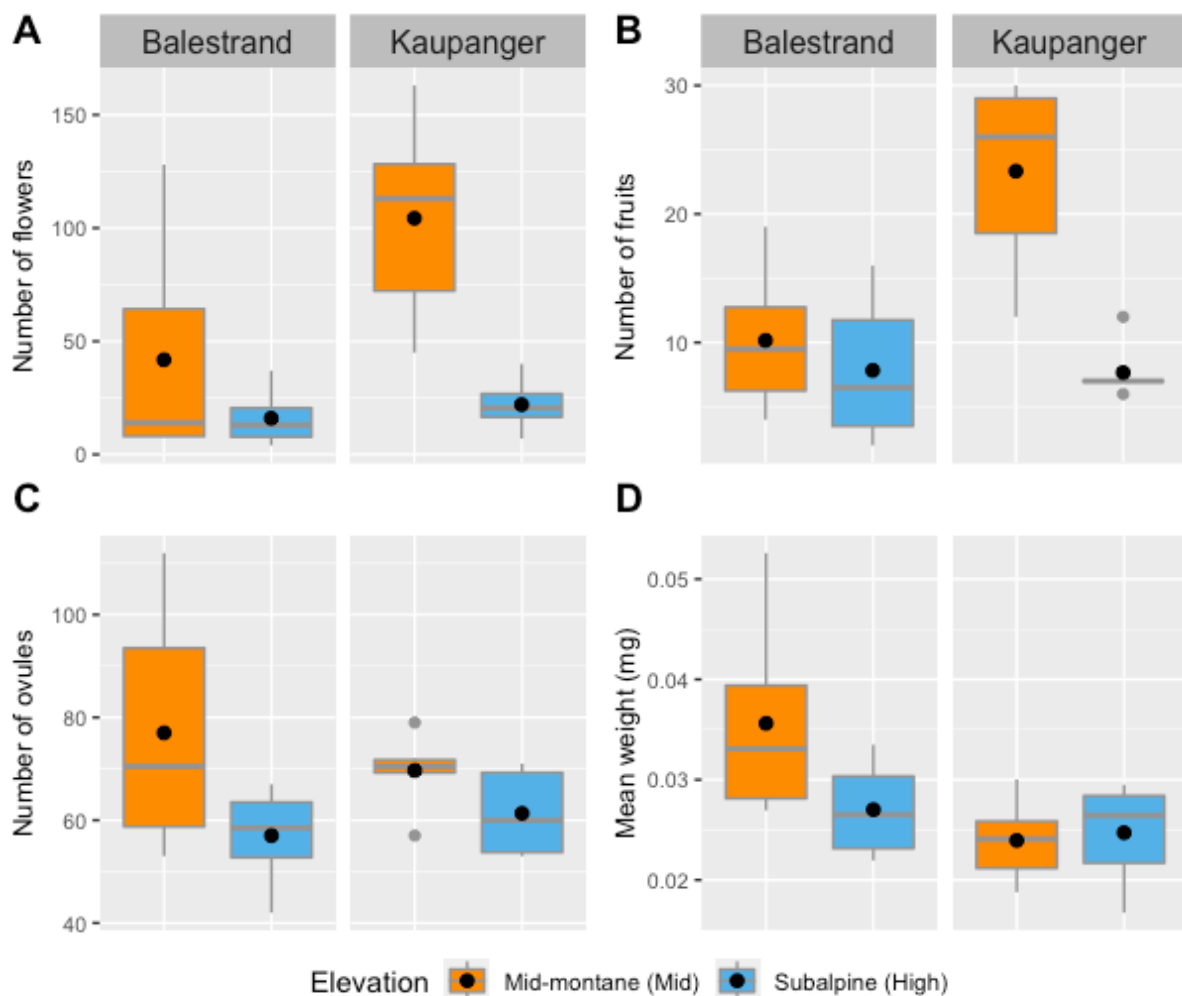


Figure 5. Measures on reproductive traits in bilberries at midmontane (mid) and subalpine (high) elevation along two elevational gradients (Balestrand and Kaupanger) in western, Norway, 2020. (A) Number of max flowers per plot (B) Number of max fruits per plot, (C) Number of ovules per fruit ($n = 1$ per plot), (D) Mean fruit weight (mg) per plot ($n = 6-10$ per plot). Group mean represented by black dots.

Fruit set (the number of flowers setting fruits) varied between 15% and 100% per plot. In contrast to the decreasing effect of elevation on flower and fruit production, fruit set increased with elevation along both gradients ($z_{20} = 4.9$, $P < 0.001$, Figure 6). There was no significant interaction between elevation and gradient on fruit set, number of ovules and fruit weight.

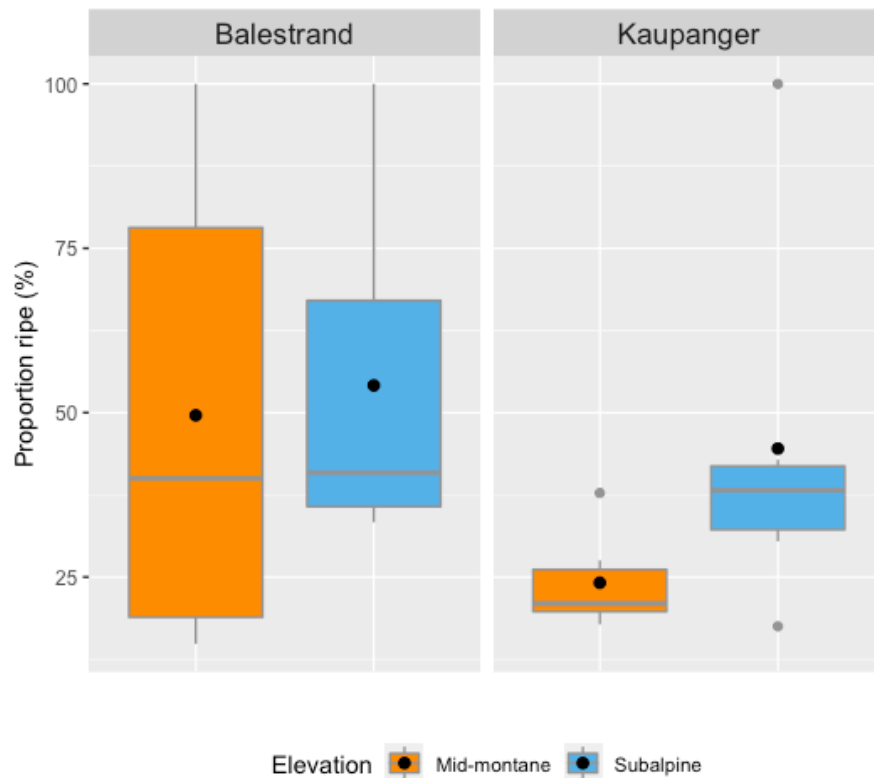


Figure 6 Fruit set (proportion of flowers setting fruits %) per plot at midmontane (mid) and subalpine (high) elevations along two elevational gradients (Balestrand and Kaupanger) in western Norway, 2020. Group mean represented by black dots.

Elevation and gradient had significant effects on the number of seeds in different development stages within fruits (Figure 7; Table VI, Appendix B). The significant interaction term between elevation and gradient for developed seeds and partly developed seed types imply that the effect of elevation depends on gradient ($P < 0.001$). The strong significant interaction is confirmed by the averaged predicted probabilities in Table VII (Appendix B) where the probability of having developed and partly developed seeds decrease with elevation in Balestrand and increases with elevation in Kaupanger.

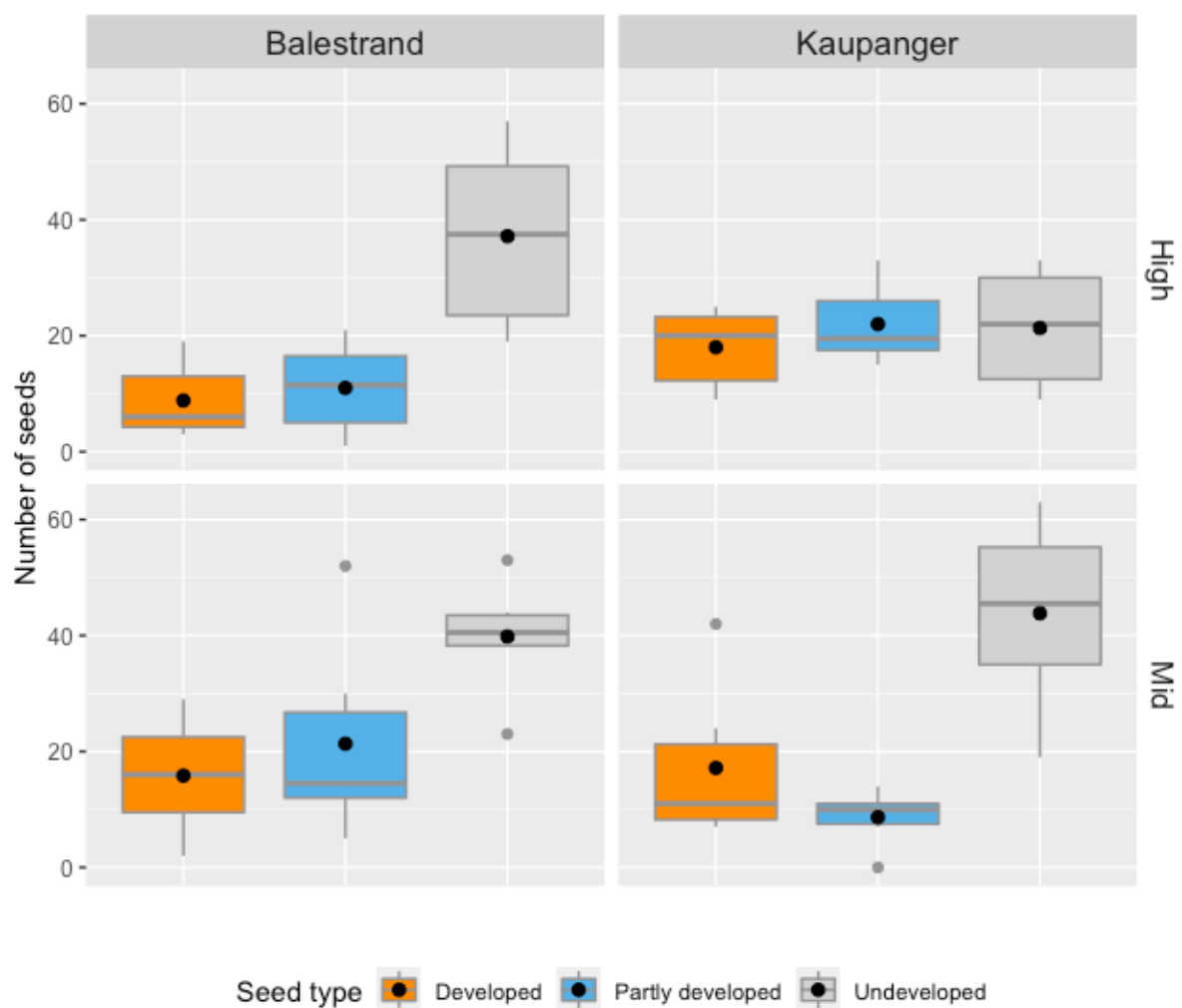


Figure 7 Number of seeds (developed, partly developed and undeveloped) per fruit ($n = 1$ per plot) at midmontane (mid) and subalpine (high) elevation along two elevational gradients (Balestrand and Kaupanger) in western Norway, 2020. Group mean represented by black dots.

A total of 71 flowers were hand cross-pollinated, in which 70.4% set fruits. Of the 71 open-pollinated flowers set 62% set fruits. However, no significant effect of treatment across elevations on fruit set were detected (Figure 8; Table VIII, Appendix B).

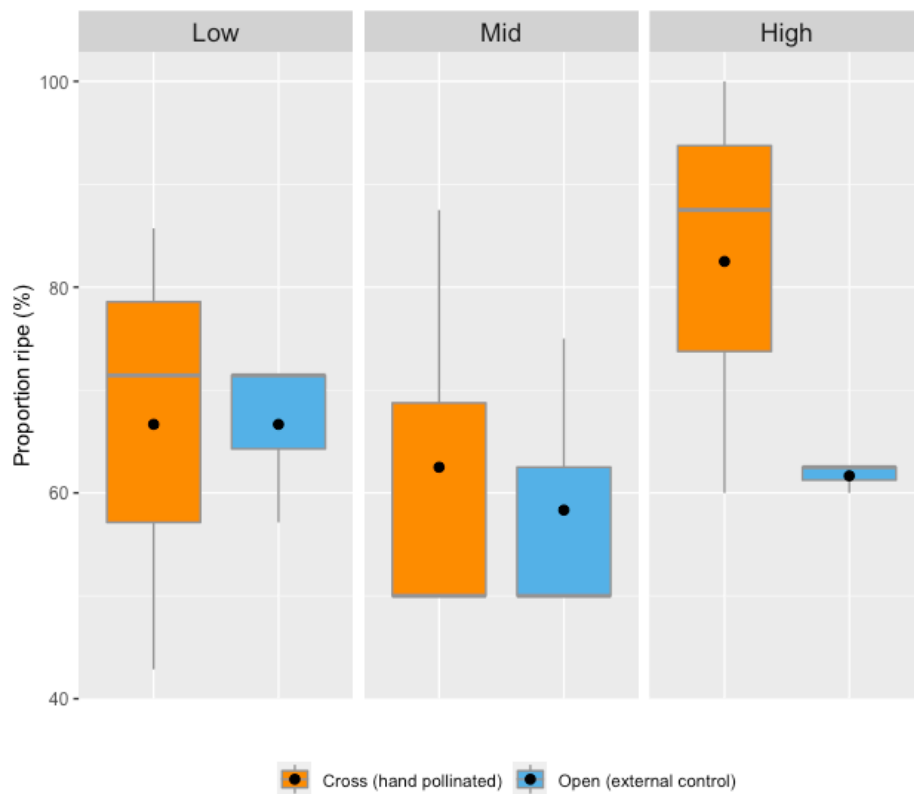


Figure 8. Fruit set (proportion of flowers setting fruits %) in hand cross-pollinated flowers ($n = 71$) and open-pollinated external control flowers ($n=71$). The experiment was conducted at submontane (low), mid-montane (mid) and subalpine (high) elevation, along an elevational gradient in western Norway (Kaupanger), in western Norway, 2021

4 Discussion

This study demonstrates relationships between elevation, and consequently variations in temperature and the timing of snowmelt, on bilberry phenology and pollinator activity, as well as underpinning complex interactive mechanisms explaining bilberry reproductive traits. Concordant with studies on plant phenology, bilberry phenology advanced with increasing temperature and snowmelt along both gradients. Even with substantial amounts of precipitation during the winter season in 2019-2020, bilberry flowering was in synchrony with pollinator activity along both gradients. *Bombus* community composition and overall pollinator activity varied between sites and gradients, with higher pollinator activity at the mid-montane sites compared to the subalpine sites. Interestingly, while the number of flowers, fruits, ovules and seed weight decreased with elevation, fruit set (proportion of flowers setting fruits) was higher at subalpine sites. Seed set (proportion of developed seeds over total amount of ovules) varied between sites, with contrasting patterns between the gradients. In the hand pollination experiment, a trend towards higher fruit set in hand cross-pollinated flowers compared to open-pollinated flowers across elevations was observed, although these differences were not significant and thus no clear sign of pollen limitation.

4.1 Bilberry phenology and flower visitation

The elevational gradient used in this study correspond to a natural climatic gradient, and consequently average temperatures together with the timing of snowmelt at the mid-montane site were higher and earlier compared to the subalpine site. Elevation had a strong effect on the onset of bilberry flowering and peak flowering dates, as well as flowering duration along both gradients (Figure 2). As hypothesized, an overall shift of plant phenology towards earlier occurrences and longer flowering periods was observed with decreasing elevation. We found that plant phenology is sensitive to temperature and snowmelt patterns, in accordance with other literature on plant phenology (Sparks *et al.* 2000; Wipf *et al.* 2009).

As expected, flower visitation during the flowering period of bilberry was higher at mid-montane elevations compared to subalpine elevations along both gradients. This is concordant with the assumption that the abundance of pollinators decreases with elevation, as the variable climatic conditions and tougher environmental conditions in the mountains restrict flight

activity (Totland 1993; Blionis & Vokou 2001; Totland *et al.* 2013). Interestingly, we found no differences in flower visitation between mid-montane and subalpine sites in Kaupanger during the flowering peak of 2021, however, there was a trend of higher activity at the submontane site (low site in 2021). Flower visitation rates varied between sites and was probably partly affected by the variable numbers of flowers within plots. Flower visitation rates was highest at the mid-montane site in Balestrand, and lowest at the mid-montane site in Kaupanger, which can be explained by the different numbers of flowers between the sites discussed in 4.3.3. As pollinator activity patterns are strongly determined by temperature and wind conditions, unfavorable weather conditions may have affected the results (Corbet 1990; McCall & Primack 1992). Even though the duration of bilberry flowering decreased with higher elevation, the overall duration of pollinator activity did not show any significant differences between elevations but was overall longer along the gradient in Kaupanger. The differences between the gradients may also be explained by unfavourable weather conditions, as more windy conditions at sites in Balestrand during observations were recorded (pers. obs.), however not tested for in the analysis. *Bombus* species were the most frequent visitor on bilberry flowers across all sites in both years, highlighting the relationship between bumblebees and bilberry in these habitats (Nuortila *et al.* 2002; Andresen 2019).

Flowering of bilberry and pollinator activity strongly overlapped along both gradients, indicating phenological synchrony, and no climate-induced (elevational) mismatch effect. Taking into account that year 2020 was unusual in terms of high amounts of precipitation with a thicker snowpack, these results indicate that the temporal synchrony between bilberry and its pollinators at these sites are resistant to fluctuations in snowmelt patterns, and that onset of bilberry flowering as well as insect emergence may be driven by the same cues (Bartomeus *et al.* 2011; Rafferty & Ives 2011). However, Kudo (2014) observed disrupted phenological synchrony between plants and bees during an unusually warm spring in Japan, suggesting different responses to environmental fluctuations. In addition, Weaver and Mallinger (2022) found altered phenology between other *Vaccinium* species and a specialist bee species changing in different rates in the eastern United States, indicating that phenology in these species may change over time. Because of the short period of the observational study (one season) and the fact that bilberry is a generalist with many possible pollinator species, this study may fail to include all pollinators dependant on bilberry flower resources. Moreover, due to the short generation time of pollinators and their high reproductive capacity, climatic factors

in addition to plant resources may have substantial effects on year-to-year variations in pollinator population sizes (Totland *et al.* 2013), which this study do not monitor.

4.2 *Bombus* diversity

Bombus communities along both gradients, represented by individuals sampled in vane traps, was different across all sites, both in abundance, richness and evenness. Interestingly, as we expected the abundance to decrease with elevation, the pattern was only observed along the gradient in Kaupanger, where abundance of *Bombus* species was also higher (Figure 3B). At this site, evenness was also lowest, with some dominating species (3A). In Kaupanger, higher abundance of *Bombus* species at the mid-montane site compared to subalpine site could be due to climatic conditions (McCall & Primack 1992; Heinrich 2004; Iserbyt & Rasmont 2012), availability of nesting sites (Hatfield & LeBuhn 2007), or that floral resources are higher at lower elevations (Mallinger *et al.* 2016). The influence of flower resource availability over time is especially important for eusocial insects, where spring resources are crucial for overwintering queens making colonies, mid-season flower resources for workers foraging and later season resources for the production of reproductive queens and males. They therefore rely on a continuous supply of floral resources to sustain the colony during the flight season (Goulson 2009). In Balestrand, heterogeneity of flower resources at the mid-montane site was lower compared to the mid-montane site in Kaupanger (pers. obs.), consequently affecting availability of flowers throughout the growing season. In addition, low flower numbers may increase inter- and intra-specific competition among the individuals in the bumblebee community (Soltz 1987). The overall low numbers of bilberry flowers at sites in Balestrand compared to sites in Kaupanger might explain the lower numbers of *Bombus* individuals here.

Bombus species collected during the flowering period of bilberry are all among early emerging species, as bilberry is an early flowering plant in this habitat. Along both gradients, only nest-building bumblebees were collected. Both short tonged and long tonged species, also including possible nectar robbers with strong mandibles, were among the captured individuals. Traces of nectar robbing on bilberry flowers was observed in the field in 2020 (pers. obs.), and may have influenced the quality of pollination, as the nectar robber bites holes in the corolla of flowers to consume nectar without touching the stigma, and therefore inhibits adequate deposition of pollen loads (Inouye 1980). Psithyrus species (parasitic bumblebees, nectar robbing normal)

were only observed during the peak flowering period along the gradient in Kaupanger in 2021, but we can therefore assume they were present in 2020 as well. Abundant species along both gradients includes common species (*B. pratorum*, *B. soroeensis*, *B. pascuorum*) as well as the subgenus *B. sensu stricto*, of which some species are considered nectar robbers (Irwin *et al.* 2010). The highest relative abundance of the subgenus *B. sensu stricto* was found at the subalpine site in Balestrand, which could have influenced the quality of pollination at this site.

Small-bodied bumblebees with short tongues found in this study (*B. pratorum*, *B. monticola*, *B. lapponicus* and *B. jonellus*) have previously been reported to forage on bilberry in spring (Yalden 1982; Moquet *et al.* 2015; Moquet *et al.* 2017c; Andresen 2019). Some of the large bodied and long-tongued species found in this study (*B. balteatus* and *B. sensu stricto*) have been found to be less frequent visitors to bilberry (Andresen 2019). As bilberry have rather short corolla and tube size, short-tongued species match bilberry flowers which may increase foraging efficiency (Klumpers *et al.* 2019). However, these results might be highly site specific and dependent on available flower resources, as flower visitor observations in this study also observed flower visits from long-tongued species (*B. sensu stricto* and *B. pascuorum*) on bilberry flowers (pers. obs.).

Species richness increased with elevation along the gradient in Balestrand and decreased along the gradient in Kaupanger. Higher richness at the subalpine site in Balestrand can be explained by higher numbers of species adapted to colder environments found at this site (*B. monticola*, *B. lapponicus* and *B. balteatus*; Figure 4A). Here, a higher number of individuals of these species were found compared to the subalpine site in Kaupanger. This might be a result of the topography between the gradients, where the mountain area is bigger around the gradient in Balestrand compared to the gradient in Kaupanger, as well as a difference in vegetation at these subalpine locations; where the subalpine site in Balestrand were located at the edge of the tree limit with less sheltered vegetation, and the subalpine site in Kaupanger was located just below the treeline. These findings is in concordance in a recent thesis by Andresen (2019), who found increased bumblebee richness in low alpine areas in midwest Norway. Higher species richness of pollinators is associated with higher robustness in generalist plant-pollinator networks, as densities in pollinator populations are highly variable over short time periods with year-to-year variation (Memmott *et al.* 2004; Totland *et al.* 2013). In habitats with higher richness, other pollinator populations may then serve as a buffer in pollination services if some pollinator populations respond negatively to influencing factors. In Norway, not many studies are

available on the status of bumblebee populations and communities at local scales (but see: Løken 1973; Totland *et al.* 2013; Ødegaard *et al.* 2015; Fourcade *et al.* 2018; Andresen 2019; Bengston *et al.* 2019), and population fluctuations are hard to determine and quantify without yearly monitoring. The short period of sampling, only during the flowering period of bilberry, might not be enough to fully describe the *Bombus* community associated with bilberry at these locations, only considering one flowering season. The species accumulation curve shows that the sampling effort during the flowering period did not fully describe *Bombus* species richness at each site (Figure 4B). In addition, activity and consequently abundance are influenced by climatic factors like precipitation, wind and temperature, not tested here (McCall & Primack 1992; Sanderson *et al.* 2015). However, considering that vane traps are highly efficient capturing *Bombus* species, we can assume that the abundance of individuals caught reflect the abundance of individuals at each site, as well as that the species caught are related to the flowering period of bilberry.

4.3 Bilberry reproductive traits

4.3.1 Flowers and fruits

Because both availability of nutrients (limitation on higher elevations) and water (limitation on lower elevations) limits bilberry growth (Woodward 1986; Boscutti *et al.* 2018; Filippi *et al.* 2021), we expected that mid-montane elevations in this area would represent the optimum for bilberry performance, with higher potential for reproduction. However, a strong effect of elevation on flower and fruit production was only seen along the gradient in Kaupanger (Figure 5A, 5B). Recent studies on bilberry fruit production in the same area, close to the gradient in Kaupanger, also observed a strong effect of elevation on fruit production (Berge 2018; Benevenuto *et al.* 2020b), and the climatic optimum of bilberry vegetative performance has been proposed to be at mid-montane elevations here (Benevenuto *et al.* 2020b). Studies on bilberry flower and fruit production along an elevational gradient in northern Spain found no variation in density of flowers and fruits with elevation (Pato & Obeso 2012), and a long-term study in Lapland Finland recorded greater numbers of flowers at alpine sites, but also greater number of fruits at the forest sites (Boulanger-Lapointe *et al.* 2017), suggesting substantial differences in flower and fruit production across locations and environments. Differences in

local abiotic factors, nutrient availability, as well as biotic interactions play important roles in bilberry performance and can explain the differences between the gradients.

Because both biotic and abiotic factors have been proposed to affect annual bilberry flower and fruit numbers, several explanations can be made to explain the differences in flower and fruit production between the gradients. Herbivory has been found to affect bilberry, inducing defense responses as a tradeoff over both growth and reproduction, also with multiannual effects (Hegland *et al.* 2016; Benevenuto *et al.* 2019; Benevenuto *et al.* 2020b). No substantial effects of herbivory were recorded in plots across sites in this study, however previous years grazing might have influenced flower and fruit reproduction. Eckerter *et al.* (2019) found increased number of fruits at locations with greater light conditions and more active pollinators, and Boulanger-Lapointe *et al.* (2017) attributed increased fruit production to pollinator networks. Overall, this might indicate that high abundances of bumblebees are important for sufficient pollination, as availability of bumblebees was higher along the gradient in Kaupanger. The clear effect of elevation on availability of pollinators along the gradient in Kaupanger might be an explaining factor in fruit production, however not on the differences in potential reproduction (number of flowers produced) between the gradients.

Nielsen *et al.* (2007) and found increased bilberry performance in younger, less mature stands after clear cutting, and proposed that disturbance history should be considered when studying plant performance in bilberry. Considering that the mid-montane site in Kaupanger is located in a previously clear-cut area compared to the natural open mid-montane site in Balestrand, it seems that bilberry performance is influenced in this regard. Climatic factors, including low temperatures in autumn and spring, thin snow cover and low or high amounts of precipitation during berry ripening in summer are also associated with decreased bilberry production (Selås 2000; Selås *et al.* 2015). In 2019-2020, precipitation was high during winter months, and created a rather deep snowpack, which may have shielded bilberry from frost damages of both vegetative and reproductive parts (Taulavuori *et al.* 2013). Also, both previous years and current-year spring temperatures, which may influence bud formation and flowering in bilberry, as well as soil nutrient availability on bilberry reproductive performance (Fernández-Calvo & Obeso 2004; Pato & Obeso 2012; Nybakken *et al.* 2013), are not considered here. In addition, masting habits (the production of many seeds in cycles, in regional synchrony) are found in bilberry, with cycles of 2-5-year intervals (Selås 2000). Such cycles may affect the results in this study, with contrasting effects between the gradients.

4.3.2 Fruit weight and number of ovules

While total numbers of flowers and fruits differed between the gradients, elevation had a strong effect on both fruit weight and the number of ovules, with the highest numbers at mid-montane elevations along both gradients (Figure 5C, D). Fruit weight was also heavier along the gradient in Balestrand compared to sites in Kaupanger. These findings may be explained by bigger ramets located at mid-elevations having potentially more energy to produce larger and heavier fruits with more seeds compared to subalpine plants. Berge (2018) found no differences in fruit weight along an elevational gradient in the same area. Similar contrasting observations within regions were observed in northern Spain, where Fernández-Calvo and Obeso (2004) and Pato and Obeso (2012) found differing patterns on fruit weight and number of ovules in bilberry along similar elevational gradients, highlighting the importance of annual variation on bilberry reproductive traits.

4.3.3 Fruit and seed set

Because of limiting climatic conditions in the subalpine, possibly yielding lower pollination services, we expected fruit set to be higher at mid-montane elevations. Conversely, fruit set (proportion of flowers setting fruits) was highest at the subalpine sites, where temperatures are lower and the growing season shorter. Other factors might be responsible for this pattern. In the alpine plants have evolved several adaptations to ensure successful reproduction, where climate is more limiting for reproduction compared to pollination availability, and species may therefore exhibit spatial variation on plant traits, due to different selection pressures. The time period that single flowers are open and susceptible of pollen loads decrease with higher temperature and also with sufficient amounts of pollen deposit (Bingham & Orthner 1998; Lundemo & Totland 2007). Flowers are thus susceptible for insect pollination for longer time periods when experiencing lower temperatures, potentially until pollinated sufficiently, compared to locations with higher temperatures. In addition, as availability of pollinators may decrease with decreasing temperatures, the adaptation allows flowers in colder environments to be open and available for flower visits for a longer time, compared to locations with higher temperatures. Variable selection pressures in response to different pollinator communities

along elevational gradients may also change morphological structures in flowers and some plants have exhibited elevational differences in floral characters (Pérez-Barrales *et al.* 2007), however these traits are not measured here, and no variation on bilberry corolla size and depth was found along an elevational gradient in Spain (Pato & Obeso 2012). Because elevational differences on resource allocation in plants may be evident on reproduction traits (Ramsey 1995), bilberry in the subalpine might allocate more resources to fruit and seed production in the pollinated flowers or reproductive organs, compared to lower elevations where climate is less limiting (Kawano & Masuda 1980; Hemborg & Karlsson 1998; Fabbro & Körner 2004). Although flower visitation was higher at mid-montane sites compared to subalpine sites, the proportion of flower visits to the total amount of flowers might be lower at mid-montane areas, because there is more competition between flowers here. Bilberry growing at mid-montane elevations therefore experience pollen limitation due to higher competition for flower visits. However, in this study, this pattern was only explained by visitation rates along the gradient in Kaupanger, which might be explained by the difference in flower resources between these sites and the variable nature of pollinator activity during field observations. Because reductions in fruit and seed set after self-pollination in bilberry has been shown in several studies on bilberry (Jacquemart & Thompson 1996; Jacquemart 1997; Nuortila *et al.* 2002), the size of ramets and the overall clone size in the area might play essential roles in bilberry fruit and seed set, as pollination by insects might deposit pollen from flowers within the same clone or ramet (Nuortila *et al.* 2002). Even though measures of ramets and clones are not included in this study, it is reasonable to assume that the size of the ramets and clones are bigger at mid-montane elevations, as vegetative performance is often higher at these elevations. Therefore, there may be less outcrossing in bilberry at mid-montane elevations, as successive distances between flower visits by bumblebees are found to be rather short (less than 1m; Nuortila *et al.* 2002).

Conversely, seed set (number of ovules developing to fully matured and developed seeds) differed substantially between sites, with opposite trends between the gradients (Figure 7). The opposite trends, where seed set increased with higher elevation in Kaupanger and decreased with higher elevation in Balestrand, might again indicate site specific differences relating to abiotic factors. Berge (2018) found the same pattern as we found along the gradient in Kaupanger, with increasing seed set with increasing elevation. Self-compatibility have also been assumed to be favored in alpine areas, as a response to low pollinator numbers (Richards & Richards 1997), and may affect seed numbers here. As the subalpine site in Balestrand were

slightly colder and wetter compared to the subalpine site in Kaupanger, an explanation may be that seed set in bilberry increase with elevation until climatic constraints are too limiting for seed production, however further investigation is needed. In addition, higher abundances of bumblebees at the subalpine site in Kaupanger compared to Balestrand was observed, which may have influenced pollination services, consequently influencing seed set. Lack of elevational differences in fruit and seed set found by Pato and Obeso (2012) in northwest Spain, indicate again site-specific differences on bilberry reproductive traits.

4.3.5 Hand-pollination experiment

A non-significant trend of increased fruit set in the hand cross-pollinated flowers compared to open-pollinated flowers, with a larger effect at the subalpine elevation compared to lower elevations was observed (Figure 8). In this study, no clear sign of pollen limitation could be seen. Pollen limitation has been proposed to explain self-compatible features, as well as clonal reproduction (Richards & Richards 1997), and Larson and Barrett (2000) found lower pollen limitation in self-compatible species compared to self-incompatible species. Considering that bilberry is considered partly self-pollinated, this could explain the lack of differences. However, in a pilot study conducted in 2020 at the subalpine site in Kaupanger, both bagged ramets and single flowers did not produce any viable fruits (unpubl.dat), which further suggests that pollination is important for bilberry reproduction. The trend found in this study follows findings from Jacquemart (1997) who found increased fruit set in bilberry after hand pollination and Nuortila *et al.* (2002) who found that bagged flowers yielded almost no fruits. Preliminary results from a study along an elevational gradient in Norway (Olsen *et al.* 2017) also found that pollination is important for fruit production in bilberry, and that the effect of pollination seems to be higher with higher elevation in the case of bilberry. However, as this study only included fruit set across the different treatments, it would be interesting to see how seed set in fruits varied across treatment and elevation, as both measures should be considered when measuring pollination success (Knight *et al.* 2006). In addition, in this study, only single flowers were allocated treatments, which due to reallocation processes might affect the results (Knight *et al.* 2006; García-Camacho & Totland 2009).

4.4 Future research

As bilberry reproductive traits vary across elevational gradients and regions as well as within regions, further investigation of regional annual and interannual effects on bilberry performance, across several elevational gradients, is needed. Adding more elevations to the gradients, from lowland habitats to higher alpine areas, as well as including several abiotic parameters, may further help understand the varying plant traits in bilberry at a finer scale, and may provide valuable information about climatic effects on bilberry reproduction. Because the effect of insect pollination on bilberry seems to be variable across elevations, further experimental studies along elevational gradients could result in a clearer picture of the importance of insect pollination on this species. Also, as pollinator populations vary over short time periods as well as short distances, further investigation of the mutualistic partnership between pollinators and bilberry should be conducted, also in parallel with later co-flowering species within the same community, to further analyse network structures. In the case of bumblebees, measuring the numbers of queens in relation to the number of workers within the community and bumblebee populations might be a more direct way of measuring phenological match/mismatch between bumblebees and early flowering species. Because anthropogenic climate change increase seasonal variability in climate parameters, the use of elevational gradients to predict species responses in the future may underestimate species evolutionary adaptations. Climatic differences may also not be the main determinant of variation among communities along gradients (Dunne *et al.* 2004). Therefore, as elevational gradients are limited to predict longer term dynamics, experimental studies should complement elevational studies for further investigation of short-term changes.

5 Conclusions

In summary, elevation and climatic conditions are important factors determining bilberry phenology and, in combination with pollinator availability, important in determining bilberry reproduction. Our results show that the optimum elevation for bilberry vegetative performance might reflect the optimum for bilberry yields, but not for fruit and seed set. Moreover, elevation had opposing effects on different reproductive parameters along the elevational gradients, suggesting that other factors are important for bilberry reproduction. In addition, fruit production was higher along the gradient with the highest pollinator abundance. Global

warming may produce opposite effects on bilberry reproductive parameters. Future studies on expected changes in boreal forests, and consequently impacts on bilberry phenology and reproduction, should be seen in parallel with expected changes in pollination availability and environmental variability. Extending observations across several elevational gradients with additional elevations, in combination with experimental warming to predict short term changes, could help fully understand the interactive processes between climatic conditions and pollination services on bilberry reproduction, in relation to a changing climate.

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Appendix A – Additional data

Flowering phenology and pollinator activity

Table I. Calculated mean per transect of first, peak, last dates (Julian) and the duration of flowering and observed flowervisits at midmontane (mid) and subalpine (high) elevation along two elevational gradients (Balestrand and Kaupanger) in western Norway, 2020

| Estimate | Balestrand | | | | Kaupanger | | | |
|----------|------------|-------------|---------|-------------|-----------|-------------|---------|-------------|
| | Mid | | High | | Mid | | High | |
| | Flowers | Pollinators | Flowers | Pollinators | Flowers | Pollinators | Flowers | Pollinators |
| First | 146. | 151. | 163. | 163. | 147. | 147. | 168. | 161. |
| Peak | 158. | 159. | 174. | 174. | 156. | 156. | 173. | 174. |
| Last | 169. | 167. | 178. | 178. | 182. | 168. | 177. | 182. |
| Duration | 23. | 16. | 15. | 15. | 35. | 21. | 9. | 21. |

Appendix B – Model summaries

Flowering phenology and pollinator activity tested in LM models

Table II. Effects of elevation and gradient on flowering phenology 2020, flower visitation during the flowering period of 2020 and flowering peak of 2021, and flowering and pollinator activity synchrony during the flowering peak of 2020, tested in LM models

| | First flowering date, 2020 | | | Peak flowering date, 2020 | | |
|--|--|----------|-------------------|--|----------|------------------|
| | Effect ± se | <i>t</i> | <i>P</i> | Effect ± se | <i>t</i> | <i>P</i> |
| Intercept, (Mid Balestrand) | 146. ± 1.55 | 94.46 | <0.001 | 157.73 ± 1.39 | 113.77 | <0.001 |
| Elevation (High) | 21. ± 2.19 | 9.61 | <0.001 | 14.82 ± 1.96 | 7.56 | <0.001 |
| Gradient (Kaupanger) | 4.33 ± 2.19 | 1.98 | 0.083 | -1.5 ± 1.96 | -0.76 | 0.466 |
| Elevation (High) *Gradient (Kaupanger) | -1.67 ± 3.1 | -0.54 | 0.605 | 1.84 ± 2.77 | 0.66 | 0.526 |
| | Flowering duration, 2020 | | | Flower visitation duration, 2020 | | |
| | Effect ± se | <i>t</i> | <i>P</i> | Effect ± se | <i>t</i> | <i>P</i> |
| Intercept, (Mid Balestrand) | 19. ± 2.73 | 2.73 | < 0.001 | 8. ± 1.68 | 4.78 | <0.001 |
| Elevation (High) | -9.33 ± 8.86 | 3.87 | <0.05 | 0. ± 2.34 | 0. | 1. |
| Gradient (Kaupanger) | 1. ± 3.87 | 3.87 | 0.802 | 6.67 ± 2.37 | 2.81 | <0.05 |
| Elevation (High) *Gradient (Kaupanger) | -6. ± 5.47 | 5.47 | 0.304 | -6.33 ± .35 | -1.89 | 0.095 |
| | Total number of flower visits, 2020 | | | Peak diff between gradients, 2020 | | |
| | Effect ± se | <i>t</i> | <i>P</i> | Effect ± se | <i>t</i> | <i>P</i> |
| Intercept, (Mid Balestrand) | 1.98 ± 0.38 | 5.2 | <0.001 | -0.8 ± 1.35 | -0.59 | 0.569 |
| Elevation (High) | -1.28 ± 0.51 | -2.51 | <0.05 | 1.71 ± 1.91 | 0.89 | 0.399 |
| Gradient (Kaupanger) | 0. ± 0.51 | 0.07 | 0.944 | 1.47 ± 1.91 | 0.77 | 0.463 |
| Elevation (High) *Gradient (Kaupanger) | 0.15 ± 0.7 | 0.22 | 0.828 | -3.16 ± 2.71 | -1.17 | 0.277 |
| | Total number of flower visits, 2021 | | | Correlation peak flowering and peak pollinator activity, 2020 | | |
| | Effect ± se | <i>t</i> | <i>P</i> | Effect ± se | <i>t</i> | <i>P</i> |
| Intercept, Elevation (Low) | 15.59 ± 2.18 | 7.16 | <0.001 | 11.33 ± 12.26 | 0.92 | 0.377 |
| Elevation (Mid) | -5.98 ± 3.03 | -1.97 | 0.056 | 0.93 ± 0.07 | 12.54 | <0.001 |
| Elevation (High) | -5.79 ± 3.58 | -1.62 | 0.113 | | | |
| | Flower visitation rate, 2020 | | | | | |
| | Effect ± se | <i>t</i> | <i>P</i> | | | |
| Intercept, (Mid Balestrand) | -4.11 ± 0.3 | -13.63 | <0.001 | | | |
| Elevation (High) | -1.32 ± 0.043 | -3.08 | <0.05 | | | |
| Gradient (Kaupanger) | -1.23 ± 0.43 | -2.87 | <0.05 | | | |
| Elevation (High) *Gradient (Kaupanger) | 1.4 ± 0.6 | 2.31 | <0.05 | | | |

Bold text indicates significant values $P < 0.05$

Bombus diversity tested in permanova analysis and GLM models

Table III. Pairwise permanova analysis results of *Bombus* community based on Bray-Curtis matrices

| | DF | Sum Of Sqs | R2 | F | P |
|------------------------|----|------------|------|------|--------------|
| Elevation | 1 | 0.48 | 0.17 | 7.45 | 0.001 |
| Gradient | 1 | 0.46 | 0.16 | 7.08 | 0.001 |
| Elevation, Gradient | 1 | 0.24 | 0.09 | 3.75 | 0.01 |
| Residual | 25 | 1.61 | 0.58 | | |
| Total | 28 | 2.80 | 1. | | |

Bold text indicates significant values $P < 0.05$

Table IV. The effect of elevation on *Bombus* diversity sampled in vane traps during the flowering period of bilberry at midmontane (mid) and subalpine (high) elevations along two elevational gradients (Balestrand and Kaupanger) in western, Norway, tested in GLM models

| | Richness | | | Abundance | | | Evenness | | |
|---|-------------|-------|------------------|--------------|-------|------------------|--------------|-------|------------------|
| | Effect ± se | t | P | Effect ± se | t | P | Effect ± se | t | P |
| Intercept, (Mid Balestrand) | 3.4 ± 0.59 | 5.72 | <0.001 | 2.42 ± 0.3 | 8.17 | <0.001 | 0.86 ± 0.05 | 17.45 | <0.001 |
| Elevation (High) | 1.98 ± 0.76 | 2.61 | <0.05 | -0.02 ± 0.38 | 0.05 | 0.962 | -0.04 ± 0.06 | -0.58 | 0.568 |
| Gradient (Kaupanger) | 1.6 ± 0.81 | 1.99 | 0.057 | 1.58 ± 0.32 | 4.92 | <0.001 | -0.16 ± 0.66 | -2.48 | <0.05 |
| Elevation (High) *Gradient (Kaupanger) | -3.31 ± 1. | -3.28 | <0.01 | -1.21 ± 0.43 | -2.83 | <0.01 | 0.19 ± 0.08 | 2.29 | <0.05 |

Bold text indicates significant values $P < 0.05$

Bilberry reproductive traits tested in GLM and LM models

Table V. Effects of elevation and gradient on different response variables related to bilberry plant reproduction at midmontane (mid) and subalpine (high) elevations along two elevational gradients in western Norway, 2020, tested in GLM and LM model

| GLM models | | | | | | |
|------------------|-----------------------|----------|------------------|----------------------|----------|------------------|
| | Max number of flowers | | | Max number of fruits | | |
| | Effect ± se | <i>t</i> | <i>P</i> | Effect ± se | <i>t</i> | <i>P</i> |
| Intercept, (Mid | | | | | | |
| Balestrand) | 3.73 ± 0.31 | 12.14 | <0.001 | 2.32 ± 0.2 | 11.41 | <0.001 |
| Elevation (High) | -0.96 ± 0.59 | -1.64 | 0.116 | -0.26 ± 0.31 | -0.85 | 0.408 |
| Gradient | | | | | | |
| (Kaupanger) | 0.91 ± 0.36 | 2.51 | <0.01 | 0.83 ± 0.24 | 3.41 | <0.01 |
| Elevation (High) | | | | | | |
| *Gradient | | | | | | |
| (Kaupanger) | -0.6 ± 0.75 | -0.8 | 0.435 | -0.85 ± 0.41 | -2.08 | 0.051 |
| LM models | | | | | | |
| | Fruit set | | | Ovules per fruit | | |
| | Effect ± se | <i>z</i> | <i>P</i> | Effect ± se | <i>t</i> | <i>P</i> |
| Intercept, (Mid | | | | | | |
| Balestrand) | -1.83 ± 0.14 | -13.26 | <0.001 | 4.34 ± 0.08 | 56.64 | <0.001 |
| Elevation (High) | 1.1 ± 0.25 | 4.87 | <0.001 | -0.3 ± 0.2 | -2.56 | <0.05 |
| Gradient | | | | | | |
| (Kaupanger) | -0.11 ± 0.17 | -1.66 | 0.511 | -0.1 ± 0.11 | -0.9 | 0.379 |
| Elevation (High) | | | | | | |
| *Gradient | | | | | | |
| (Kaupanger) | -0.48 ± 0.29 | -1.62 | 0.105 | 0.17 ± 0.17 | 1.04 | 0.310 |
| LM models | | | | | | |
| | Fruit dry weight | | | | | |
| | Effect ± se | <i>t</i> | <i>P</i> | | | |
| Intercept, (Mid | | | | | | |
| Balestrand) | -3.36 ± 0.09 | -39.04 | <0.001 | | | |
| Elevation (High) | -0.26 ± 0.12 | -2.13 | <0.05 | | | |
| Gradient | | | | | | |
| (Kaupanger) | -0.38 ± 0.12 | -3.11 | <0.01 | | | |
| Elevation (High) | | | | | | |
| *Gradient | | | | | | |
| (Kaupanger) | 0.28 ± 0.17 | 1.64 | 0.116 | | | |

Bold text indicates significant values $P < 0.05$

Seed set tested in MLR model

Table VI. The log of odds of seed types relating to predictor variables elevation (midmontane (mid) and subalpine (high)) and gradient (Balestrand and Kaupanger) in the multinomial regression model

| | Developed vs. undeveloped | | | Partly developed vs. Undeveloped | | |
|--|---------------------------|-------|------------------|----------------------------------|-------|------------------|
| | Effect ± se | z | P | Effect ± se | z | P |
| Intercept, (Mid Balestrand) | -0.92 ± 0.12 | -7.61 | <0.001 | -0.62 ± 0.11 | -7.61 | <0.001 |
| Elevation (High) | -0.51 ± 0.2 | -2.64 | <0.01 | -0.59 ± 0.18 | -2.64 | <0.001 |
| Gradient (Kaupanger) | -0.02 ± 0.17 | -0.09 | 0.929 | -0.99 ± 0.19 | -0.09 | <0.001 |
| Elevation (High) *Gradient (Kaupanger) | 1.28 ± 0.26 | 4.89 | <0.001 | 2.25 ± 0.27 | 4.89 | <0.001 |

Bold text indicates significant values $P < 0.05$

Table VII. Averaged predicted probabilities of seed types relating to midontane (mid) and subalpine (high) elevation and gradient (Balestrand and Kaupanger), calculated from odd ratios in the multinomial regression model (Table VI)

| | Balestrand | | Kaupanger | |
|------------------|------------|------|-----------|------|
| | Mid | High | Mid | High |
| Developed | 0.27 | 0.16 | 0.12 | 0.29 |
| Partly developed | 0.21 | 0.19 | 0.25 | 0.36 |
| Undeveloped | 0.52 | 0.65 | 0.63 | 0.35 |

Fruit set in the hand pollination experiment tested in GLM model

Table VII. *Effects of elevation and treatment on fruit set in a hand pollination experiment. Cross = hand pollinated, Open internal = Open pollinated internal control, Open external = Open pollinated external control. Tested in GLM model*

| | Effect ± se | <i>z</i> | <i>P</i> |
|---|--------------|----------|----------|
| Intercept elevation (low), treatment (open, control) | 0.69 ± 0.46 | 1.49 | 0.134 |
| Treatment (cross) | 0. ± 0.66 | 0. | 1. |
| Elevation (mid) | -0.36 ± 0.62 | -0.57 | 0.566 |
| Elevation (high) | -0.23 ± 0.61 | -0.36 | 0.716 |
| Treatment (cross), elevation (mid) | 0.17 ± 0.88 | 0.2 | 0.843 |
| Treatment (cross), elevation (high) | 0.97 ± 0.92 | 1.05 | 0.292 |

Bold text indicates significant values $P < 0.05$

Appendix C - Species recorded and sampled in the field

Pollinator species recorded during flower visitor observations

Table IX. List of pollinator species recorded during flower visitor observations made during bilberry flowering period of 2021 and flowering peak of 2021 at submontane (low), midmontane (mid) and subalpine (high) elevation along two elevational gradients in western Norway

| Order/Family | 2020 | | | |
|-----------------------------|------------|------|-----------|------|
| | Balestrand | | Kaupanger | |
| | Mid | High | Mid | High |
| Hymenoptera/Bumblebees | 226 | 28 | 240 | 44 |
| Hymenoptera/Solitary bees | 47 | 2 | 9 | 5 |
| Hymenoptera/Wasps | 1 | 0 | 9 | 3 |
| Diptera/Hoverflies | 1 | 0 | 0 | 0 |
| Total number of individuals | 275 | 30 | 258 | 52 |

| Order/Family | 2021 | | |
|-----------------------------|------|-----------|------|
| | Low | Kaupanger | |
| | | Mid | High |
| Hymenoptera/Bumblebees | 202 | 86 | 88 |
| Hymenoptera/Solitary bee | 62 | 63 | 10 |
| Hymenoptera/Wasps | 16 | 19 | 19 |
| Diptera/Hoverflies | 1 | 24 | 0 |
| Total number of individuals | 281 | 192 | 117 |

The table indicates the abundance of each species at each site. Solitary bees have several families not listed here

Species sampled in vane traps

Table X. List of *Bombus* species sampled with vane traps during the flowering period of bilberry at midmontane (mid) and subalpine (high) elevation along two elevational gradients (Balestrand and Kaupanger), in western Norway 2020

| Order/Family | Species | Balestrand | | Kaupanger | |
|-------------------------------|-------------------------|------------|------|-----------|------|
| | | Mid | High | Mid | High |
| Hymenoptera/ | | | | | |
| Bumblebees | <i>B. soroeensis</i> | 6 | 4 | 166 | 64 |
| | <i>B. pratorum</i> | 18 | 35 | 61 | 73 |
| | <i>B. pascuorum</i> | 27 | 10 | 56 | 33 |
| | <i>B. sensu stricto</i> | 4 | 19 | 25 | 13 |
| | <i>B. consorinus</i> | 0 | 1 | 3 | 4 |
| | <i>B. monticola</i> | 0 | 5 | 0 | 2 |
| | <i>B. hortorum</i> | 0 | 6 | 1 | 0 |
| | <i>B. hypnorum</i> | 1 | 2 | 2 | 1 |
| | <i>B. lapponicus</i> | 0 | 5 | 0 | 0 |
| | <i>B. balteatus</i> | 0 | 1 | 1 | 0 |
| | <i>B. jonellus</i> | 0 | 0 | 0 | 1 |
| Hymenoptera/Solitary bees | | | | | |
| | | 5 | 2 | 8 | 8 |
| Total of individuals | | 61 | 90 | 323 | 199 |
| Total Bomubs species richness | | 5 | 10 | 8 | 8 |

The table indicates the abundance of each species at each site. Solitary bees have several families not listed here

Appendix D – Specimens from the Natural History collection

| <i>Table XI. Specimens used for comparison borrowed from the Natural History Collections, University of Bergen</i> | | |
|--|-----------------------|-----------------------|
| Order/Family | Species | Accession nb |
| Hymenoptera/Bombus | <i>B. sporadicus</i> | Hym 35596 (kasse 312) |
| | | Hym 35605 (kasse 312) |
| | <i>B. soroeeensis</i> | Hym 30313 (kasse 294) |
| | | Hym 30324 (kasse 294) |
| | <i>B. apidae</i> | Hym 47909 (kasse 355) |
| | | Hym 47888 (kasse 355) |
| | | Hym 47900 (kasse 355) |
| | <i>B. hortorum</i> | Hym 51443 (kasse 372) |
| | | Hym 51447 (kasse 372) |
| | <i>B. consobriuns</i> | Hym 48694 (kasse 360) |
| | | Hym 48698 (kasse 360) |
| | <i>B. muscorum</i> | Hym 56507 (kasse 378) |
| | | Hym 56496 (kasse 378) |
| | | Hym 56623 (kasse 378) |
| | <i>B. pascuorum</i> | Hym 57470 (kasse 383) |
| | | Hym 57485 (kasse 383) |
| | | Hym 57418 (kasse 383) |
| | <i>B. cingulatus</i> | Hym 41636 (kasse 318) |
| | | Hym 41664 (kasse 318) |
| | | Hym 41602 (kasse 317) |
| | <i>B. hypnorum</i> | Hym 41870 (kasse 319) |
| | | Hym 41768 (kasse 319) |
| | <i>B. jorellus</i> | Hym 44366 (kasse 328) |
| | | Hym 44340 (kasse 328) |
| | <i>B. lapponicus</i> | Hym 38044 (kasse 336) |
| | | Hym 38033 (kasse 336) |
| | <i>B. balteanus</i> | Hym 48533 (kasse 359) |
| | Hym 48541 (kasse 359) | |
| <i>B. lucorum</i> | Hym 38872 (kasse 305) | |
| | Hym 33888 (kasse 305) | |
| <i>B. monticola</i> (<i>B. lapponicus scandinavicus</i>) | Hym 38520 (kasse 338) | |
| | Hym 38704 (kasse 338) | |
| | Hym 38529 (kasse 338) | |

Appendix E – Additional figures

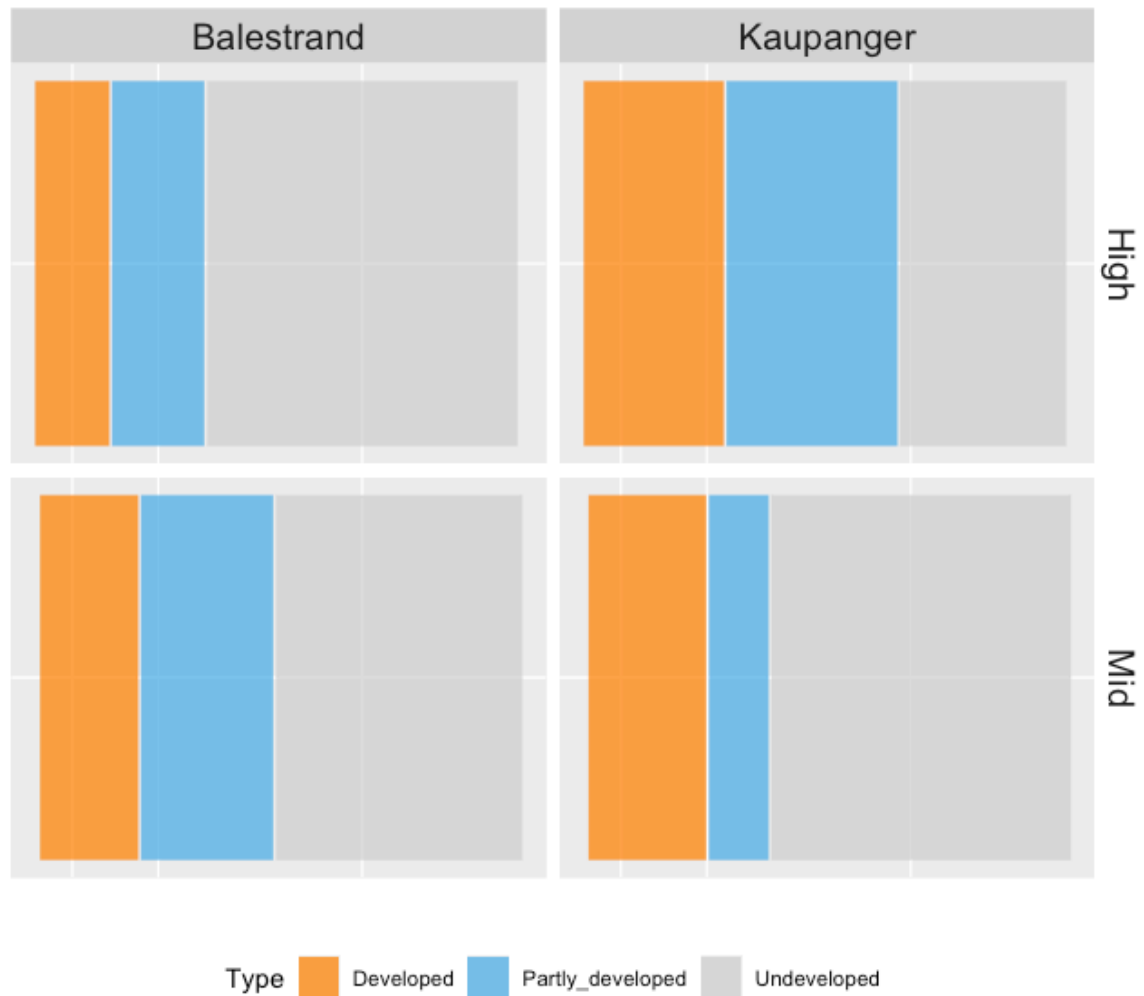


Figure I. Mosaic plot of seed type category proportions of *V. myrtillus* seedlings at midmontane (mid) and subalpine (high) elevations along two elevational gradients (Balestrand and Kaupanger) in western Norway, 2020. Seed type category; developed = large and filled, partly developed = large and partly filled, undeveloped = small and unfilled.

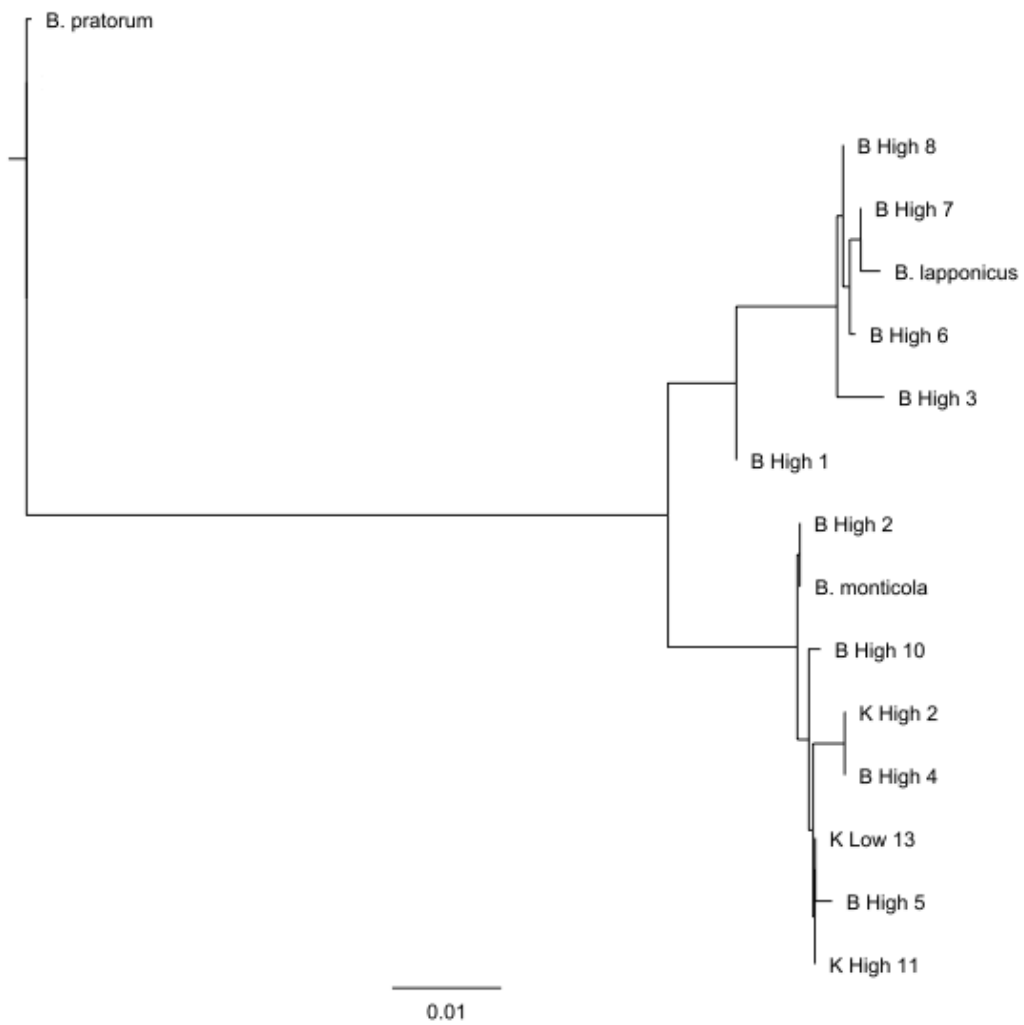


Figure II. Gen tree generated from the mitochondrial COI region for representatives of *B. monticola* and *B. lapponicus* species from the *Bombus* dataset ($n = 12$). Computed using Geneious Tree Bulder (version 11.1.5). *B. pratorum* (acc. nb. MBF00070), *B. lapponicus* (acc. nb. BMB229), and *B. monticola* (acc. nb. MZH_GP.65740), as reference from GenBank.