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Seasonal food availability and its influence on the number of generations of *Bombus terrestris* in the Greek Island of Lesvos

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Abstract

In temperate climates, bumblebee colonies typically persist for a single season, with their life span averaging 3 to 6 months depending on the species. However, certain bumblebee species exhibit bivoltinism, producing two generations within one season. This study investigates the seasonal food availability and its impact on the number of *Bombus terrestris* generations on Lesvos Island. Data were collected across 16 locations, categorized into forests, olive groves, abandoned fields, and coastal areas, conducted over nine weeks in winter (October 2020 to January 2021) and nine weeks in spring (March to May 2021). The emergence of the queens at early October and at the end of March indicates the presence of two distinct generations annually. The winter generation is sustained mainly by *Arbutus unedo* flowering in forested areas, which exhibited significantly higher bumblebee activity compared to other habitats. Also a correlation test showed that there is no significant correlation between temperature and bumblebee activity. This study highlights the ecological flexibility of *B. terrestris* and its potential adaptation to Mediterranean conditions.

Key Words

Bivoltinism, *Bombus terrestris*, ecological flexibility, Lesvos Island, Mediterranean, pollinators, seasonal food availability

Introduction

Bumblebees (Hymenoptera: Apidae: *Bombus* Latreille, 1802), are eusocial insects that depend on a steady supply of floral resources for colony growth and survival (Alford 1975). They play a crucial role as pollinators in both wild and agricultural ecosystems, contributing significantly to biodiversity and food production (Goulson 2010). In temperate regions, mated queens that have overwintered emerge in early spring to forage for pollen and nectar, establish nests, and begin laying eggs. As the colony develops, workers take over foraging duties, ensuring the continuous growth and reproductive success of the colony. The availability and continuity of floral resources are essential for sustaining bumblebee populations, influencing their life cycles and reproductive strategies (Heinrich 2004). In Mediterranean regions, where floral availability fluctuates seasonally, some *Bombus* species have adapted by exhibiting bivoltinism, producing two generations within a single year (Potapov et al. 2018). This phenomenon allows populations to exploit different seasonal resources, potentially enhancing survival and reproduction. However, the underlying factors driving this adaptation remain insufficiently explored. This study focuses on *B. terrestris* on Lesvos Island, aiming to investigate how seasonal food availability influences the number of generations produced annually.

Materials and methods

Field observations were conducted across 16 locations in Lesvos Island (Suppl. material 1: fig. S1), categorized into four categories: forests, olive groves, abandoned fields, and coastal areas. These locations were specifically selected to capture ecological variability due to differences in altitude, distance from the sea, habitat type, vegetation diversity and proximity to human activities.

Coastal areas represented low-altitude, maritime-influenced environments, while forested regions were located further inland at higher altitudes. This selection aimed to investigate how varying environmental conditions influence bumblebee activity and seasonal food availability. The locations were mapped using ArcMap 10.5, with the Greek Geodetic Reference System GGRS87/Greek Grid - EPSG: 2100. Each transect was 500 meters long and 3 meters wide, with a duration of 30 minutes per walk to ensure consistent observation efforts. Observations were carried out weekly over nine weeks in winter (October 2020 to January 2021) and nine weeks in spring (March to May 2021), totaling 288 transect walks (144 hours of observation). No transects were carried out on rainy days to ensure consistent observation parameters.

Data collection included: bumblebee abundance (Queens, workers, young workers and males), some other species like *Xylocopa violacea*, *Xylocopa pubescens*, and syrphids, floral resource availability (species that bombus were foraging), and environmental variables such as temperature and humidity. During each transect, the number of individuals foraging on the flowers of each visited species was recorded. This allowed the assessment of both the richness of floral resources effectively used by the bumble bees and the frequency of their visits to different plant species. Despite the rich insect fauna of Lesvos (Germann and Braunert 2016; Petanidou et al. 2020; Lesvos Geopark 2023), the genus *Bombus* is represented solely by *B. terrestris*, and no other species have been observed on the island. Identification was straightforward and based on the author's prior experience in bumblebee field surveys and sampling.

Equipment such as digital thermometers and hygrometers were used to measure temperature and humidity. The temperature and the relative humidity were recorded at the beginning and end of each transect walk to capture temporal variability. To assess if there is an effect in bumblebee abundance by the temperature, a linear regression was performed. Data analysis and visualization were performed in R software and excel.

Results

A total of 168 bumblebees were observed, with 83 sightings in winter and 85 in spring. Arbutus unedo was identified as the primary winter food source in forest habitats, where bumblebee activity was significantly higher than in other habitats, the data is presented in a table (Suppl. material 1: table S1) and a graph (Fig. 1).

Analysis of floral visitation indicated a strong preference for *A. unedo* in winter and *Lavandula stoechas* and *Vicia sp*. in spring. In total, 30 plant species were recorded as foraging resources. The abundance of plant species represents only those plant species that were observed to be visited by bumblebees.The next graph (Fig. 2) represents the abundance of the bumblebees in relation with the plants they visited during the two observation periods.

It is well known that the colony starts with the emergence of the queens which lay a small number of eggs which they going to be the first workers of the colony, in the field they are easy recognizable from their small body size and also young workers may display a narrow strip of brown (or orange) hairs at the base of the white stripe, as noted in field identification guides (BWARS 2025). (Fig. 3) The "second generation" of workers appeared 1–2 weeks later and they were significantly larger. The Queens in the field when they search for a suitable nest, they hover low in the ground for cavities, that's atypical behavior. This behavior (in the present research)

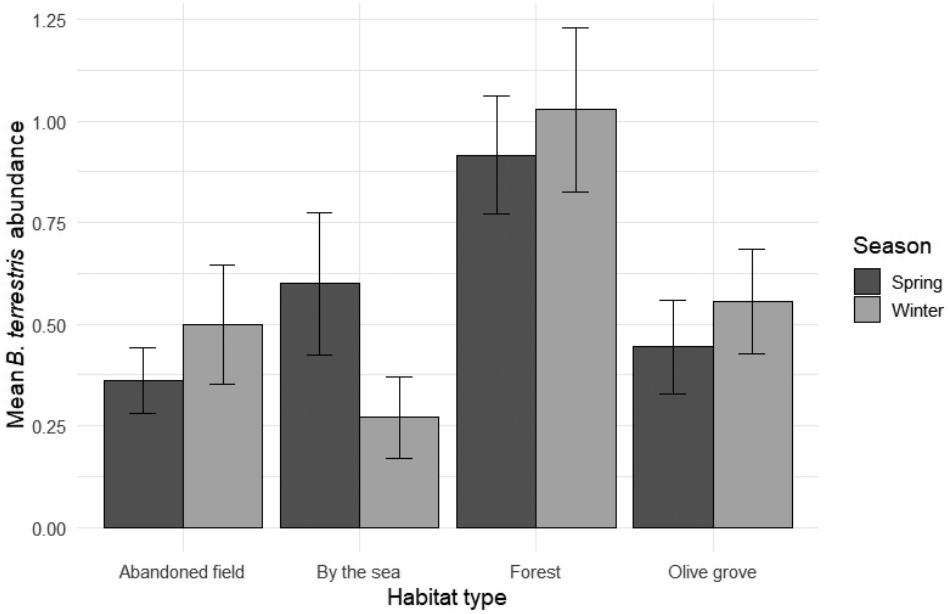

Figure 1. Mean bumblebee abundance by habitat type and season.

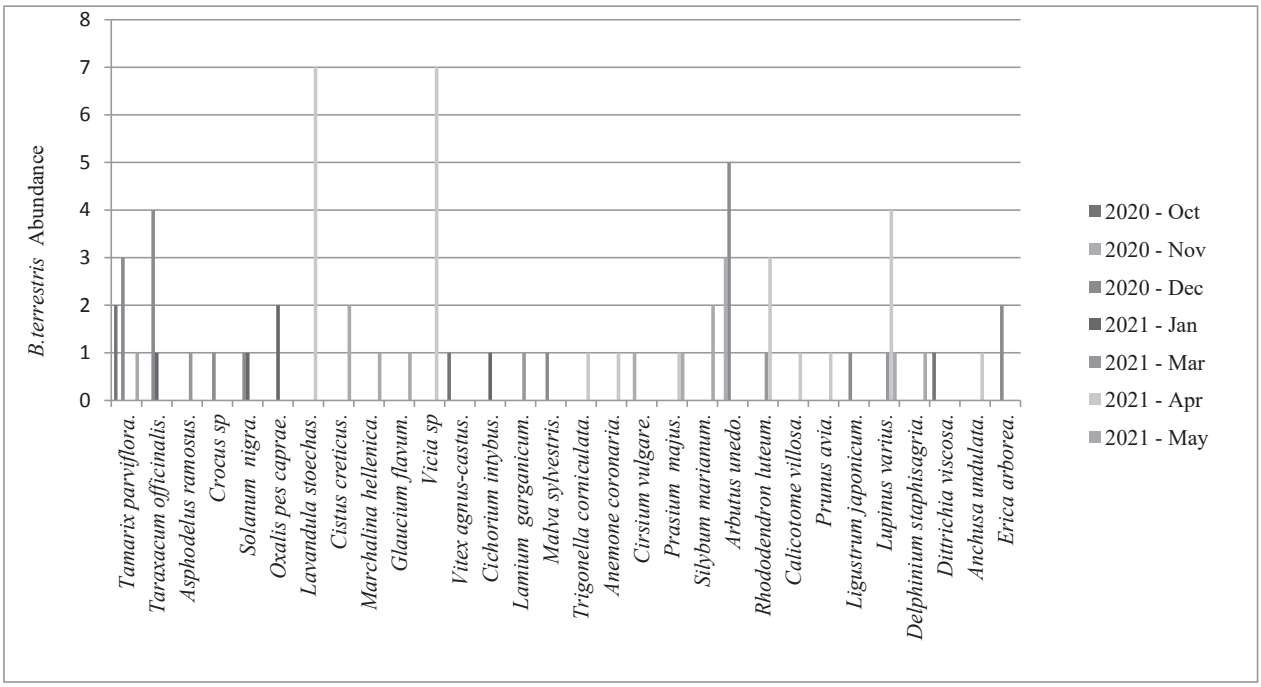

Figure 2. Bumblebee abundance relative to floral visitation per plant species.

Figure 3. Young worker *B. terrestris*; the brown-orange stripe at the base of the white stripe is visible.The image is under a CC BY 2.0 licence. Photographer: Gail Hampshire.

of the queens was observed around mid-October, and a similar behavior was recorded again at the end of March and early April. In the field the Queens were documented separately from the workers, the young workers and the males. The following graph (Fig. 4) shows the first queens of *B. terrestris* that was observed in every location on a weekly basis. Some queens appeared later in some locations, this could be due to some particular characteristic of the location (altitude, microclimatic conditions) or to a random factor. Nevertheless the observations of the first queens show that in the winter season four queens who represents four locations they was observed a couple of weeks later than the others, these four locations happen to represent all the categories of land cover: seaside areas, forests, olive groves and abandoned fields, thus that could be due to random factors. The data that was collected clearly indicates that two generations of *B. terrestris* are observed annually. This phenomenon represents a case of bivoltinism, which we mentioned earlier.

To assess if there is an effect in bumblebee abundance by the temperature, a linear regression was performed. The adequacy of the model was evaluated using the coefficient of determination (R^2), and the statistical significance of the correlation was assessed with the Pearson p-value, that was visualized by a scatter plot (Fig. 5). The linear regression analysis yielded an R^2 of −0.013, indicating that temperature does not explain the variation in bumblebee abundance. A small or negative R^2 suggests a very weak or no relationship. The Pearson p-value was 0.82, which is greater than 0.05, indicating that the relationship is not statistically significant. Therefore, these results suggest that temperature does not have a measurable effect on bumblebee abundance.

Discussion

Statistical analysis showed no significant correlation between temperature and bumblebee activity (R^2 = -0.013, p = 0.82), despite initial assumptions that warmer temperatures would enhance bumblebee foraging activity, indicating that temperature is not a determining factor in bumblebee abundance on Lesvos Island. This finding aligns with the observed year-round activity of *B. terrestris*, suggesting that food availability rather than temperature drives the species' bivoltinism. The presence of two generations per year reflects the ecological flexibility of

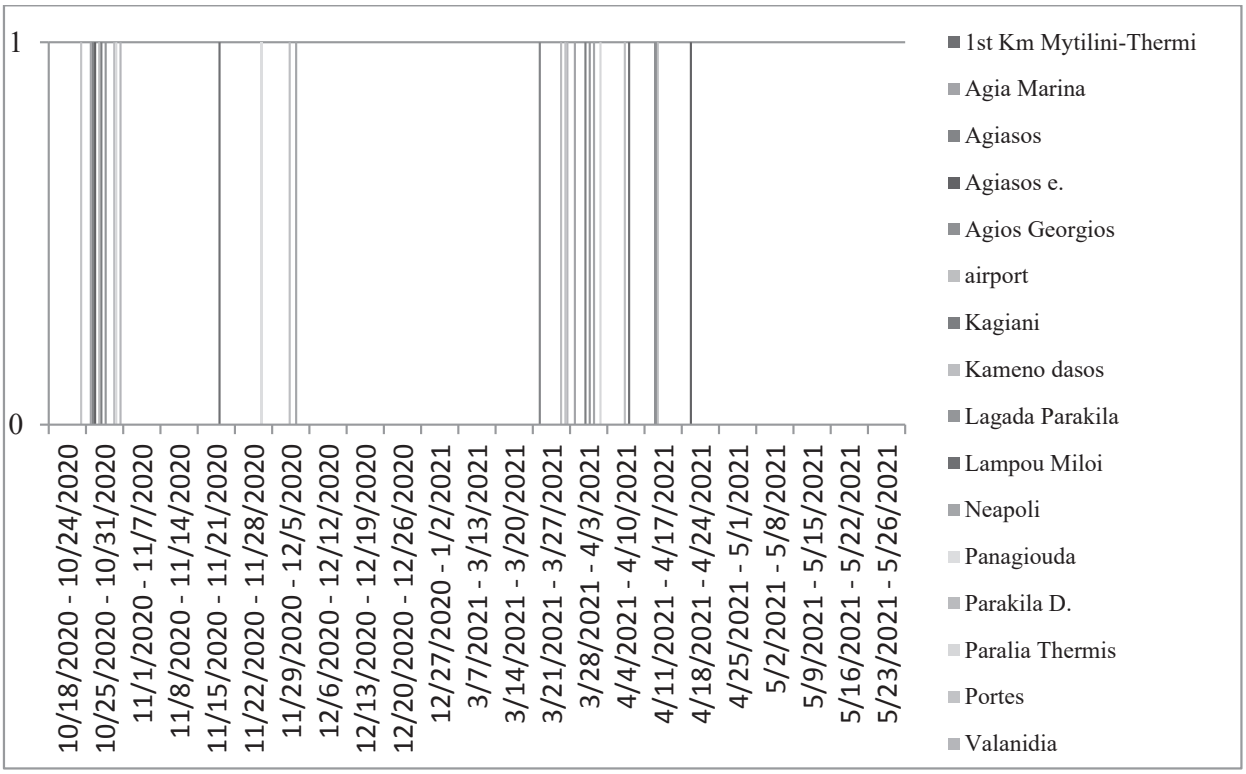

Figure 4. The figure shows the first queens of *B. terrestris* that was observed in every location on a weekly basis. The x-axis is up to 1 because, what's interesting is when the first queens appeared. The figure represents the winter and the spring documentation.

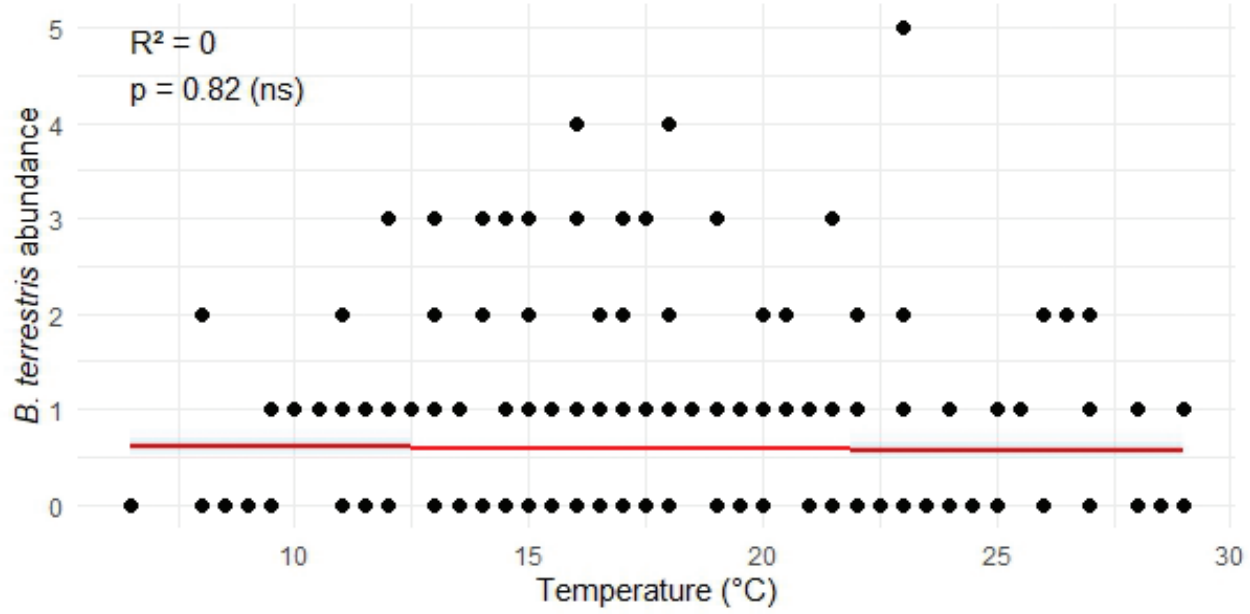

Figure 5. Bumblebee abundance plotted against temperature. The red line shows the linear regression trend. The model explains almost none of the variation in abundance ($R^2 = -0.013$), and the relationship is not statistically significant (Pearson p-value = 0.82).

B. terrestris in adapting to Mediterranean conditions. The availability of *A. unedo* during winter months provides crucial sustenance, enabling the survival and reproduction of bumblebee populations outside the typical season. The significant variation in abundance between habitats highlights the importance of forested areas in maintaining winter populations. The winter populations have scarce food resources compared to the spring populations when all plants are in their bloom. Additionally, anthropogenic factors such as land-use changes could impact resource availability and, consequently, the bivoltinism of *B. terrestris*.

A similar study conducted in Crete by Potapov, Kolosova, and Bolotov (2018) on several bumblebee species reported evidence of possible bivoltinism, where *B. terrestris* was observed as late as November. This observation indicates the absence of a mandatory diapause period, suggesting that continuous food availability may play a crucial role in sustaining bumblebee activity throughout the year, similar to the findings on Lesvos Island.

Research on the life cycles of bumblebees remains relatively limited. Most studies focus on one or two species typical of specific regions (Meidell 1968; Rasmont 1985; Corbet and Prys-Jones 1987). Comprehensive overviews are scarce, with notable exceptions of Radchenko and Pesenko (1994), who explored bee biology and gave examples of bumblebee life cycles in various climates. Additionally, Sakagami (1967) provided an insightful review of bumblebee life cycles across different climatic zones, highlighting how environmental conditions influence their phenology. In contrast to the bumblebee populations studied in tropical or lower latitude regions, where colonies can reach sizes of several thousand individuals under optimal conditions (Michener and LaBerge 1954; Dias 1958; Husband 1977), the bumblebees on Lesvos Island exhibit a markedly different life cycle. Due to the temperate Mediterranean climate, two distinct generations emerge annually: a winter and a spring generation, limiting the time available for colony expansion. This seasonal constraint likely prevents the formation of large colonies comparable to those in tropical environments, where stable year-round temperatures and continuous resource availability support prolonged colony development. The very hot and dry summers on Lesvos Island might be the reason why bumblebees reappear in October, when some plant species start blooming again. In recent years, however, this autumn appearance may be happening later, possibly due to increased drought linked to climate change, which seems to delay the blooming of the plants. These differences highlight how environmental factors, such as seasonal drought and temperature extremes, shape colony dynamics and social organization within Bombus species across different geographic regions.

Further research is recommended to explore genetic and climatic factors influencing this adaptation. In addition, future climate change scenarios could potentially impact the phenomenon of bivoltinism by altering food availability and phenology, which may, in turn, affect the survival and reproductive cycles of *B. terrestris*.

Conclusions

This study confirms the bivoltinism of *B. terrestris* on Lesvos Island is attributed to seasonal food availability. The findings underscore the need to preserve forested habitats, particularly those with *A. unedo*, to support winter generations. Further research is recommended to explore genetic and climatic factors influencing this adaptation.

Data availability statement

Data supporting the findings of this study are available from the corresponding author upon reasonable request.

Disclosure Statement: The authors report there are no competing interests to declare.

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Supplementary material 1

Supplementary table and figure

Authors: Eleni Tsiripli
Data type: docx
Explanation note: **table S1.** Mean bumblebee abundance (± SD) recorded across different habitat types during winter and spring observation periods. Forest habitats exhibited the highest bumblebee abundance in both seasons, particularly in winter, largely due to the presence of *Arbutus*; **figure S1.** Map of Lesvos Island with 16 Survey Locations categorized in Land types.

Link: https://doi.org/10.3897/bull.insectology.164673.suppl1