



UMEÅ UNIVERSITET

# **DO BUMBLEBEES PARTITION AN ELEVATIONAL GRADIENT BY BODY SIZE?**

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## Abstract

As the climate warms, Arctic bumblebee species face the loss of habitat and must deal with increased competition from southern species tracking their thermal and habitat niches north, for example *Bombus terrestris*. Previous studies demonstrate that bumblebees follow Bergmann's rule, i.e., larger body sizes at higher latitudes, despite bumblebees not being considered truly ectothermic, as they can generate heat through muscular activity (i.e., beating their wings). This study seeks to confirm and understand the relationship between body size and temperature using an elevational gradient as a proxy for climate. In this study, I examined 13 plots (420-1164 m.a.s.l.) set along the 3.4 km transect up the slope of Mt. Nuolja in Abisko National Park, Sweden. For body size, I chose to use the commonly accepted proxy distance between the base of the wings (i.e., intertegular distance). For temperature, I chose the mean temperature at time of visitation. Results show that climate is a significant explanatory variable for bumblebee body size, with an overall increasing body size with increasing elevation (i.e., colder climate), although most of the variance is explained by caste, i.e., queens having a larger body size than workers. Body size also shows some correlation with day of capture, which can be explained by changes in environmental conditions (e.g., temperature, flowering plant species) during the growing season experienced by the different emerging times for the castes. Given that caste was the most useful explanatory variable for body size, future studies could look at a larger environmental gradient, for example, by sampling at multiple locations along the entire Scandes mountain range to see if the effects found are localized. Further, specific habitat and specific traits of preferred plants may also help to elucidate body-size differences between species and castes. For example, many bumblebee species' castes emerge at a specific time of year when only certain flowering plant species in specific habitats are available. This important research would also help to illuminate whether bumblebees and the species of plants they pollinate remain synchronous as climate warming accelerates. Nevertheless, my results show an overall positive relationship between bumblebee body size and elevation, indicating that a warming climate will result in reduced body sizes among bumble bee species. Future studies will have to investigate what consequences this will have for Arctic bumblebee populations – and for the plants that rely on bumblebee visits for their pollination.

Key Words: *Bombus*; Bergmann's rule; Gradient study; Arctic climate change.

# Contents

1. Introduction.....	1
1.1 Background .....	1
1.2 Hypothesis and predictions .....	3
2. Methods.....	4
2.1 Area description .....	4
2.2 Bumblebee sampling .....	5
2.3 Bumblebee processing and body size measurements .....	7
2.4 Temperature measurements.....	7
2.5 Data analysis .....	8
3. Results.....	9
3.1 Hypothesis 1.....	11
3.2 Hypothesis 2.....	12
4. Discussion.....	14
5. References.....	17
Supplementary Materials .....	

# 1. Introduction

## 1.1 Background

Species have through time developed different strategies to survive and adapt to a range of environmental conditions. Within biomes such as the Arctic or the tundra, a diversity of ecosystems, habitats, and microclimatic conditions dictate the distribution of species and their interactions. Today, our world is warming rapidly coupled with rapid loss of biodiversity due to land-use changes and degradation caused by humans (IPCC, 2021). The effects of human-induced planetary upheavals and loss of biodiversity has led us into a new era called “the Anthropocene”, where it is also believed by some that we are entering the “Sixth Mass Extinction” (Kluser and Peduzzi, 2007). Recent alarms over the loss of insect biodiversity, especially pollinators, has driven interest in factors that limit insect populations in the Anthropocene (Klein *et al.*, 2007; Rasmont *et al.*, 2015). For pollinators such as bumblebees a range of factors determine their distributions and ability to adapt to this changing world.

Animals regulate their metabolic needs in different ways relative to their environment. Broadly, animals can be seen as either endothermic or ectothermic. Endothermic animals are able to regulate body temperature internally with different methods such as increasing metabolic rates or shivering (Hammel and Pierce, 1968; Whittow, 1986). Ectothermic animals, in comparison, gain their heat from the surroundings which means that their activity is limited by ambient temperatures (Stevenson, 1985; Hertz, Huey and Stevenson, 1993). Body size in many taxonomic groups, as observed by Carl Bergmann nearly two centuries ago, correlates with ambient temperature and latitude (as a proxy for temperature; Bergmann 1847). Bergmann and others observed that having a larger body sizes helps to preserve heat (Bergmann, 1847; Blackburn, Gaston and Loder, 1999; Ashton, Tracy and Queiroz, 2000; Meiri and Dayan, 2003). The trend of larger body sizes at higher latitudes is generally shown for birds and mammals, but has also been observed for some insects such as bumblebees (Ashton, Tracy and Queiroz, 2000; Meiri and Dayan, 2003; Osorio-Canadas *et al.*, 2016; Scriven *et al.*, 2016).

Insects can be viewed as either thermoconformers or thermoregulators, the formers body temperatures naturally fluctuates with the ambient environmental conditions (i.e., ectothermic), and the latter is able to regulate their body temperature within a range regardless of ambient temperature (i.e., endothermic; Sanborn, 2005). For very small insects which are generally thermoconformers, body temperatures quickly exchange heat with their surroundings (Sanborn, 2005). The often larger body sizes of thermoregulators require capabilities to generate heat through other mechanisms, such as muscular activation, e.g., wing movements and shivering (Heath *et al.*, 1971; Heinrich, 1974, 1975).

Understanding the different mechanisms insects use to adapt to a rapidly warming climate is important as thermoconformers will have different constraints than thermoregulators. If the climate warms faster than their ability to adapt, then the future of the population and species is uncertain. Bumblebees, although not the most abundant pollinator, are particularly important

because some wild plants are predominantly, or exclusively, pollinated by them (Kevan, 1972; Egawa and Itino, 2020). Studies have shown that a reduction in highly linked pollinators, such as bumblebees, can negatively impact plant species diversity (Goulson, 2003; Memmott, Waser and Price, 2004). The importance of bumblebees as pollinators is amplified in the Arctic, due to their ability to withstand the harsh conditions (Bernd, 1979; Peat *et al.*, 2005; Martinet *et al.*, 2021). For example, due to their insulating pile, ability to generate heat with their wings, larger body size compared to other pollinators, and the overwintering (i.e., hibernation) of the queens, bumblebees appear to be particularly adapted to arctic and alpine environments (Bernd, 1979; Peat *et al.*, 2005). Considering bumblebee's mechanisms of adaptation to arctic habitats, it is important to understand how they might adapt to the rapidly warming Arctic.

The global average temperature has increased by approximately 1.1 °C since the beginning of the industrial revolution (IPCC, 2021). Alarmingly, the temperature at high latitudes is rising at three times the global rate (IPCC, 2021). In Sweden, the mean winter temperature increased by 2 °C in the period 1991-2000 compared to 1961-1990 (Räisänen and Alexandersson, 2003). If bumblebees found in the high latitudes and altitudes are specifically adapted to cold harsh environments, how will their populations be impacted by this rapid warming and other warming associated impacts, e.g., increased precipitation? Arctic specialists could track their thermal niche to higher altitudes or latitudes, but what happens when southern (boreal) species that are better adapted to warmer conditions move north or upslope? Recent studies indicate that the Mediterranean *Bombus terrestris* and *B. lapidarius* have extended their habitats north of the Arctic Circle (Martinet *et al.*, 2015). How will the bumblebees and the plant communities that rely on their pollinator's services be affected as the climate continues to warm? Soroye *et al.* (2020) suggests that bumblebee species richness is on a decline as a result of increasing global temperatures.

Bumblebee queens emerge as the snow melts across the Arctic each spring and begin by collecting pollen and nectar resources to lay the foundation for a new colony (Söderström, 2013). The first members of the colony to emerge are workers, who tend to have the largest intraspecific variation in body size, with the first emerging workers being smaller than those later in the growing season due to the scarcity of energetic resources (i.e., pollen and nectar) and more extreme weather conditions early in the season (Bernd, 1979; Scaven and Rafferty, 2013; Söderström, 2013). Queens tend to have the largest body size, followed by drones, and finally workers being on average the smallest (Goulson, 2010).

Studies have shown that bumblebees with larger body sizes, found at higher altitudes, can tolerate a lower threshold of extreme minimum temperatures (Oyen, Giri and Dillon, 2016). Conversely, bumblebees with smaller body sizes, found in lower altitudes, are able to tolerate higher maximum threshold of temperatures (Oyen, Giri and Dillon, 2016; Peters *et al.*, 2016). This is explained by bumblebees ability to thermoregulate through muscular activity (Heinrich, 1974, 1975). This could explain why queens have a larger body size than workers and drones, due to them emerging at the early of the season when the temperatures are at their coldest (Goulson, 2010).

To better understand how species and populations are adapted to a wide range of environmental conditions, gradient studies can be a powerful approach (Sundqvist, Sanders and Wardle, 2013). For example, as temperatures and precipitation vary along an elevational gradient, species community composition and their interactions vary as well. Therefore, gradient studies can be used as a space-for-time substitution, rather than having a long term study, to for example illustrate how rapid warming will impact species, communities, and ecosystems (Sundqvist, Sanders and Wardle, 2013).

## 1.2 Hypothesis and predictions

Bumblebees are large and easy to find pollinators and with experience identified to both species and caste, which is important due to intraspecific size difference between castes. The aim of this study is to answer how temperature limits the distribution of bumblebee species in space and time, specifically if bumblebees follow Bergmann's Rule and if body size differences between castes is larger at higher altitudes.

Using an environmental gradient in the mountains of northern Sweden that represents different climates, I ask, is body size reflected in how bumblebees are partitioned along the mountain slopes, reflecting the temperature gradients along the slope, i.e., reflecting thermal constraints?

**Hypothesis 1:** Bumblebee body size is correlated with elevation regardless of species or caste (Fig.1).

**Prediction 1:** Body size for bumblebees is positively correlated with elevation, meaning that the largest bumblebees will be found higher on the gradient and the smallest lower on the gradient.

**Hypothesis 2:** The intraspecific variation in body size between castes (i.e., queens and workers) is correlated with their distribution along the elevation gradient (Fig.1).

**Prediction 2.1:** Alpine specialists found in higher altitudes, where we expect to find the largest variation in temperature, will have the largest variation in body size between castes.

**Prediction 2.2:** Generalist species found all along the gradient are expected to have an intermediate variation in body size between castes.

**Prediction 2.3:** Species only found in the forest zones (lower altitudes) are expected to have the smallest variation in body size between castes.

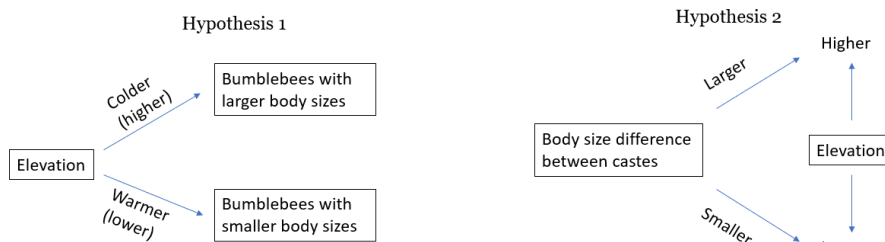


Figure 1: Conceptual model showing that larger bumblebees are expected to be found on higher elevations (hypothesis 1) and that difference in body size between castes increases (i.e., queens – largest - to workers – smallest) at higher elevations (hypothesis 2).

## 2. Methods

### 2.1 Area description

The study site was situated along a 3.4 km transect (marked with 79 poles approximately 45 m apart), that runs up the eastern slope of Mount Nuolja in Abisko National Park, Sweden (Fig. 2; est. 1916; Fries, 1925). The transect starts at a low elevation birch forest zone (420 m.a.s.l.) rising to the alpine at the summit of Mount Nuolja (1164 m.a.s.l.). The transect is characterized by three distinct habitat types or zones, birch forest, willow shrubs, and alpine. Each of these broad zones can be further subdivided based on the structure of the vegetation. For example, the lower birch forest is dominated by a field layer of low stature woody plants, such as *Empetrum nigrum* and *Vaccinium vitis-idaea*. The upper birch forest has a field layer comprised of primarily herbaceous plants, including *Geranium sylvaticum*, *Solidago virgaurea*, and *Trollius europaeus*. The shrub zone is dominated by tall willows, such as, *Salix myrsinifolia* and *S. phyllicifolia* in the lower reaches. While in the upper reaches with short stature willows, such as, *S. lanata* and *S. glauca*. The alpine zone contains both meadow and heath habitats, with snow beds, rocky cliffs, and wind-blown stone fields. Thirteen bumblebee plots are situated along the transect, covering the entire transect (Table 1; Fig. 2). The forest zone has five plots, two in the lower birch forest and three in the upper birch forest. The shrub zone has another five plots with three plots in the tall shrubs and two in the upper low shrub zone. The final three plots are placed in the alpine zone.

Table 1: Pole elevation for each plot.

Altitude of plot poles (m a.s.l.)	Plot poles
432.37 - 436.56	4 - 5
456.56 - 463.13	9 - 10
538.55 - 540.13	21 - 22
577.98 - 587.13	25 - 26
641.04 - 655.13	29 - 30
681.43 - 699.13	32 - 33
740.45 - 757.13	35 - 36
818.61 - 834.26	40 - 41
897.31 - 904.8	45 - 46
917.63 - 925.03	47 - 48
1063 - 1077.2	60 - 61
1086.1 - 1088.21	65 - 66
1157.23 - 1161.39	73 - 74

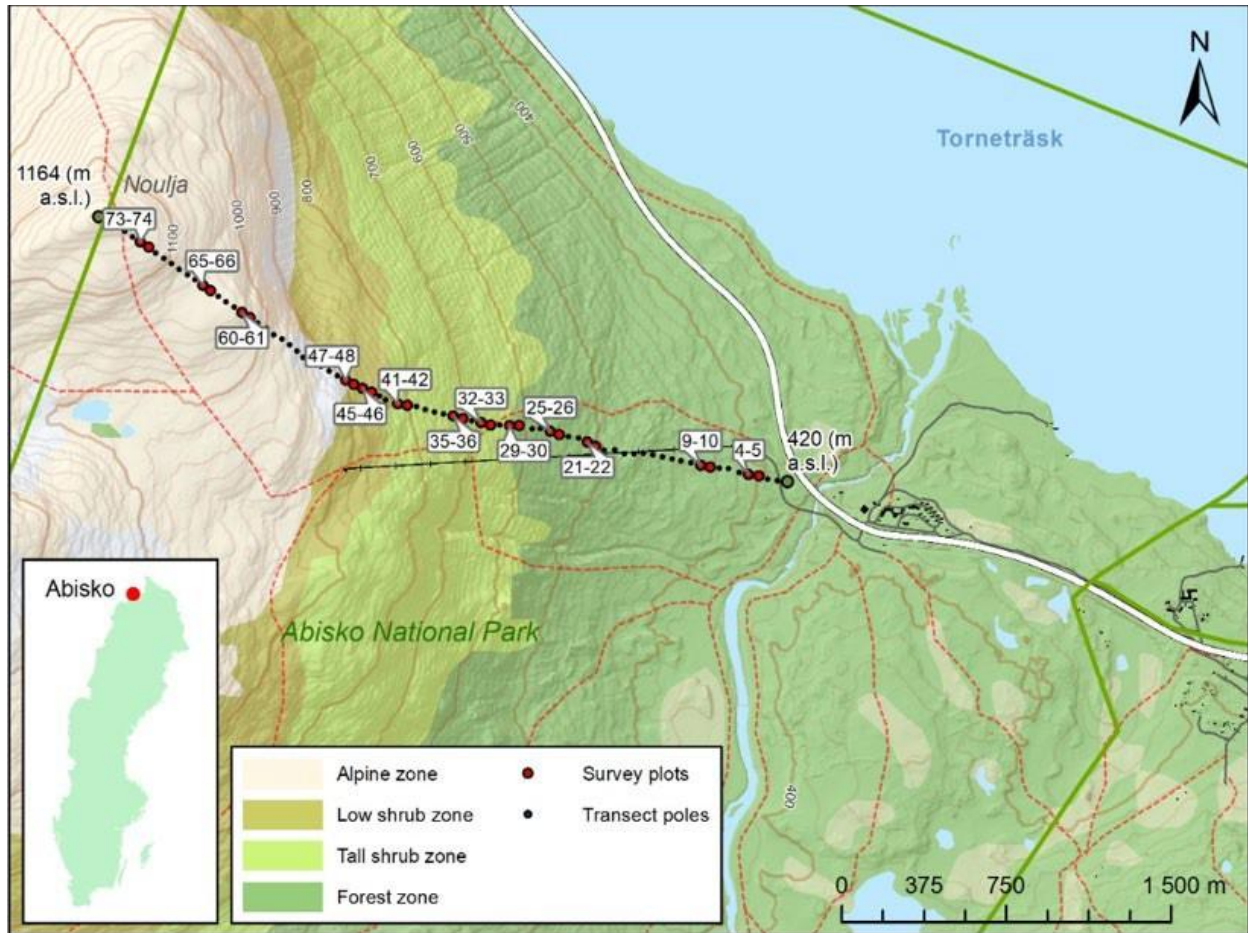


Figure 2: Transect along Mt. Nuolja in Abisko National Park with a plot (N=79) approximately every 45 meters. Each plot is marked on the map (red) coinciding with the adjacent pole numbers. Colours represent the different vegetation zones (source of base layers: Lantmäteriet 2022, edited by Johan Svedin).

## 2.2 Bumblebee sampling

Sampling along the transect had previously been conducted in 2018 and 2019, and again in 2021. The 2020 field season was lost due to the global pandemic. Fieldwork began in May each year, as the snow melted, and queens emerged from hibernation. In 2018 and 2019 fieldwork ended in mid-July, while in 2021, sampling covered the entire growing season. Each plot was visited twice each week, resulting in four sampling days per week. In 2018 and 2019, sampling ended in mid-July due to logistical constraints, whereas, in 2021, the field season ended once the number of observations in a week were less than 5% of the daily maximum observed bumblebees (i.e., early September).

The plots were divided into two groups, where each group included at least one plot from each vegetation zone and daily sampling alternated between the groups. The starting plot was randomly selected each day to avoid consistently sampling specific plots at the same time of the day. The plot grouping and the randomized start were done to ensure that all vegetation zones



were sampled four times a week, and that if day one had poor weather conditions, then the other day would be sampled. Sampling did not occur under poor weather conditions which were defined as rain greater than 0.2 mm/hour or low visibility (i.e., if the entire plot was not visible). Sampling was always conducted by two people.

The starting point of the plot was the middle right or middle left, picking the point closest to the transect, and the first loop was walked in a figure-8 and the perimeter of the plot for the second loop (Fig. 3).

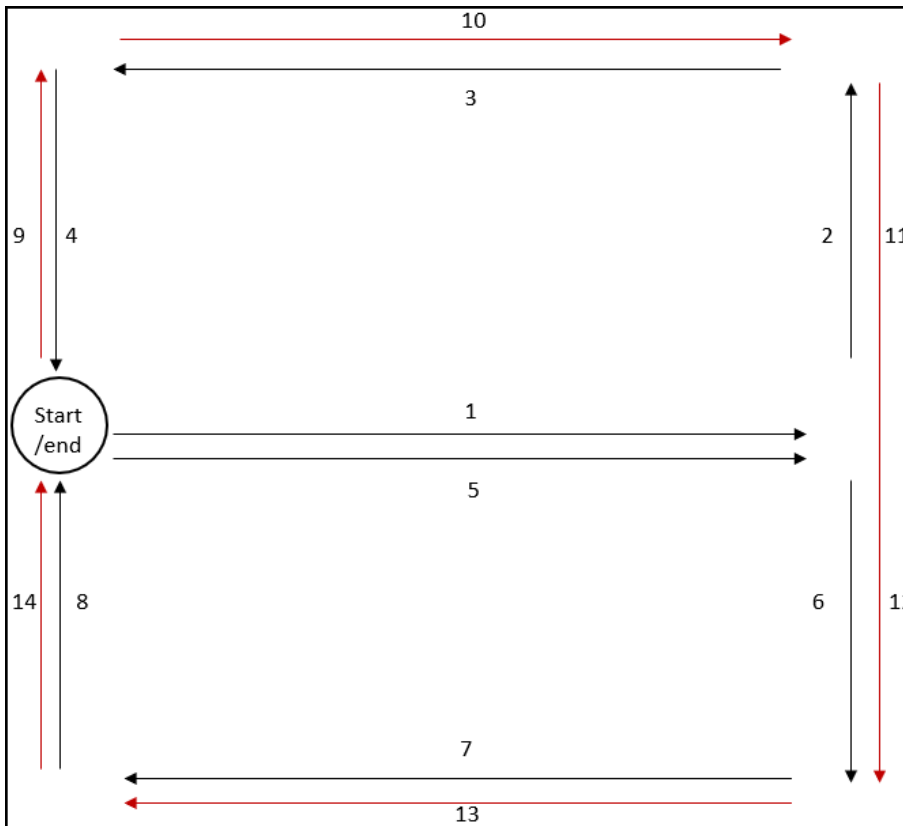


Figure 3: Typical bumblebee study plot, approximately 45 x 45 m. The black lines represent the first loop, and the red lines represent the second loop. The numbers help the reader follow the arrows in the right order.

Sampling stopped once both criteria of 20 minutes and at least two loops were made. Total number of loops and time were noted at the end. Butterfly nets (45 cm diameter) were used to capture the bumblebees. When captured, the time was paused for processing or to note observations of non-captured bumblebees. Species and caste along with a degree of identification certainty were recorded on the datasheet with each observation receiving a unique identification number. Captured bumblebees were stored in capture pots and placed into a cooling bag for processing after the survey was completed.

### 2.3 Bumblebee processing and body size measurements

After sampling each plot, individual bumblebees were transferred to a cage (28 mm inside diameter) with a reference scale for size and a dorsal photo was taken with a digital camera (Canon SX720 HS) for standardized body size measurements. All individuals were released after processing was completed.

The intertegular distance (ITD) was chosen to represent body size measurement and was measured using the program TPSdig version 2.31 (Rohlf, 2006). This program allows the user to put landmarks on a photo and set a scale. The landmarks were placed at the inner side of the proximal end of each wing base and the resulting coordinates were then transformed to length units (cm) using the package “geomorph” in R (Fig. 4; Adams *et al.*, 2022).



Figure 4: An example of where the landmarks (red markings) were placed on the thorax of the bumblebees, e.g., this *B. lucorum*.

### 2.4 Temperature measurements

iButton Thermochron temperature loggers were installed at each of the 79 poles (approximately 45 m apart) along the 3.4 km transect in 2017, to measure the above-ground temperature (10 cm) throughout the growing season (measurements taken every 15 minutes). In 2019, TSM4 temperature and soil moisture loggers (Tomst s.r.o.) were added alongside the iButtons at each pole along the transect. Starting in 2020, the TMS4 loggers were used exclusively. The TMS4 loggers provide temperature measurements below ground, at the surface, and above ground, in addition to soil moisture. To get the best approximation of the above-ground temperature at the time of capture of each bumblebee. The mean temperature was calculated from the two loggers found at the upper and lower poles along the transect where the bumblebee plot was located, i.e., Plot 1 found in the lower forest, the average of the above-ground temperature would be calculated from Pole 4 and 5 (see Fig. 2).

## 2.5 Data analysis

To test whether temperature varied along the Nuolja transect elevational gradient, I fitted a linear mixed effects model with temperature as a response variable and elevation and day as explanatory variables with random intercepts for each altitude and day. I used a mixed effects model as it allows seasonal change in temperature as well as how temperature changes by elevation to have random intercepts. Model assumptions of normality of the data and random distribution of the residuals were met. For this, the model was built using the ‘lmer’ from the R package ‘lme4’ (Bates *et al.*, 2022).

Only bumblebees that were reliably identified to species and caste were used to test Hypothesis 1 and 2 (Table 2).

Multiple models for each hypothesis were built and the best model for each hypotheses were chosen using Akaike Information Criterion (Ramachandra Murthy *et al.*, 2019).

The analysis was conducted using R version 4.1.2 (R Core Team, 2021).

### 2.5.2 Hypothesis 1

To test the hypothesis that body size varies with elevation, I used a linear mixed effects model that fitted ITD (body size measurement) as a response variable and elevation as an explanatory variable with species and caste as random effects. Model assumptions of normality of the data and random distribution of the residuals were met. The model was built using the ‘lmer’ from the R package ‘lme4’ (Bates *et al.*, 2022).

### 2.5.3 Hypothesis 2

In order to test the hypothesis that the difference in body size between castes increases along the elevation (i.e., size variation between queens and workers was larger on higher elevations), I tested used a linear model. Here, ITD is a response variable, with elevation and the interaction of caste are explanatory variables. Caste from each species was used as a random effect to allow them to have their own intercepts. The random effect is important as body size for larger species could vary greatly from that of smaller species. Model assumptions of normality of the data and random distribution of the residuals were met. For this, the model was built using the R function ‘lm’ (Wilkinson and Rogers, 1973).

Table 2: Bumblebees reliably identified to species and castes captured and measured in 2018, 2019, and 2021 (n = 2030).

<b><i>Bombus</i> species</b>	<b>Queens</b>	<b>Workers</b>	<b>Drones</b>	<b>Total</b>
<i>B. alpinus/polaris</i>	67	17	6	90
<i>B. balteatus</i>	81	100	33	214
<i>B. bohemicus</i>	21	-	2	23
<i>B. cingulatus</i>	8	3	1	12
<i>B. flavidus</i>	10	1	2	13
<i>B. hortorum</i>	22	28	7	57
<i>B. hyperboreus</i>	15	-	8	23
<i>B. hypnorum</i>	-	-	1	1
<i>B. jonellus</i>	54	114	55	223
<i>B. lapponicus</i>	144	129	102	375
<i>B. lucorum</i>	61	112	19	192
<i>B. monticola</i>	130	141	101	372
<i>B. pascuorum</i>	27	74	12	113
<i>B. pratorum</i>	59	193	67	319
<i>B. soroensis</i>	-	1	-	1
<i>B. wurflenii</i>	-	-	2	2
<b>Total</b>	<b>699</b>	<b>913</b>	<b>418</b>	<b>2030</b>

### 3. Results

Temperature along the transect gradient varied by both elevation and day of year where the highest recorded temperatures were found at the bottom of the transect in the birch forest and the lowest temperatures found in the alpine zone at higher elevations (Fig. 5). Temperature is highly dependent on both elevation with temperature decreasing with elevation (d.f. = 8354, t-value = -29,97,  $p < .001$ ) and increasing with the day of the year (d.f. = 8656, t-value = 34,57,  $p < .001$ ) (Fig.5, Table 3). However, the effect sizes showed that most of the variation in observed temperatures is likely explained by other factors (Fig. S1).

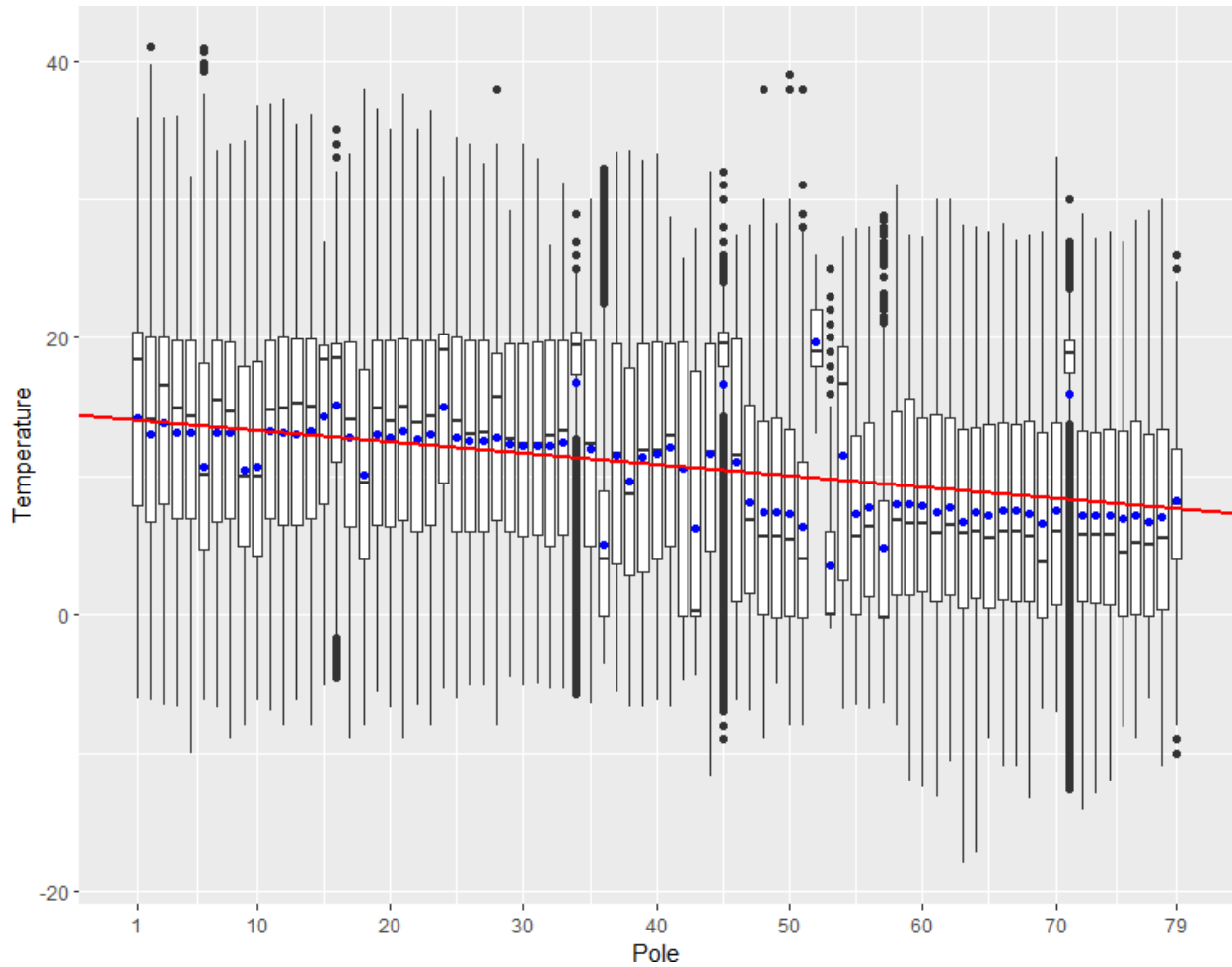


Figure 5: Recorded temperatures (May – September) across the Nuolja transect at each pole for the years 2018, 2019 and 2021. Pole 1 is at the bottom of the transect in the birch forest (436 m.a.s.l.) and Pole 79 at the alpine summit of Nuolja (1161 m.a.s.l).

Table 3: Results from mixed effects model for temperature as the response variable and elevation and altitude as the explanatory variables with random intercepts for each altitude and day of year.

<b>Coefficient</b>	<b>est. (<math>\pm</math> s.e.)</b>	<b>d.f.</b>	<b>t-value</b>	<b>p-value</b>
Intercept	3.90 $\pm$ 0.32	8654	12.15	<.001
Elevation	-0.01 $\pm$ 0	8354	-29.97	<.001
Day of year (Julian)	0.05 $\pm$ 0	8656	34.57	<.001
<b>Random effects</b>	<b>Var.</b>	<b>S.D.</b>		
Elevation:Day of year (intercept)	27.15	5.21		
Residual	16.05	4.01		

### 3.1 Hypothesis 1

Relationship between body size and elevation showed no significance (estimate  $\pm$  s.e. =  $0.01 \pm 0$ , d.f. = 2019,  $t = 0.894$ ,  $p = .371$ , Table 4, Fig. 6).

Table 4: Test results from the mixed effects model for ITD as the dependent variable and elevation as the explanatory variable with species and castes as random intercepts.

Coefficient	estimate ( $\pm$ s.e.)	d.f.	t-value	p-value
Intercept	$0.58 \pm 0.08$	2.275	7.569	.012
Elevation	$0.01 \pm 0.00$	2019	0.894	.371
Random effects	Var.	S.D.		
<i>Bombus</i> species	0.01	0.07		
<i>Bombus</i> caste	0.02	0.13		
Residual	0.00	0.06		

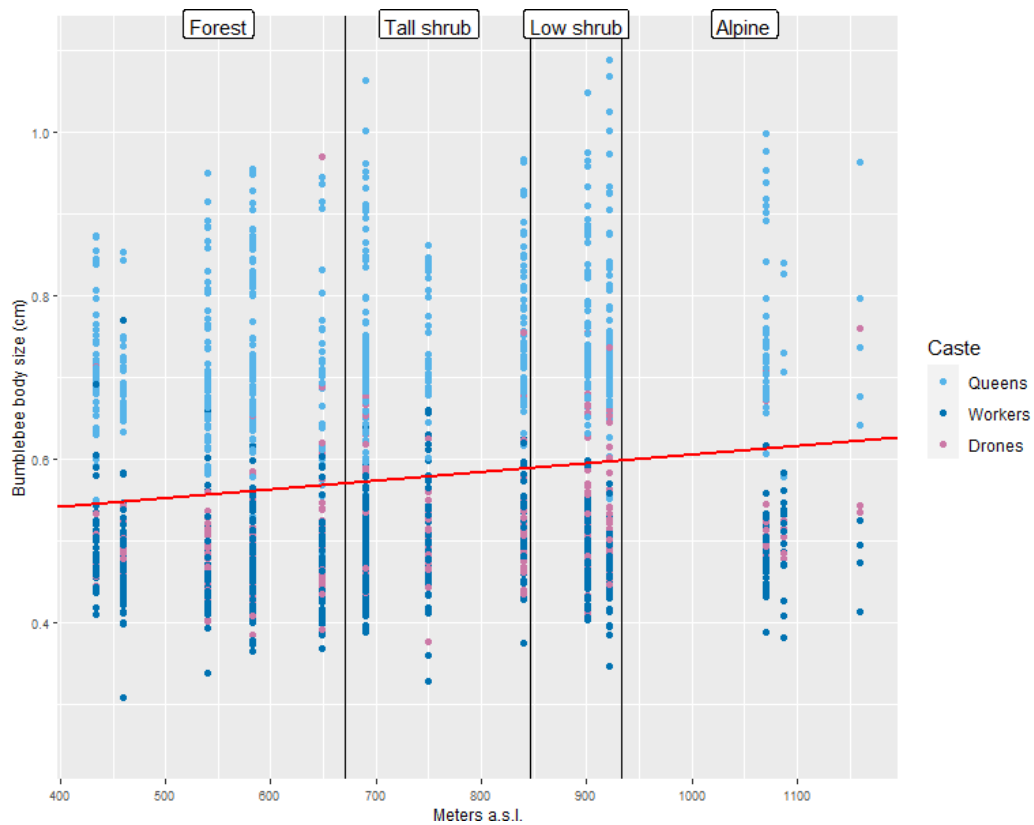


Figure 6: Bumblebee body size (years 2018, 2019 and 2021) plotted over elevation (mid-point) on each bumblebee sampling plot. Red line is the close-to-significant ( $p < .001$ ) trend line for ITD ~ elevation. The four habitat zones are divided by a black line.

However, conducting a *post hoc* analysis, most of the variation in body size was explained by caste (Fig. 7). Therefore, an ANOVA was performed to compare the effects of caste on body size, to reveal a statistically significant difference between at least two groups ( $F = 2386$ , d.f. = 2,  $p < .001$ ). Tukey's HSD test found that the mean value of ITD was statistically significant for all groups (Table 5).

Table 5: Results from Tukey's HSD test for ITD (response) over caste (explanatory).

Group	<i>p</i> -value	95% C.I. lower	upper
Queens - Drones	<.001	0.21	0.24
Workers - Drones	<.001	-0.03	-0.01
Workers - Queens	<.001	-0.26	-0.24

### 3.2 Hypothesis 2

The relationship between body size and elevation and the interaction of caste, showed that elevation was significant (estimate  $\pm$  s.e. =  $0 \pm 0$ ,  $t = 4.37$ ,  $p < .001$ ) along with queens (estimate  $\pm$  s.e. =  $0.23 \pm 0.01$ ,  $t = 24.29$ ,  $p < .001$ ), the interaction between elevation and workers also showed significance (estimate  $\pm$  s.e. =  $0 \pm 0$ ,  $t = -2.367$ ,  $p = .018$ ). Other factors showed no significance (Table 6, Fig. 7).

Table 6: Results from linear regression model where ITD is the response variable and elevation and its interaction with caste as the explanatory variable. Caste levels from each species were introduced as random effects.

Coefficient	Est. ( $\pm$ s.e.)	<i>t</i> -value	<i>p</i> -value
Intercept	$0.48 \pm 0.01$	60.63	<.001
Elevation	$0.00 \pm 0.00$	4.37	<.001
Queens	$0.23 \pm 0.01$	24.29	<.001
Workers	$0.00 \pm 0.01$	-0.38	0.702
Elevation:Queens	$0.00 \pm 0.00$	-0.37	0.712
Elevation:Workers	$0.00 \pm 0.00$	-2.367	0.018
Coefficient	d.f.	adjusted R <sup>2</sup>	
Elevation	2024	0.71	

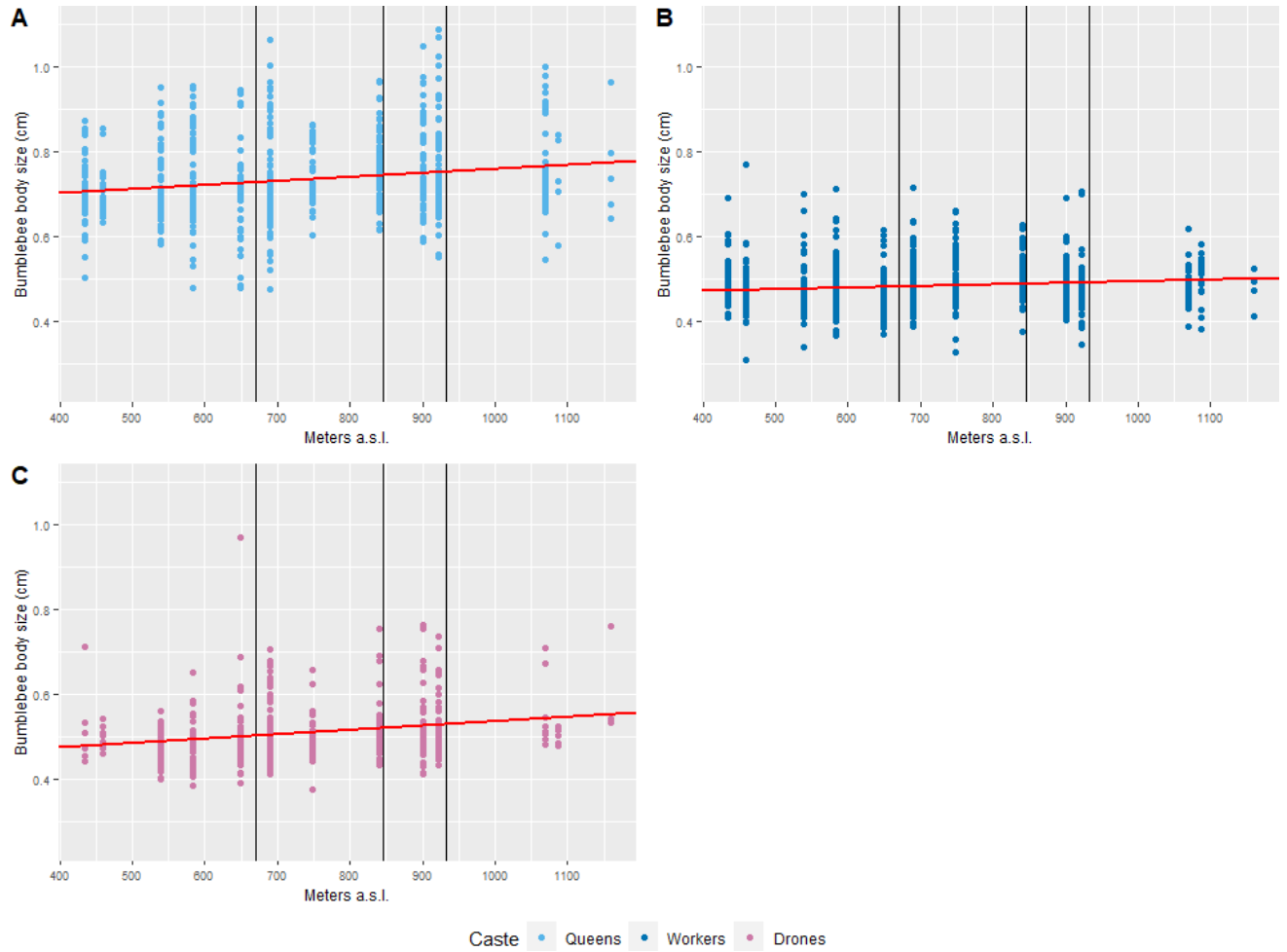


Figure 7: Bumblebee body size (ITD) plotted over elevation for each caste. Red line is the close-to-significant ( $p < .001$ ) trend line for  $ITD \sim \text{elevation}$ . The four habitat zones are divided by a black line.



## 4. Discussion

At the spatial scale of the transect along the gradient on Mt. Nuolja, elevation is not sufficient to explain the variation in species body size. However, *post hoc* results suggest caste better explains the variation in body size (Table 4, Table 5). Further results demonstrate that the interaction between elevation and caste best explained the variation in body size. These results suggest a positive relationship between body size and elevation, but it is most clear when considering the caste rather than species (Fig. 6, fig. 7).

The transect along Nuolja is an elevational gradient that captures two dimensions of temperature, both spatial (elevation), and temporal (day of year), in relation to the distribution and interaction of plant and pollinator communities. Temperature is warmest in the low elevation birch forest and coolest in the high elevation alpine zone. However, there is a significant seasonal component to temperature with the coldest temperatures early and late in the season and warmest during the peak of the growing season (Fig. S2). Day of year and temperature both interact to constrain the body size of bumblebees (Oyen, Giri and Dillon, 2016; Peters et al., 2016).

While analysis showed that elevation alone was unable to explain the variation in bumblebee body size along the Nuolja transect, queens had a much larger body size than both workers and drones independent of elevation (Table 5, Fig. 7). The variation likely reflects the constraints in the seasonal temperatures experienced by each caste, for example, queens emerge late in the season and hibernate over winter, then emerging in the early summer (i.e., the beginning of the growing season) when temperatures are the coldest. Whereas workers do not experience the extremes of the early season. Finally, drones start emerging half-way through the season, persisting through to the end of the season (Bernd, 1979; Goulson, 2010; Scaven and Rafferty, 2013; Söderström, 2013; Fig. S1).

Queens maybe larger than both workers and drones as they emerge late in the season when food is no longer a constraint, and the larger body size increases their survival chances during winter hibernation. Comparatively, workers may be constrained by food availability rather than temperature at the start of the growing season when the temperatures are the coolest (Bernd, 1979; Scaven and Rafferty, 2013). Geist (1986) suggests that body size follows annual productivity rather than temperature, i.e., bumblebee body size increases as the season progresses due to the increasing availability in nutrients (i.e., nectar and pollen). For example, the spring emerging queens are a product of the previous season's colony efforts during peak biomass and the large variance in worker body size coincides with the seasonal changes in nutrient resource availability (Bernd, 1979; Shpigler *et al.*, 2013). Further, Shpigler *et al.* (2013) suggested that body size is directly correlated with colony size where early workers tend to be smaller as it is most likely only the queen tending to the larvae, whilst workers emerging later in the season are larger as there are more workers tending to the larvae.

Alternatively, variation in body size may correlate with plant diversity and floral abundance, where increased diversity reflects the spatial and seasonal abundance of nectar and pollen (Vaudo *et al.*, 2015; Hass *et al.*, 2019). In the low shrub zone, we see the largest range of body

sizes along the gradient along with the first plot in the tall shrub zone (Fig. 6). Just above the shrub zones, snowbeds finish melting late in the season which keeps the soil below the snowbeds moist and nutrient rich throughout the summer. In the first plot in the low shrub zone, we observed the highest number of plant species (Fig. S3). The first plot in the tall shrub zone, located in a ski slope, has the second highest number of plant species (Fig. S3). The increased soil moisture in the low and tall shrub zones due to the deeper snow captured by the vegetation along the slope each winter, and the disturbance in the tall shrub zone, could explain the higher number of flowering species compared to other areas. Previous studies have found that an increase in soil moisture can benefit *Salix* species abundance and that disturbance can benefit biodiversity (Thom and Seidl, 2016; Scharn *et al.*, 2021).

Finally, the shrub ecotone between the birch forest and alpine shows a larger range of bumblebee's species, which could explain the large variation in body size (Fig. S4). Ecotones are well documented for their higher levels of species diversity (Smith *et al.*, 1997; Ward, Tockner and Schiemer, 1999). Therefore, microhabitat conditions along environmental gradients may be an important factor in determining the distribution of species, traits (e.g., body size) and the timing of their life history events, for example emergence date for each caste and body size (Beekman *et al.*, 1998).

In the future, it would be useful to consider additional factors including, precipitation, plant community diversity, seasonal patterns of floral abundance, below ground temperatures, and measurement of insulating pile differs (Geist, 1987; Cueva del Castillo, Sanabria-Urbán and Serrano-Meneses, 2015; Hülsmann *et al.*, 2015; Egawa and Itino, 2020).

In conclusion, the results from this study suggest that the variation of bumblebee body size along the elevation gradient of the Nuolja transect is mainly determined by caste, namely that queens are significantly larger than both workers and drones. Further, that the day of year (captured) also explains this body size variation, as castes emerge at different times of the growing season (Bernd, 1979; Goulson, 2010; Scaven and Rafferty, 2013; Söderström, 2013, Table 4, fig. S1).

In a rapidly warming world, how will bumblebees adapt to these new conditions. Given the clear body size differences by cast, timing of emergence and the conditions they encounter are important. If plants respond differently to rapid warming, will ecological mismatches occur effecting the synchronization between caste emergence and flowering resources (Scaven and Rafferty, 2013; Solís-Montero and Vallejo-Marín, 2017)? It would be interesting to explore seasonal interactions and carry-over effects by dividing the populations by year to examine if body size is more restricted by annual floral abundance and the production of floral resources (Geist, 1987). A recent study by Pardee *et al.*, (2022), suggests that bumblebees with larger body sizes in montane regions are decreasing in abundance while bumblebees with smaller body sizes are increasing in abundance. It is likely that bumblebees in Arctic regions will experience the same changes as temperatures warm. Here as winter temperatures becoming less extreme, selection may favor smaller queens.

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## Supplementary Materials

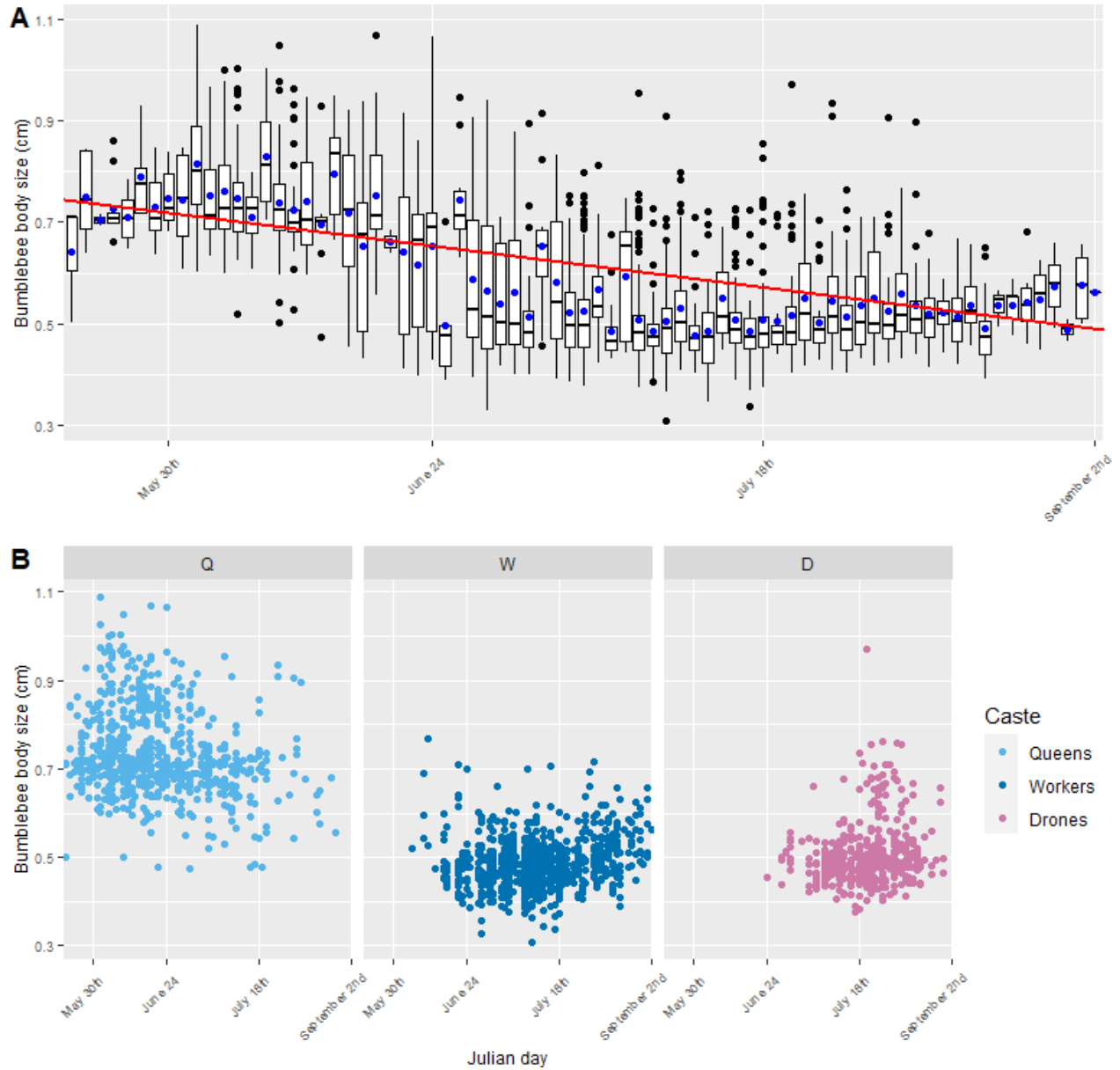


Figure S1: Bumblebee body size (ITD) plotted over Julian day. (A) with a trend line (red line) generated from the estimates, fitted onto it. Blue dots are the mean body size for each day, black are outliers. (B) has ITD over Julian day separated by caste.

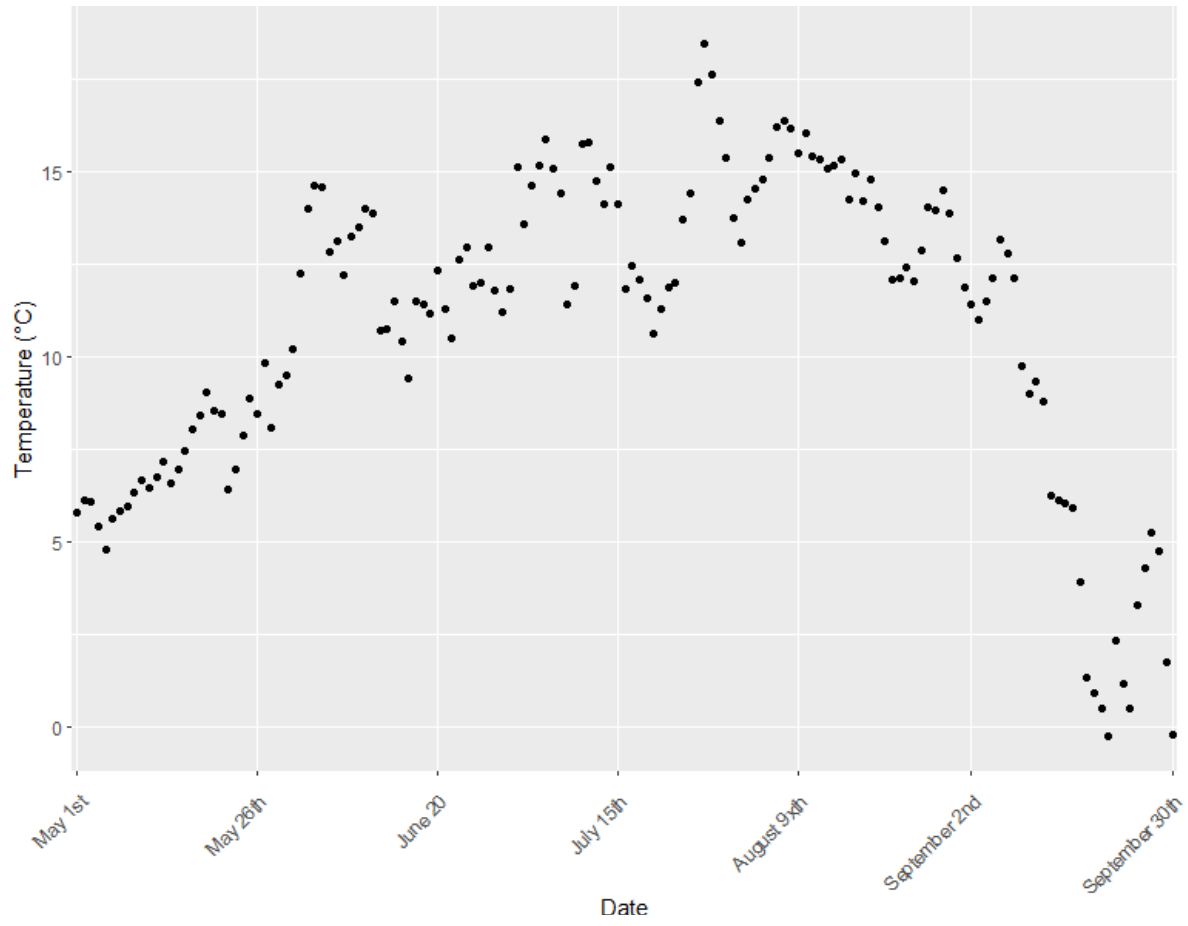


Figure S2: Daily mean temperature for 2018, 2019 and 2021 combined.

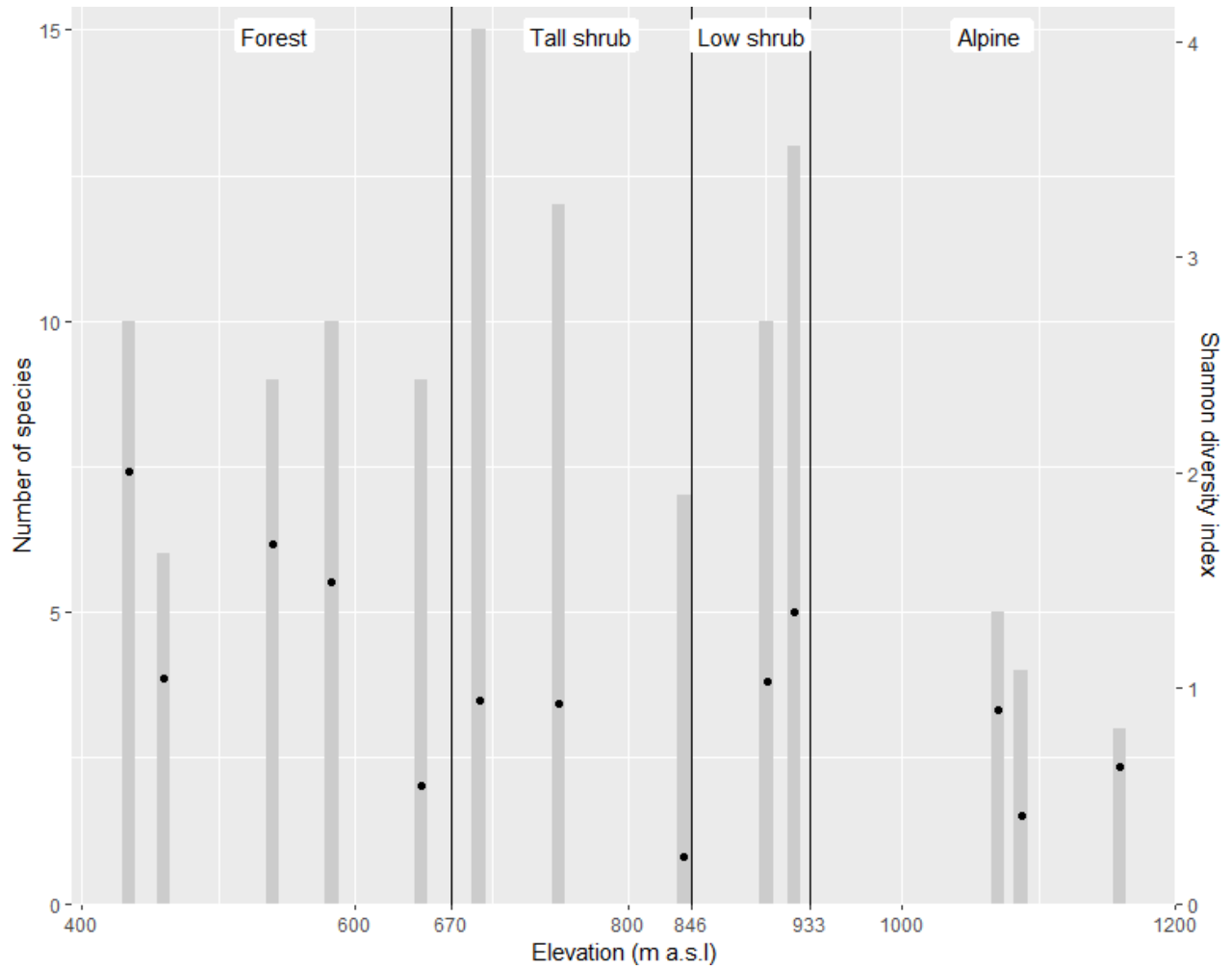


Figure S3: Plant species diversity where number of species (bars) and Shannon diversity index (black dots) are shown for each plot along the gradient.

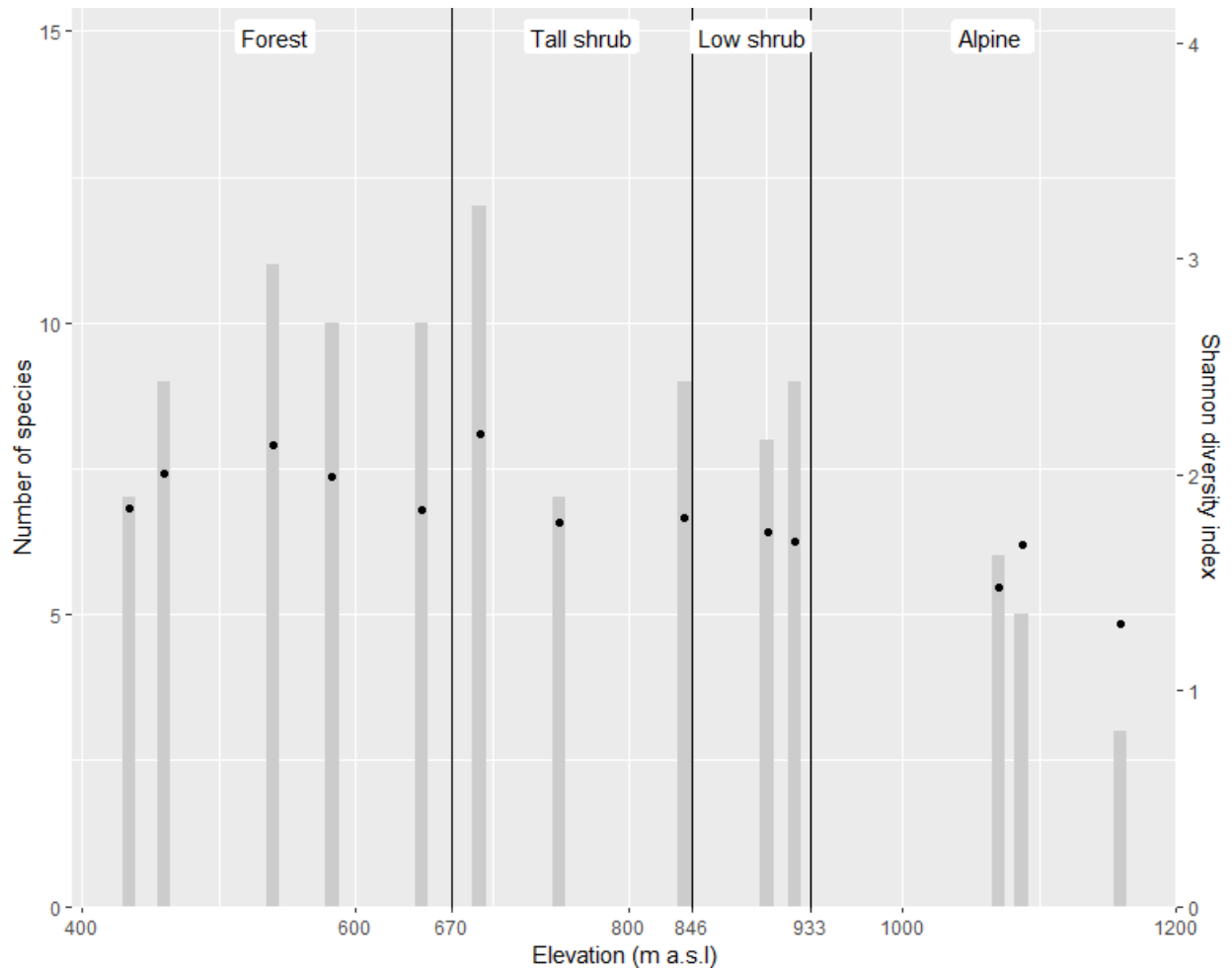


Figure S4: Bumblebee species diversity where number of species (bars) and Shannon diversity index (black dots) for each plot along the gradient.