UMEÅ UNIVERSITET

INDIVIDUAL TRAIT MATCHING OF BUMBLEBEES (*BOMBUS*) AND FLOWERS ALONG AN ENVIRONMENTAL GRADIENT

Johan Per Michael Svedin

Abstract

Insect pollinators serve a critical role in maintaining plant biodiversity and are especially susceptible to changes within their environment. To study the possible effects of seasonal variation in temperature, as well as climatic temperature increase on the plant-pollinator community, the relationship between bumblebee and flowering plant traits along an elevational gradient, representing warming-induced changes in plant community, were examined. Two hypotheses were tested; 1) if plant traits can predict visiting bumblebee proboscis length, and 2) if the relationship between plant traits and proboscis length is influenced by elevation, and the progression of the growing season. The study took place along an elevational gradient on Mt. Nuolja in Abisko National Park, Sweden. During surveys bumblebees were caught and measured. Flowers visited by captured bumblebees were collected, categorized by restrictiveness (i.e., whether or not the flower require a certain proboscis length, in order to access the nectar and pollen rewards) and floral traits measured (e.g., petal length). The results revealed that petal length was a significant predictor of bumblebee proboscis length, when taking restrictiveness into account. Furthermore, the relationship became weaker with increasing elevation for restrictive flowers but stronger for unrestrictive flowers. These findings show that trait-matching between bumblebees and flowers is an influential factor for flower selection and is affected by climatic temperature. This highlights the importance of considering individual-level traits when studying plant preference and creates a framework for assessing plant-pollinator networks. Future studies should examine additional traits that could explain the apparent size matching between unrestrictive flowers and proboscis.

Key Words: Arctic climate change; *Bombus*; Flower morphology; Plant-pollinator trait-matching

Table of contents

1 Introduction

1.1 Background

The distribution of species is determined by abiotic and biotic factors, which makes it possible to predict changes in species distribution by examining ecosystem change caused by rapid global warming. While abiotic factors such as temperature, may pose strict limits to the distribution of species, so too can biotic factors such as species interactions, i.e., between plants and pollinators, and competition (Damos and Savopoulou-Soultani, 2011; Miller-Struttmann and Galen, 2014; Rasmont et al., 2015; Kuppler et al., 2020). On a more discrete scale, functional traits, such as flower size, can be influenced by temperature, e.g., growing smaller in response to colder temperatures (Dai et al., 2017). However, the influence from biotic factors, such as visiting pollinators, have been shown to cause plants to break from the trend with temperature and instead keep or increase their flower size, even in colder temperatures, due to selection for certain complementary traits (e.g., corolla depth; Egawa, Hirose and Itino, 2020; Wei et al., 2021). This is of particular interest for insect pollinators, as they serve a critical role in maintaining plant biodiversity and are especially susceptible to changes within their environment (Burkle, Marlin and Knight, 2013; Wei et al., 2021).

Insect pollinators provide vital ecosystem functions by transporting pollen between flowering plants (Daily, 1998). These plant-pollinator interactions serve the reproduction of flowering plants and as food resources for the pollinator and, in turn, increases food availability to other organisms in the form of fruits and seeds (Klein et al., 2007). For insect pollinators, flower preference can be partially explained by the relationship between feeding parts (e.g., proboscis and head size) and floral morphology (e.g., petal length, position of the inflorescence, and corolla length), suggesting that individuals are more likely to visit flowers where floral and pollinator morphology correlates (bumblebees (*Bombus*): Ranta and Lundberg, 1980; Klumpers, Stang and Klinkhamer, 2019; butterflies (*Rhopalocera*): Szigeti et al., 2020). Mismatch between insect and flower morphologies increase the handling time when foraging, and too large of a mismatch can impose a functional threshold where foraging is no longer practical (Stang, Klinkhamer and van der Meijden, 2007; Klumpers, Stang and Klinkhamer, 2019). Insects with long proboscises relative to floral morphology can still visit plants with short corollas. However, these tend to have nectar with lower sugar concentration compared to plants with long corollas: Therefore, insects with a long proboscis have to spend more time foraging to receive the same amount of energy reward from short-corolla plants (Klumpers, Stang and Klinkhamer, 2019).

These examples of trait matching are hypothesised to have come about due to fecundity driven co-evolution of flowering plants and pollinators that have occurred over millions of years of climatic variation (Goulson, 2010). However, with the recent unprecedented rapid warming of the planet, it is important to understand the possible impacts on these relationships, especially from an anthropocentric point of view, as a majority of our food crops are insect pollinated (Daily, 1998; Klein et al., 2007; IPCC, 2021). One of the places where global warming is particularly impactful today is the Arctic, where short growing seasons and long, harsh winters can put extreme selection pressure on species adapted to these environments (Gilg et al., 2012; IPCC, 2021).

One such group are the bumblebees (genus *Bombus),* which have, unlike most insects that are generally ectothermic, the ability to generate and maintain a much higher body temperature than their surroundings (Bernd, 1979). Recently, several alarming studies have pointed to a rapid decrease in pollinator biomass, and that many historical plant-pollinator interactions may have been lost. Here a warming climate in addition to loss of spatial, and temporal co-occurrence between pollinator and plant as a result of habitat loss are among the most cited reasons (Hegland et al., 2009; Burkle, Marlin and Knight, 2013; Hallmann et al., 2017). While warming is likely to affect plant-pollinator interactions globally, the rapid warming of the Arctic is hypothesized to cause changes that may be too fast for many species to adapt to (Overland et al., 2014; Rasmont et al., 2015; Ballinger et al., 2020; IPCC, 2021).

In the Arctic and at higher elevations, where the growing season is particularly short, autumn and winter warming has led to a longer growing season (Miller-Rushing and Inouye, 2009). This change in timing and length of the growing season can shift flowering and pollinator phenology that can, in turn, lead to a mismatch, resulting in a reduction in pollinator visitation and consequently, reduced seed production in plants (Hegland et al., 2009; Miller-Rushing and Inouye, 2009; Gallagher and Campbell, 2020). Additionally, Arctic bumblebee species with long proboscis visit a wider range of flower sizes than their lower latitude counterparts, where increased competition for resources drive specialization (Miller-Struttmann and Galen, 2014). Therefore, could rapidly warming temperatures influence interspecies competition throughout the season as southern species track their thermal niches northwards, and, in turn, influence competition and specialization? If so, there is a potential for mismatches to occur between plant and pollinator species where trait matching has coevolved.

Seasonal temperature difference affects the size of emerging bumblebees, and the castes vary in size and the timing of activity during the growing season (Scaven and Rafferty, 2013). Queens are the first to emerge in late spring or early summer and are on average larger than the other castes (Goulson, 2010). The drones have an intermediate size and emerge later together with the new generation of queens (Goulson, 2010). Workers have the most intraspecific morphological variation and are on average the smallest of the castes (Bernd, 1979). Worker size also varies over the season, stemming from development speed being affected by temperature and food availability, with workers early in the season being smaller than later in the season due to more extreme weather and limited food availability (Bernd, 1979; Scaven and Rafferty, 2013). Additionally, workers are generally the most numerous caste within a colony, increasing in numbers over the early summer, until the production of drones and queens start (Bernd, 1979). The bumblebee annual cycle therefore provides a natural case study to understand how global temperature increases, and competition driven niche partitioning, affect pollinator traits and their interactions with flowering plants. This is especially true at high latitudes where the rate of temperature change is three times the global average (IPCC, 2021).

Indeed, traits of both plants and pollinators can vary depending on their environment, either through trait plasticity or local adaptation. For example, functional traits of the plant *Pedicularis siphonantha* were found to grow larger in response to being transplanted to a warmer location at lower elevation (Dai et al. 2017). Additionally, Egawa, Hirose and Itino (2020) found that the corolla of *Prunella vulgaris* were shorter at higher elevations, representing a colder climate. However, Egawa, Hirose and Itino (2020) found longer corolla lengths where local bumblebee populations had long proboscises, similar to those at lower

elevations. Elevated temperatures have been shown to reduce the size of adult bumblebees (Guiraud et al., 2021). While size-matching between bumblebee body size and the distance between plant reproductive parts have been shown to be an important factor for pollen transfer, this relationship creates opportunities for a mismatch in either direction, that can result in reduced seed production when either plant or pollinator morphology changes independently to other abiotic or biotic conditions (Solís-Montero and Vallejo-Marín, 2017). Therefore, a reduction in body size as the climate warms could influence the rate of successful pollen transfer of plants, if the plants do not adapt simultaneously, causing reduced fecundity of Arctic specialist species, and may lead to eventual species loss (Hegland et al., 2009; Burkle, Marlin and Knight, 2013; Scaven and Rafferty, 2013; Solís-Montero and Vallejo-Marín, 2017).

To study the impacts of warming on organisms and their interactions, environmental gradients offer a powerful study method (Sundqvist, Sanders and Wardle, 2013; Miller-Struttmann and Galen, 2014; Egawa, Hirose and Itino, 2020). An elevational gradient can serve as a space-for-time substitution used to predict changes in climate over large latitudinal distances, due to the predictable way temperature changes with both elevation and latitude (Sundqvist, Sanders and Wardle, 2013). In the context of global warming, gradients can be a powerful way to predict changes over time (Likens, 1989). Therefore, examining plant-pollinator interactions over an elevational gradient has the potential to reveal the possible impacts of global warming on both plant and pollinator communities, such as, phenological and trait mismatches, and both northward and upslope spread of species better adapted to warmer environments (Scaven and Rafferty, 2013; Sundqvist, Sanders and Wardle, 2013; Miller-Struttmann and Galen, 2014; Martinet et al., 2015; Rasmont et al., 2015).

1.2 Hypothesis and predictions

This study explores the possible relationship between the matching of bumblebee and flowering plant traits along an elevational gradient in the Swedish mountains. Here a diversity of bumblebee and flowering plant species form a range of communities along the elevational gradient. I ask if there is a relationship between plant and pollinator traits that track elevation (which is a proxy for temperature change). I hypothesize that if there is a relationship then it should also track seasonal changes along the gradient.

The first hypothesis (H_1) is that plant floral traits predict visiting bumblebee proboscis length along the elevational gradient, with the predictions that ($P_{1.1}$) plants with restrictive flower morphology (i.e., flower shapes which require a certain proboscis length, in order to access the nectar and pollen rewards), are positively correlated with bumblebee body size (a proxy for proboscis length), and ($P_{1.2}$) plants traits (i.e., petal length) for those with unrestrictive floral morphology (i.e., flower shapes which do not require a certain proboscis length, in order to access the nectar and pollen rewards), does not correlate with bumblebee body size (a proxy for proboscis length; Figure 1a, 1b). The second hypothesis (H_2) is that trait matching between flower morphology and proboscis length is influenced by time of year (growing season) along the elevational gradient. The predictions for H_2 are that ($P_{2.1}$) the correlation between flower restrictiveness and bumblebee body size (proxy for proboscis length) gets stronger over the growing season until flowers disappear, and ($P_{2.2}$) the correlation between flower petal length and body size (a proxy for proboscis length) gets weaker with increasing elevation (Figure 1c, 1d).
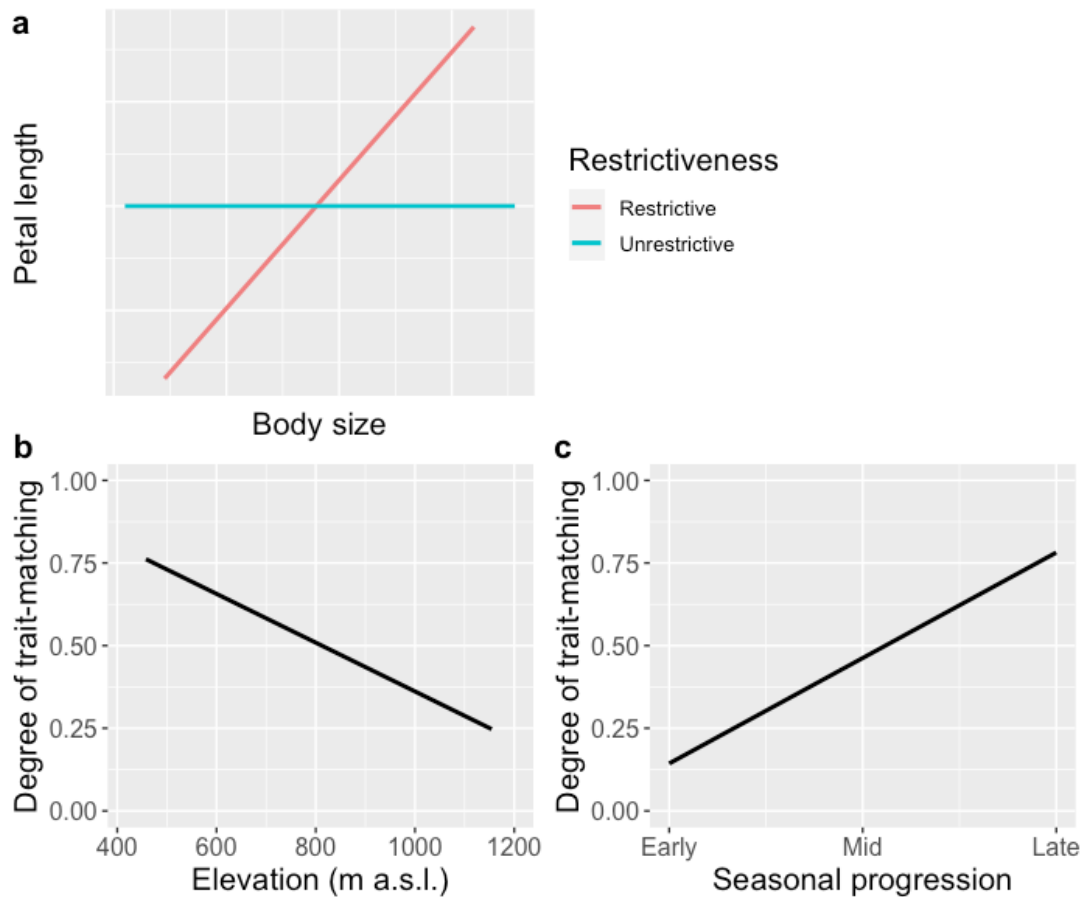
3

Figure 1. Conceptual model of hypotheses 1 and 2. Showing the relationship between petal length and body size for **a** restrictive flowers and unrestrictive flowers. Additionally, showing how trait-matching will change **b** with elevation and **c** over the season.

2 Methods

2.1 Field site

The research was conducted in Abisko National Park, Sweden, along an elevational transect (420-1164 m a.s.l.; est. 1916; Fries, 1925), located on the eastern slope of Mt. Nuolja (Fig. 2). The transect transitions from a gradient of mountain birch forest at the lowest elevations to a shrub transition zone and reaches the alpine zone at its highest elevations, representing large scale latitudinal changes in vegetation caused by the progressively colder climate closer to the poles.
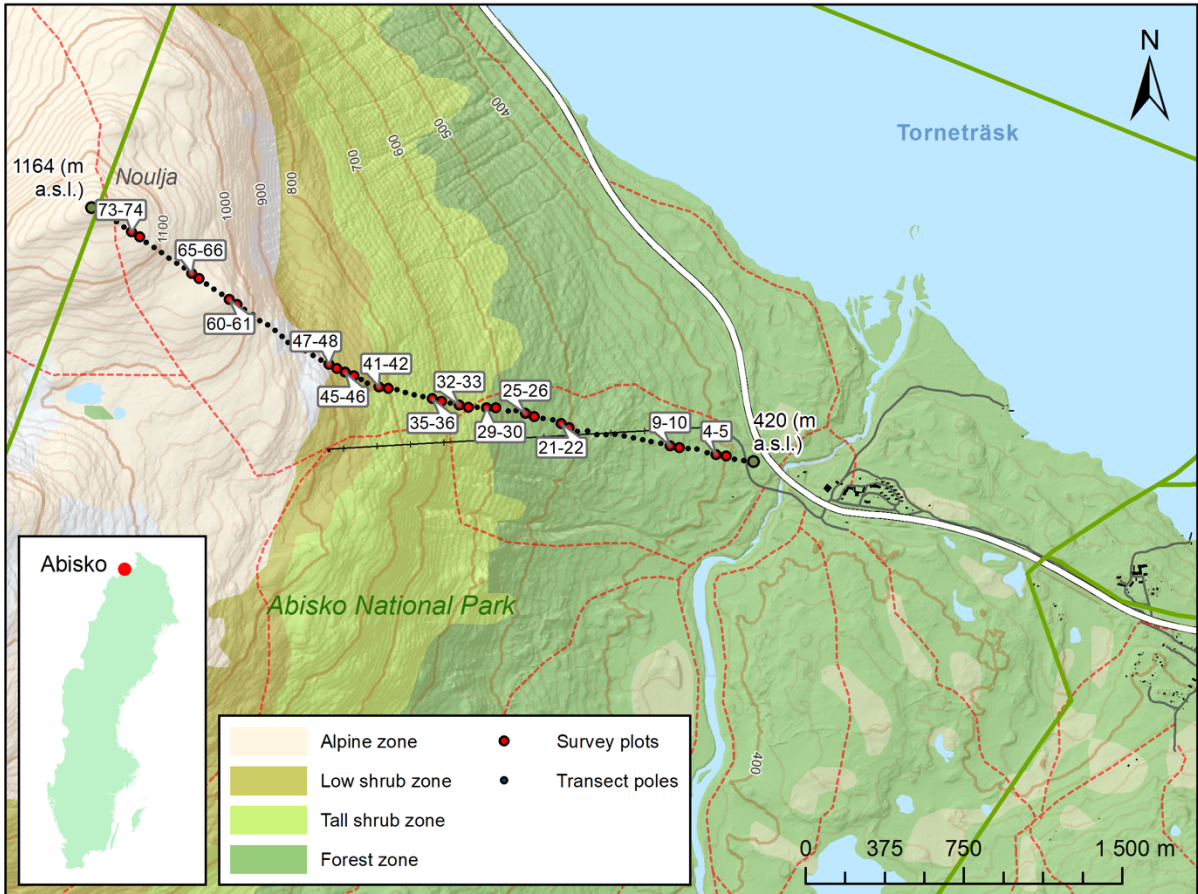
Figure 2. Map of vegetation zones along the transect, leading up Mt. Nuolja in Abisko National Park. The boundary of the national park is represented in green. The permanent survey plots are named after the poles they are located between. The map was created using elements from the Property map, and Terrain model grid 1+ © The Swedish Mapping, Cadastral and Land Registration Authority.
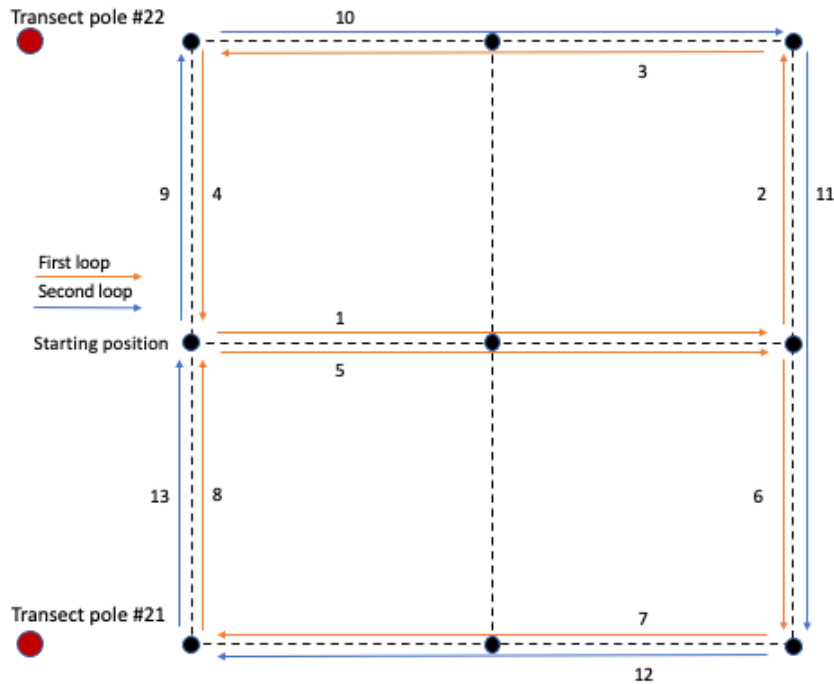
Figure 3. A sketch of the survey plots, with numbered arrows indicating walking direction.

Thirteen permanent survey plots along the transect were surveyed between 25 May and 3 September 2021. The plots are 45 x 45 m and divided into four equal quadrants (Fig. 3). The mountain birch (*Betula pubescens*) forest has a field layer dominated by heath (*Empetrum nigrum, Vaccinium myrtillus*, *V. uliginosum,* and *V. vitis-idaea)* and at the higher elevations the field layer is dominated by forbs (e.g., *Geranium sylvaticum, Myosotis decumbens and Trollius europaeus*) and grasses. In the lower (tall) shrub zone, *Salix myrsinifolia* and *S. phylicifolia,* are the dominating species, whereas in the upper reaches, the low shrubs, *S. glauca* and *S. lanata,* are dominant (Figure 4). The alpine zone can be divided into meadow and heath habitats, with a diversity of other habitats (e.g., snow beds, wind-blown rocky fields, and cliffs). Following the natural gradient of the vegetation, the transect is divided into five zones, with the survey plots split between them (lower birch forest; $n = 3$, upper birch forest; $n = 2$, tall willow shrub; $n = 3$, low willow shrub; $n = 2$, and alpine; $n = 3$; Fig. 2).

Figure 4. Pictures of the typical vegetation of each zone. **(a)** Plot 09-10 (460 m a.s.l.), taken 7 June 2021, shows mountain birches and a *Vaccinium*-dominated ground layer. **(b)** Plot 35-36 (749 m a.s.l.), taken 21 August 2021, shows a dense layer of tall *Salix* shrubs, with forbs scattered at the ground layer. **(c)** Plot 45-46 (901 m a.s.l.), taken 19 July 2021, shows patches of short Salix shrubs, and a mixture of forbes and heath. **(d)** Plot 65-66 (1087 m a.s.l.), taken 1 August 2021, shows the patchy vegetation cover of grasses and heath, typical for the higher elevation plots.

2.2 Field survey

Surveys began late May, as soon as the snow started to melt and continued through the end of the growing season (defined to be when less than 5% of the maximum number of bees, spotted in a day, had been spotted over the course of the sampling week; Stemkovski et al., 2020). When conducting the field survey, every other plot was surveyed each field day, to increase the chance of getting samples from each of the vegetation zones in case adverse weather prevented sampling. All the plots were surveyed twice a week, spread over four days. The starting plot of each day were randomized, to prevent the plots being surveyed at the same time each day. The surveys took place between 0800 and 2300 to coincide with the active foraging hours of the bumblebees in this region (Stelzer and Chittka, 2010).

Each plot was observed for 20 minutes by two people, walking a standardized route around the plot – first, a figure of eight around the outside and through the middle of the plot, followed by a second loop around the outside (Fig. 3). All bumblebees observed flying or foraging inside the plot during the survey were recorded, and the species and caste were recorded with certainty of 1-3. If foraging, the visited plant species, and height was noted, and, if known, the visited flower was taken as a sample for later measurement. If the exact flower on a plant was uncertain, up to three of the most likely candidates were taken, and later two were randomly discarded. An attempt was made to catch all observed bumblebees

with a 45 cm diameter butterfly net. If caught, the time was paused and the bumblebee was transferred into a plastic holding pot and put into a dark, insulated bag. The time was resumed when the survey was resumed. After the 20-minute survey was completed, the captured individuals were identified, the caste determined, and photographed in a marking cage with a standardized ruler for later size measurements (Söderström, 2017). Observations where bumblebee species or caste were unknown, were excluded. Additionally, *Bombus alpinus/polaris, B. hortorum/jonellus,* and *B. norvegicus/sylvestris,* were grouped as species aggregates (Williams et al., 2015). A total of 1443 bumblebees were caught out of 2723 observed during the 2021 field season, with 416 flying (obs. $n = 1223$) and 1027 foraging (obs. $n = 1500$), out of these, 318 could not be assigned to a species (Table 1).

Table 1. Total number of bumblebees caught, by species and caste.

Bombus Species	Queen	Drone	Worker	*Total*
B. alpinus/polaris	27	6	5	*38*
B. balteatus	36	31	77	*144*
B. bohemicus	10	1	-	*11*
B. cingulatus	3	-	3	*6*
B. flavidus	2	1	-	*3*
B. hortorum/jonellus	22	61	122	*205*
B. hyperboreus	11	8	-	*19*
B. hypnorum	-	1	-	*1*
B. lapponicus	52	74	90	*216*
B. lucorum	31	18	91	*140*
B. monticola	29	88	77	*194*
B. norvegicus/sylvestris	7	11	-	*18*
B. pascuorum	14	10	66	*90*
B. pratorum	5	43	89	*137*
B. soroeensis	-	-	1	*1*
B. wurflenii	-	2	-	*2*
Total	**249**	**355**	**621**	*1125*

2.3 Bumblebee traits

As measuring proboscis length is a destructive sampling method, it could not be measured during the field surveys, intertegular distance (ITD; distance between the two wing bases) was used as a proxy for proboscis length as ITD has been shown to predict proboscis length of bees (Cariveau et al., 2016; unpublished data from this study). To establish a relationship between ITD and proboscis for the local population, individuals of all castes from 11 species were collected outside of Abisko National Park (Table 2). Bumblebee body size was measured using ITD, a standardized method, by placing two landmarks onto each image of bees in the marking cage with a ruler for scale (Cane, 1987; Fig. A1). The landmarking was done with 'TPSDig' (version 2.31) and using the 'geomorph' R-package (version 4.0.2) to measure the distance between the landmarks (Rohlf, 2006; Adams et al., 2021; Baken et al., 2021).

Table 2. The number of individuals of each species and caste that were used for allometry measurements.

Bombus species	Queen	Worker	Drone	*Total*
B. alpinus/polaris	10	6	1	17
B. balteatus	13	7	13	33
B. hortorum	6	12	-	18
B. hortorum/jonellus	8	8	-	16
B. hyperboreus	7	-	-	7
B. jonellus	4	1	5	10
B. lapponicus	13	9	10	32
B. lucorum	4	12	-	16
B. monticola	10	9	9	28
B. pascuorum	10	11	-	21
B. pratorum	4	11	3	18
Total	**89**	**86**	**41**	**216**

The bumblebees were also photographed in a marking cage for size measurement (using the same method for the other parts of the study), after which the proboscis was removed and photographed under a stereomicroscope fitted with a camera. The prementum of the proboscis was then measured using 'TPSdig' (version 2.31), and a scaling equation for each species and caste were used to determine the relationship between body size and proboscis length (Rohlf, 2006; Cariveau et al., 2016). Prementum was used as a measurement of proboscis length since it has been found to be a good predictor of total proboscis length (Cariveau et al., 2016). The allometric scaling was done using a mixed effects model with species and caste as random effects, with the R package 'lme4' (version 1.1-28; Bates et al., 2015; Fig. 4). Additionally, mixed effects models are less restrictive in sample size of random effects, and, thus, *Bombus* species did not have to be excluded on the basis of insufficient sample size (Bates et al., 2015). Given the strong allometric relationship between proboscis length and ITD, ITD is used as a proxy for all further analysis of the relationship between floral and bumblebee traits.
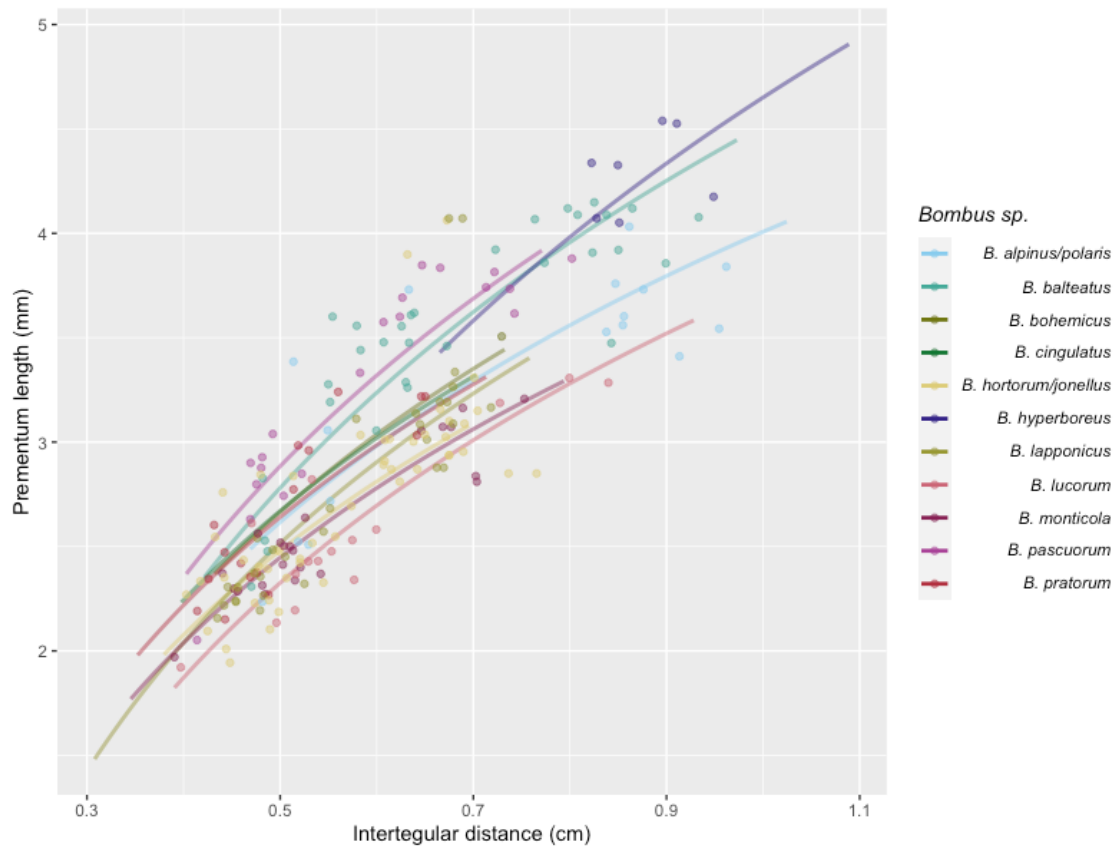
Figure 5. Relationship between intertegular distance (ITD) and prementum length in *Bombus* species. Coloured dots represent observed measurements, and the coloured lines represent the predicted relationship between prementum length and ITD.

2.4 Plant traits

Photos of petals, stamen, and pistil were taken of the dissected flowers, and measured in ImageJ (version 1.53k; Schneider, Rasband and Eliceiri, 2012) with the line tool or segmented line tool for curved objects. Petal length was used instead of measuring corolla directly because it was much more repeatable, due to the number of samples collected (N = 534) and that the corolla rapidly degrades making it impossible given remote fieldwork. Petals were measured from the tip of the petal to the nectaries. For plant species that had flowers which opens up part-way up the petals (e.g., *Silene dioica),* length was measured from the flower opening to the nectaries. For plant species with non-uniform petal types (e.g., *Astragalus alpinus*), the least restrictive petal type was used (Fig. A2). The sepals of *Trollius europaeus* were measured in place of the petals, as they function the same way as most other species' petals (Zhao and Wang, 2015). Restrictiveness was determined for each of the collected plant species, using the classification of flower shape, made by Wei et al. (2021), as a proxy for restrictiveness (Table 3).

Table 3. The sampled plant species used for modelling. Flower shapes of collected species based on the classifications used by Wei et al., (2021), sorted by restrictiveness (N = 434).

Plant species	Flower shape	Restrictiveness	*n*
Epilobium angustifolium	open	unrestrictive	3
Geranium sylvaticum	open	unrestrictive	86
Potentilla crantzii	open	unrestrictive	1
Pyrola rotundifolia	open	unrestrictive	1
Rhodedendron lapponicum	funnelform	unrestrictive	3
Viola biflora	labiate	unrestrictive	6
Andromeda polifolia	bell-like	restrictive	4
Astragalus alpinus	pea-like	restrictive	46
Cassiope tetragona	bell-like	restrictive	1
Diapensia lapponica	salverform	restrictive	1
Hieracium sp.	aster-like	restrictive	1
Melampyrum pratense	labiate	restrictive	2
Pedicularis lapponica	labiate	restrictive	17
Rhinanthus minor	labiate	restrictive	6
Saussurea alpina	aster-like	restrictive	6
Silene acaulis	salverform	restrictive	1
S. dioica	salverform	restrictive	4
Solidago virgaurea	aster-like	restrictive	107
Vaccinium myrtillus	bell-like	restrictive	26
V. uliginosum	bell-like	restrictive	15
V. vitis—idaea	bell-like	restrictive	32

Since there were no plants with flowers that resembled the bells of the heath species (Ericaceae, e.g., *Andromeda polifolia, V. vitis-idaea*) in Wei et al. (2021), the "bell-like" shape was added and classified as restrictive because their narrow opening. The unique shape of *T. europaeus* made it difficult to fit into either the restrictive or unrestrictive group due to them being able to both be open and closed (Ibanez, Dujardin and Després, 2009). As a result of this uncertainty, *T. europaeus* (*n* = 5) were excluded from the analysis. *Salix* spp. *(n* = 48) were also excluded from the analysis due to their unique flower morphology (which makes them difficult to classify) and the fact that they were visited almost exclusively by queens in the early season when no other flowers are in bloom. Further, *T. europaeus* have different petal structures making comparisons with other plant species visited in this study impossible. In total, 434 flowers were measured, with 326 being restrictive, and 108 being unrestrictive.

2.5 Statistical analyses

2.5.1 H$_1$: Plant floral traits predict visiting bumblebee proboscis length

To examine the relationship between petal length and ITD, first, the intra- and interspecies bumblebee size difference needed to be known. To do this a linear model was fitted, using *Bombus* species and caste as independent variables, and ITD as the dependent variable. All captured bumblebees were used in this analysis, regardless of whether the bumblebees were foraging or flying. The analysis excluded species with less than five observations. The

relationship was evaluated using 1225 bumblebees (249 queens, 355 drones, 621 workers). A linear model was also used to test for differences in petal length of restrictive versus unrestrictive flowers.

Next, the ITD of bumblebees by restrictiveness of the visited flower was assessed, using a mixed effects model fitted with the interaction between species and caste as random effects. Using *Bombus* species and caste as random effects allows the mixed effect model to account for the differences in ITD between species and caste.

Finally, the log-ratio of petal length to ITD regarding restrictiveness was examined using a mixed effects model. The model was fitted with *Bombus* species and caste as random intercept, and restrictiveness as random slope. Including restrictiveness as random slope in the model allows for evaluating the difference in response, between species and caste, to restrictive and unrestrictive petals. For this model 369 observations and eleven species were used, while 144 samples were excluded due to unknown species, caste or missing ITD measurements.

2.5.2 H₂: Trait matching is influenced by time of year and elevation

When analysing the spatiotemporal effects on the relationship between petal length and ITD, time was expressed as 'days since onset of sampling (25 May)'. The scaling was done to make model intercepts meaningfully visualize petal length at the beginning of the season. Time and elevation were fitted using mixed effects model that, like for the first hypothesis, used the log-ratio of petal length to ITD as response variable. Additionally, the relationship between restrictiveness and both time, and elevation were assessed in the model. Divergence of the mean ratio of petal length to ITD away from zero would be interpreted as a decrease, and the inverse would be interpreted as an increase in trait-matching. To acquire the desired intercepts for elevation at the lowest plot instead of at sea level mentioned in the methods section, a *post hoc* scaling of elevation using the model results was done.

For the mixed effects models, two coefficients of determination (R^2) were generated using the 'MuMIn' R-package (version 1.43.17; Bartón, 2020). These R^2-values were Marginal R^2, representing the R^2 of the fixed effects, and conditional R^2, representing the R^2 of the full model, including both fixed and random effects (Nakagawa, Johnson and Schielzeth, 2017). Models were assessed based on Akaike Information Criterion (AIC). Due to collinearity with ITD, the interaction between *Bombus* species and caste were not assessed in any linear models. The assumptions of normality and random distribution of the residuals were met for all models.

3 Results

3.1 H₁: Plant floral traits predict visiting bumblebee proboscis length

There was a significant difference in ITD, interspecies, intraspecies, and between castes, with caste explaining the greatest amount of variation (Caste; $F = 1645$; $p < .001$; Species; $F = 223$; $p < .001$). The model used to examine the ITD of bumblebees by flower restrictiveness showed that bumblebees visiting unrestrictive flowers were significantly smaller than those visiting restrictive flowers (lme: restrictive flowers; mean ($\pm$ s.e.) ITD = 0.59 $\pm$ 0.023 cm; $n = 566$; $t(31.75) = 25.16$; $p < .001$; unrestrictive flowers; 0.57 $\pm$ 0.004 cm; $n = 207$; $t(739.18) = -4.25$; $p < .001$). Additionally, the variation of ITD was larger for restrictive petals than

unrestrictive petals. There was a significant difference in petal length between restrictive flowers (mean (± s.e.) = 0.74 ± 0.015 cm; n = 326) and unrestrictive flowers (1.55 ± 0.04 cm; n =108; Difference = 0.82 cm, $t(161.95)$ = -25.20, $p < .001$).

Finally, when examining the effect of restrictiveness on the ratio of petal length to ITD, the mean (± s.e.) ratio for restrictive flowers were 0.22 ± 0.06 log(cm^{-2}), and 0.95 ± 0.07 log(cm^{-2}) for unrestrictive flowers (Table 4).

Table 4. Summary of a linear mixed-effects model investigating the log-ratio between petal length of visited plants, and bumblebee (*Bombus spp.*) intertegular distance (ITD). The random term 'Rest' is short for restrictiveness. Values in bold were considered statistically significant (p ≤0.05). AIC = 171.43, marginal R^2 = 0.38, conditional R^2 = 0.71. N = 369.

Coefficient	estimate (± s.e.)	*n*	*t*-value	*p*-value	variance of random term
Intercept	0.22 ± 0.06	269	3.71	**0.001**	
Unrestrictive	0.73 ± 0.071	100	10.26	**< 0.001**	
1|Species:Caste					0.078
Rest|Species:Caste					0.047
Residual					0.072

3.2 H$_2$: Trait matching is influenced by time of year and elevation

No change over elevation or across time was found for ITD; there was, however, a significant change in petal length over both elevation and time (elevation; Δ (± s.e.) = -0.045 ± 0.014 cm 100 m^{-1}; t = -3.17; p = .002; time; -0.34 ± 0.13 cm 100 m^{-1}; t = -2.52; p = .012). Model results revealed significant effects on the ratio of petal length to ITD, for both elevation and restrictiveness (elevation; F = 21.61; $p < .001$; restrictiveness F = 50.90; $p < 0.001$), as well as the interaction between the two (F = 43.757; $p < .001$). The ratio of petal length to ITD changed by 0.039 ± 0.079 log(cm^{-2}) 100 m^{-1} (p = .001) for restrictive flowers, and by -0.22 ± 0.26 log(cm^{-2}) 100 m^{-1} ($p < .001$) for unrestrictive flowers, important to note is that the standard error remains untransformed (Fig. 6). No relationship was found between the ratio of petal length to ITD and date.
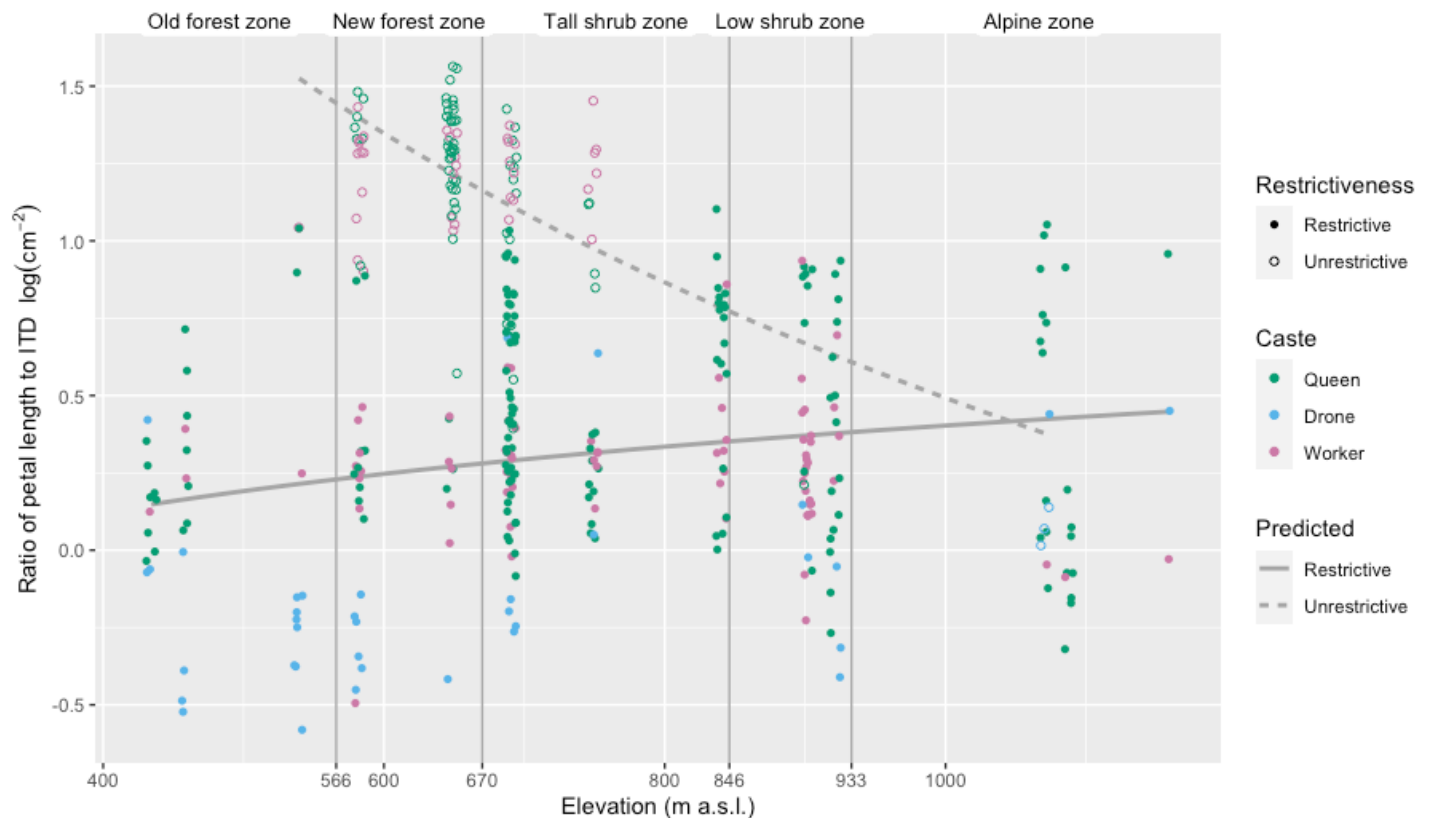
Figure 6. The change in ratio of petal length: ITD with elevation for restrictive and unrestrictive flowers. Plotted points are the recorded observation, and the predicted lines for restrictive and unrestrictive flowers were generated by the mixed effects model. Marginal $R^2 = 0.57$ and conditional $R^2 = 0.74$. Vertical lines signify the approximate extend of the lower birch forest (420 m - 556 m a.s.l.), upper birch forest (556 m - 670 m a.s.l.), tall shrub zone (670 m - 846 m a.s.l.), low shrub zone (846 m - 933 m a.s.l.), and the alpine zone (933 m - 1164 m a.s.l.).

4 Discussion

The results support the hypothesis that petal length predicts visiting bumblebee proboscis length, as ITD (as a proxy for proboscis length) could be predicted by the petal length and restrictiveness of the flower. Interestingly, while it was predicted that only restrictive flower traits would be able to predict proboscis length, the results suggest that unrestrictive flower traits too could predict proboscis length, a result not previously explicitly discussed (Inouye, 1980; Stang, Klinkhamer and van der Meijden, 2006). These results build on the existing body of knowledge suggesting the importance of size matching between pollinator proboscis length and flower petal length (Ranta and Lundberg, 1980; Klumpers, Stang and Klinkhamer, 2019). Another point of interest is that the maximum ITD was larger for bumblebees visiting restrictive flowers than for those visiting unrestrictive flowers, suggesting that larger individuals tended to avoid unrestrictive flowers in favour of restrictive ones, as hypothesised by Klumpers, Stang and Klinkhamer (2019; Fig. A3). While there was a difference in maximum ITD, no such difference was found for the minimum ITD, contrary to the findings of Stang, Klinkhamer and van der Meijden (2007).

The ratio of petal length to ITD along the transect increased for restrictive flowers and decreased for unrestrictive flowers. These results support the hypothesis that trait matching between flower morphology and proboscis length is affected by elevation, but not by time. The correlation between petal length and ITD (as a proxy for proboscis length) decreased for

14

restrictive petals as predicted. Surprisingly however, the correlation increased for unrestrictive flowers. As such, an interesting question arises from these results, as the different effects of ratio with elevation between restrictive and unrestrictive petals, suggest a normalization between the two, which could be evidence of more generalist foraging patterns as it pertains to petal length of flowers (Miller-Struttmann and Galen, 2014). Furthermore, the ratio of petal length to ITD changed the most for unrestrictive petals, paired with the results that suggests that larger individuals tended to prefer restrictive flowers, also agrees with the findings of Miller-Struttmann and Galen (2014) that generalism tends to increase with elevation. That no change in ratio over time was found could be because the hypothesised specialization is more related to primary productivity, e.g., pollen and nectar availability, than seasonal progression. If productivity is a better explanation, then both elevation, as described by Miller-Struttmann and Galen (2014), and time could instead be described as two productivity gradients, decreasing with elevation, and increasing then decreasing with the progression of the growing season.

These findings show that trait-matching between bumblebees and flowers is an influential factor for bumblebees when deciding what plants to visit. Future studies should look into how other traits could explain the apparent size matching between unrestrictive flowers and proboscis, such as distance between anther and style (Solís-Montero and Vallejo-Marín, 2017). Lastly, a study examining productivity as a function of elevation and seasonal progression might better capture the change in specialization. Nevertheless, this study demonstrates the importance of considering individual level traits when looking at plant preference, it also creates a framework for future studies, assessing plant-pollinator networks.

As the climate warms, the number of pollinator specialists is likely to increase due to increased interspecies competition (Miller-Struttmann and Galen, 2014). How this increased competition will affect the more generalist Arctic species is uncertain, and a topic for future research.

Acknowledgments

References

Adams, D.C., Collyer, A., Kaliontzopoulou, A. and Baken, E.K. 2021. *Geomorph: Software for geometric morphometric analyses. R package version 4.0.2.* Available at: https://cran.r-project.org/package=geomorph.

Baken, E.K., Collyer, M.L., Kaliontzopoulou, A. and Adams, D.C. 2021. Geomorph v4.0 and gmShiny: enhanced analytics and a new   graphical interface for a comprehensive morphometric experience. *Methods in Ecology and Evolution*. 12: 2355–2363.

Ballinger, T., Overland, J., Wang, M., Bhatt, U., Hanna, E., Hanssen-Bauer, I., Kim, S.-J., Thoman, R. and Walsh, J. 2020. Arctic Report Card 2020: Surface Air Temperature.

Bartón, K. 2020. *MuMIn: Multi-Model Inference.* Available at: https://CRAN.R-project.org/package=MuMIn.

Bates, D., Maechler, M., Bolker, B. and Walker, S. 2015. Fitting Linear Mixed-Effects Models Using lme4. *Journal of Statistical Software*. 67(1): 1–48. doi: 10.18637/jss.v067.i01.

Bernd, H. 1979. *Bumblebee Economics*. Cambridge, United Kingdom: Harvard Bussiness Review.

Burkle, L.A., Marlin, J.C. and Knight, T.M. 2013. Plant-Pollinator Interactions over 120 Years: Loss of Species, Co-Occurrence, and Function. *Science*. 339(6127): 1611–1615. doi: 10.1126/science.1232728.

Cane, J.H. 1987. Estimation of Bee Size Using Intertegular Span (Apoidea). *Journal of the Kansas Entomological Society*. 60(1): 145–147. doi: 10.1098/rstb.2011.0050.

Cariveau, D.P., Nayak, G.K., Bartomeus, I., Zientek, J., Ascher, J.S., Gibbs, J. and Winfree, R. 2016. The allometry of bee proboscis length and its uses in ecology. *PLoS One*. 13(11): e0151482. doi: 10.1371/journal.pone.0151482.

Dai, W.-K., Amboka, G.M., Kadiori, E.L., Wang, Q.-F. and Yang, C.-F. 2017. Phenotypic plasticity of floral traits and pollination adaption in an alpine plant Pedicularis siphonantha D. Don when transplanted from higher to lower elevation in Eastern Himalaya. *Journal of Mountain Science*. 14(10). doi: 10.1007/s11629-017-4481-1.

Daily, G.C. (ed.). 1998. *Nature's services. Societal dependence on natural ecosystems*. Washington, DC: Island Press.

Damos, P. and Savopoulou-Soultani, M. 2011. Temperature-Driven Models for Insect Development and Vital Thermal Requirements. *Psyche*. 2012: 123405. doi: 10.1155/2012/123405.

Egawa, S., Hirose, K. and Itino, T. 2020. Geographic changes in pollinator species composition affect the corolla tube length of self-heal (Prunella vulgaris L.): Evidence from three elevational gradients. *Ecological Research*. 35(5): 819–825. doi: 10.1111/1440-1703.12146.

Gallagher, M.K. and Campbell, D.R. 2020. Pollinator visitation rate and effectiveness vary with flowering phenology. *American Journal of Botany*. 107(3): 445–455.

Gilg, O., Kovacs, K.M., Aars, J., Fort, J., Gauthier, G., Grémillet, D., Ims, R.A., Meltofte, H., Moreau, J., Post, E., Schmidt, N.M., Yannic, G. and Bollache, L. 2012. Climate change and the ecology and evolution of Arctic vertebrates. *Annals of the New York Academy of Sciences*. 1249(1): 166–190. doi: 10.1111/j.1749-6632.2011.06412.x.

Gómez, J.M., Perfectti, F., Armas, C., Narbona, E., González-Megías, A., Navarro, L., DeSoto, L. and Torices, R. 2020. Within-individual phenotypic plasticity in flowers fosters pollination niche shift. *Nature Communications*. 11(1): 4019. doi: 10.1038/s41467-020-17875-1.

Goulnik, J., Plantureux, S., Théry, M., Baude, M., Delattre, M., van Reeth, C., Villerd, J. and Michelot-Antalik, A. 2020. Floral trait functional diversity is related to soil

characteristics and positively influences pollination function in semi-natural grasslands. *Agriculture, Ecosystems & Environment*. 301: 107033. doi: 10.1016/j.agee.2020.107033.

Goulson, D. 2010. *Bumblebees: Behaviour, Ecology and Conservation*. Oxford University Press.

Guiraud, M., Cariou, B., Henrion, M., Baird, E. and Gérard, M. 2021. Higher developmental temperature increases queen production and decreases worker body size in the bumblebee Bombus terrestris. *Journal of Hymenoptera Research*. 88: 39–49. doi: 10.3897/jhr.88.73532.

Hallmann, C.A., Sorg, M., Jongejans, E., Siepel, H., Hofland, N., Schwan, H., Stenmans, W., Müller, A., Sumser, H. and Hörren, T. 2017. More than 75 percent decline over 27 years in total flying insect biomass in protected areas. *PloS One*. 12(10): e0185809.

Hegland, S.J., Nielsen, A., Lázaro, A., Bjerknes, A.-L. and Totland, Ø. 2009. How does climate warming affect plant-pollinator interactions? *Ecology letters*. 12(2): 184–195. doi: 10.1111/j.1461-0248.2008.01269.x.

Hegland, S.J. and Totland, Ø. 2005. Relationships between species' floral traits and pollinator visitation in a temperate grassland. *Oecologia*. 145: 586–594. doi: 10.1007/s00442-005-0165-6.

Ibanez, S., Dujardin, G. and Després, L. 2009. Stability of floral specialization in Trollius europaeus in contrasting ecological environments. *Journal of Evolotionary Biology*. 22(6): 1183–1192. doi: 10.1111/j.1420-9101.2009.01731.x.

Inouye, D.W. 1980. The Effect of Proboscis and Corolla Tube Lengths on Patterns and Rates of Flower Visitation by Bumblebees. *Oecologia*. 45: 197–201. doi: 10.1007/BF00346460.

IPCC. 2021. *Climate Change 2021: The Physical Science Basis. Contribution of Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge, United Kingdom: Cambridge University Press.

Klein, A.-M., Vaissière, B.E., Cane, J.H., Steffan-Dewenter, I., Cunningham, S.A., Kremen, C. and Tscharntke, T. 2007. Importance of pollinators in changing landscapes for world crops. *Proc. R. Soc. B.* 274(1608): 303–313.

Klumpers, S.G.T., Stang, M. and Klinkhamer, P.G.L. 2019. Foraging efficiency and size matching in a plant-pollinator community: the importance of sugar content and tongue length. *Ecology letters*. 22: 469–479. doi: 10.1111/ele.13204.

Kühn, P., Ratier Backes, A., Römermann, C., Bruelheide, H. and Haider, S. 2021. Contrasting patterns of intraspecific trait variability in native and non-native plant species along an elevational gradient on Tenerife, Canary Islands. *Annals of Botany*. 127(4): 565–576. doi: 10.1093/aob/mcaa067.

Kuppler, J., Albert, C.H., Ames, G.M., Armbruster, W.S., Boenisch, G., Boucher, F.C., Campbell, D.R., Carneiro, L.T., Chacón-Madrigal, E., Enquist, B.J., Fonseca, C.R., Gómez, J.M., Guisan, A., Higuchi, P., Karger, D.N., Kattge, J., Kleyer, M., Kraft, N.J.B., Larue-Kontić, A.-A.C., Lázaro, A., Lechleitner, M., Loughnan, D., Minden, V., Niinemets, Ü., Overbeck, G.E., Parachnowitsch, A.L., Perfectti, F., Pillar, V.D., Schellenberger Costa, D., Sletvold, N., Stang, M., Alves-dos-Santos, I., Streit, H., Wright, J., Zych, M. and Junker, R.R. 2020. Global gradients in intraspecific variation in vegetative and floral traits are partially associated with climate and species richness. *Global Ecology and Biogeography*. 29(6): 992–1007. doi: 10.1111/geb.13077.

Kuppler, J., Höfers, M.K., Wiesmann, L. and Junker, R.R. 2016. Time-invariant differences between plant individuals in interactions with arthropods correlate with intraspecific variation in plant phenology, morphology and floral scent. *New Phytologist*. 210: 1357–1368. doi: 10.1111/nph.13858.

Likens, G.E. (ed.). 1989. *Long-Term Studies in Ecology: Approaches and Alternatives*. New York, NY: Springer-Verlag.

Martinet, B., Rasmont, P., Cederberg, B., Evrard, D., Ødegaard, F., Paukkunen, J. and Lecocq, T. 2015. Forward to the north: two Euro-Mediterranean bumblebee species now cross the Arctic Circle. *International Journal of Entomology*. 51(4): 303–309. doi: 10.1080/00379271.2015.1118357.

Miller-Rushing, A.J. and Inouye, D.W. 2009. Variation in the impact of climate change on flowering phenology and abundance: An examination of two pairs of closely related wildflower species. *American Journal of Botany*. 96(10): 1821–1829. doi: 10.3732/ajb.0800411.

Miller-Struttmann, N.E. and Galen, C. 2014. High-altitude multi-taskers: bumble bee food plant use broadens along an altitudinal productivity gradient. *Oecologia*. 176: 1033–1045. doi: 10.1007/s00442-014-3066-8.

Nakagawa, S., Johnson, P.C.D. and Schielzeth, H. 2017. The coefficient of determination R2 and intra-class correlation coefficient from generalized linear mixed-effects models revisited and expanded. *Journal of The Royal Society Interface*. 14(134): 20170213. doi: 10.1098/rsif.2017.0213.

Overland, J., Hanna, E., Hanssen-Bauer, I., Kim, S., Walsh, J., Wang, M., Bhatt, U. and Thoman, R. 2014. Air Temperature [In Arctic Report Card 2014].

Ranta, E. and Lundberg, H. 1980. Resource Partitioning in Bumblebees: The Significance of Differences in Proboscis Length. *Oikos*. 35(3): 298–302.

Rasmont, P., Franzén, M., Lecocq, T., Harpke, A., Roberts, S.P., Biesmeijer, J.C., Castro, L., Cederberg, B., Dvorak, L. and Fitzpatrick, Ú. 2015. *Climatic risk and distribution atlas of European bumblebees*. Pensoft Publishers.

Rohlf, F.J. 2006. tpsDig, version 2.10. *http://life.bio.sunysb.edu/morph/index/*.

Scaven, VictoriaL. and Rafferty, NicoleE. 2013. Physiological effects of climate warming on flowering plants and insect pollinators and potential consequences for their interactions. *Current zoology*. 59(3): 418–426. doi: 10.1093/czoolo/59.3.418.

Schneider, C.A., Rasband, W.S. and Eliceiri, K.W. 2012. NIH Image to ImageJ: 25 years of image analysis. *Nature Methods*. 9(7): 671–675. doi: 10.1038/nmeth.2089.

Smith, S.D., Ané, C. and Baum, D.A. 2008. The role of pollinator shifts in the floral diversification of iochroma (Solanaceae). *Evolution*. 62(4): 793–806.

Söderström, B. 2017. *Sveriges Humlor - En Fälthandbok*. Stockholm, Sweden: Entomologiska Föreningen i Stockholm.

Solís-Montero, L. and Vallejo-Marín, M. 2017. Does the morphological fit between flowers and pollinators affect pollen deposition? An experimental test in a buzz-pollinated species with anther dimorphism. *Ecology and Evolution*. 7(8): 2706–2715. doi: 10.1002/ece3.2897.

Stang, M., Klinkhamer, P.G.L. and van der Meijden, E. 2006. Size constraints and flower abundance determine the number of interactions in a plant flower visitor web. *OIKOS*. 112(1): 111–121.

Stang, M., Klinkhamer, P.G.L. and van der Meijden, E. 2007. Asymmetric specialization and extinction risk in plant–flower visitor webs: a matter of morphology or abundance? *Oecologia*. 151(3): 442–453. doi: 10.1007/s00442-006-0585-y.

Stelzer, R.J. and Chittka, L. 2010. Bumblebee foraging rhythms under the midnight sun measured with radiofrequency identification. *BMC Biol*. 8(93). doi: 10.1186/1741-7007-8-93.

Stemkovski, M., Pearse, W.D., Griffin, S.R., Pardee, G.L., Gibbs, J., Griswold, T., Neff, J.L., Oram, R., Rightmyer, M.G., Sheffield, C.S., Wright, K., Inouye, B.D., Inouye, D.W.,

Irwin, R.E. and Coulson, T. 2020. Bee phenology is predicted by climatic variation and functional traits. *Ecology letters*. 23(11): 1589–1598. doi: 10.1111/ele.13583.

Sundqvist, M.K., Sanders, N.J. and Wardle, D.A. 2013. Community and Ecosystem Responses to Elevational Gradients: Processes, Mechanisms, and Insights for Global Change. *Annual Review of Ecology, Evolution, and Systematics*. 44(1): 261–280. doi: 10.1146/annurev-ecolsys-110512-135750.

Szigeti, V., Vanja, F., Körösi, Á. and Kis, J. 2020. Are all butterflies equal? Population-wise proboscis length variation predicts flower choice in a butterfly. *Animal Behaviour*. 163: 135–143. doi: 10.1016/j.anbehav.2020.03.008.

Wei, N., Kaczorowski, R.L., Arceo-Gómez, G., O'Neill, E.M., Hayes, R.A. and Ashman, T.-L. 2021. Pollinators contribute to the maintenance of flowering plant diversity. *Nature*. doi: 10.1038/s41586-021-03890-9.

Williams, P.H., Byvaltsev, A.M., Cederberg, B., Berezin, M.V., Ødegaard, F., Rasmussen, C., Richardson, L.L., Huang, J., Sheffield, C.S. and Williams, S.T. 2015. Genes Suggest Ancestral Colour Polymorphisms Are Shared across Morphologically Cryptic Species in Arctic Bumblebees. *PLoS One*. 10(12): e0144544. doi: 10.1371/journal.pone.0144544.

Zhao, Z.-G. and Wang, Y.-K. 2015. Selection by pollinators on floral traits in generalized Trollius ranunculoides (Ranunculaceae) along altitudinal gradients. *PloS One*. 10(2): e0118299–e0118299. doi: 10.1371/journal.pone.0118299.
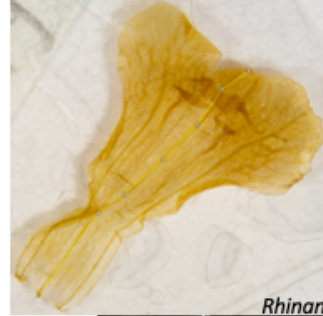
Appendix

A1. A *Bombus lapponicus* drone in a marking cage, with landmarks (red dots) placed at the wing bases for to measure intertegular distance.
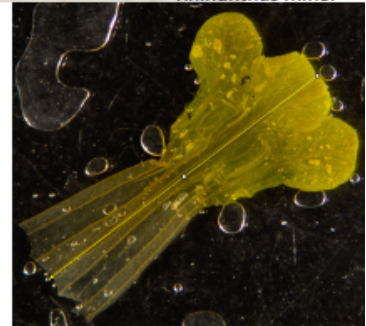
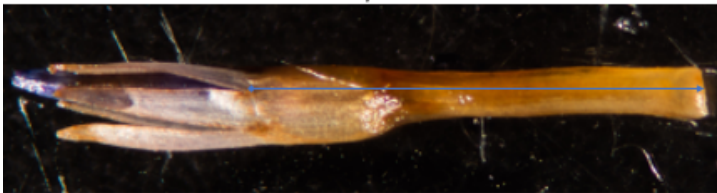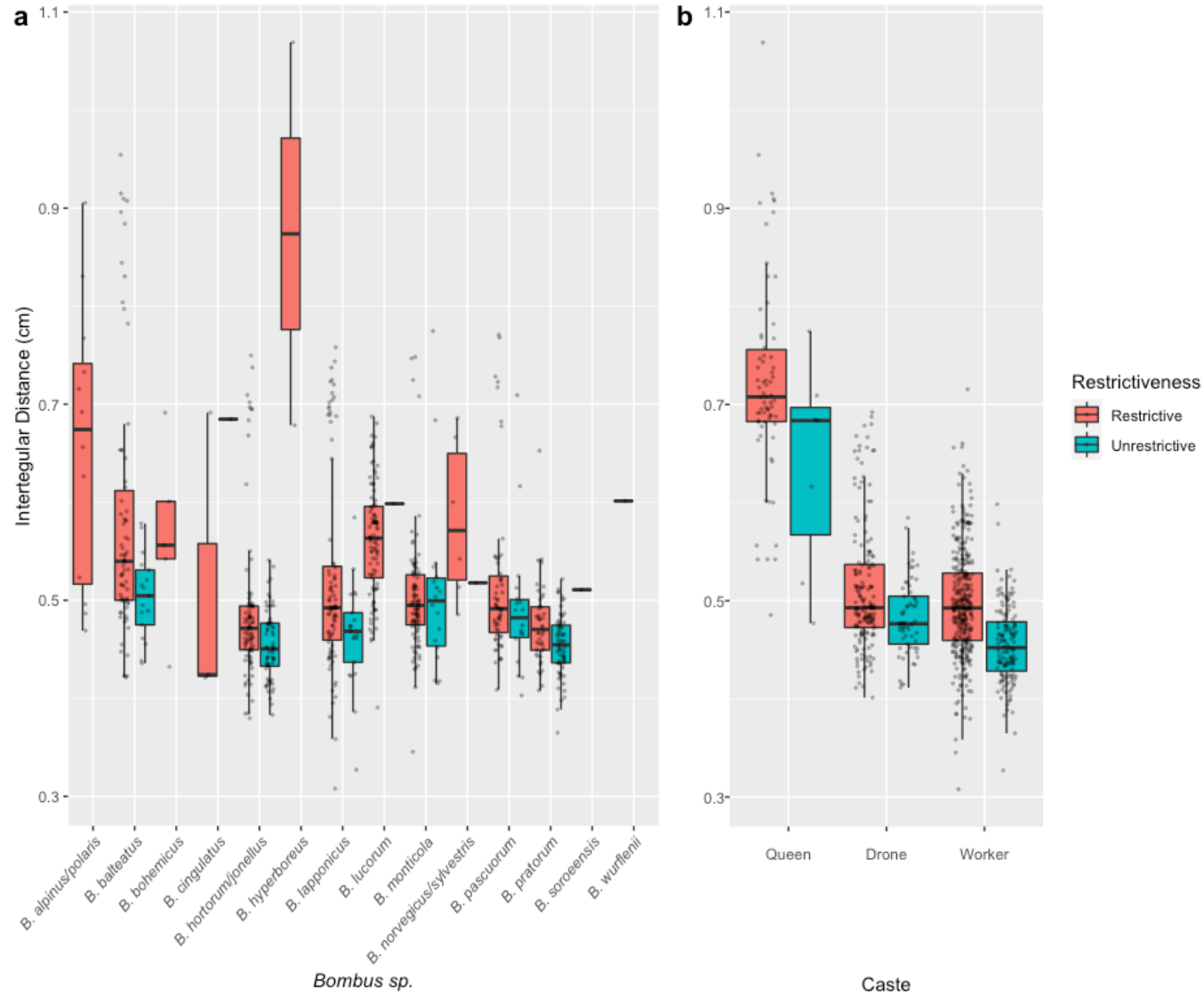A2. Petal measurements from flowers with unique shapes. For *Pedicularis lapponica*, *Melampyrum pratense, Rhinanthus minor* and *Viola biflora*, anterior petals were used. The inner floret was used in cases when an Asteraceae had differentiated inner and outer florets, e.g., *Solidago virgaurea*.

A3. Intertegular distance (ITD) of bumblebees with a red and blue column for bumblebees visiting restrictive and unrestrictive flower types, respectively. Figure **a** shows species, and **b** shows all individuals of each cast.