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Pollinator conservation with an island-wide approach: from community characterisation to demonstrating competition with honey bees

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Chapter 1. General introduction

1.1 Biodiversity declines

Global biodiversity is currently facing a severe crisis. Over the last centuries, the loss of taxa has reached alarming and unprecedented levels, leading to the wide use of the term "Earth's sixth mass extinction" among conservation biologists (Barnosky et al., 2011; Ceballos et al., 2015, 2017). Indeed, recent extinction rates of vertebrates are 1,000-10,000 times higher than the background rates estimated from fossil records (Ceballos et al., 2015, 2017; De Vos et al., 2015; Singh, 2002). Worryingly, the actual animal extinction rates are very likely higher than the measured ones, as these data are based solely on mammals without considering invertebrates (Régnier et al., 2015), which encompass most of the biodiversity on Earth and are fundamental in ecosystems functioning (Eisenhauer et al., 2019; Prather et al., 2013; Wilson, 1987). Data about invertebrates richness, abundance and distribution are scarce, in relation to their huge number of species, with many poorly known taxa (Régnier et al., 2015). Indeed, protection and conservation of these species are profoundly hindered worldwide by these knowledge gaps and by their severe underrepresentation in the IUCN Red List (Eisenhauer et al., 2019).

Among invertebrates, insects play a key role in natural environments providing several ecosystem services (Noriega et al., 2018). In this optic, the number of studies addressing the decline and extinction of insects has increased in the last years and highlighted a dramatic scenario for them. Long-term monitoring at local or regional scale reported numerical declines in many insect populations (Hallmann et al., 2017; Harmon et al., 2007; Lister & Garcia, 2018; van Strien et al., 2019). These worrying trends have been documented worldwide (Dirzo et al., 2014; Sánchez-Bayo & Wyckhuys, 2019, 2021; Wagner, 2020). The main global drivers can be reconducted to climate change, habitat loss and fragmentation, chronic exposure to pesticides and biological factors, such as the spread of pathogens or the introduction of invasive species (Goulson, 2019; Sánchez-Bayo & Wyckhuys, 2019; Wagner, 2020). Despite the increasing data availability and awareness, there are still several geographic and taxonomic gaps as most of the studies are conducted in North America or part of Europe

(Montgomery et al., 2020) or focused on a few taxa such as the Lepidoptera and Hymenoptera which also resulted the most declining (Sánchez-Bayo & Wyckhuys, 2019).

The bees (Hymenoptera Anthophila) are a charismatic insect group (Sumner et al., 2018) and due to their pivotal role in the pollination ecosystem service and crop production are largely studied worldwide (Hristov et al., 2020; Ollerton, 2017; Simpson et al., 2022; van der Sluijs & Vaage, 2016; Weissmann et al., 2021). Bee populations are globally in danger due to land-use and climate changes, impact of alien species, pollution and pesticide use that may simultaneously and synergistically act (Goulson et al., 2008; Potts et al., 2010; Zattara & Aizen, 2021). However, most of the studies on wild bees species are focused on *Bombus* species (Goulson et al., 2008; Kosior et al., 2007) and on specific regions or country such as United Kingdom (Potts et al., 2010). Despite Hymenoptera being the most studied pollinators, there are still several local knowledge gaps, especially for solitary species, due to the fragmentary nature of data and to the lack of standardised and generalised monitoring protocols (Potts et al., 2010).

For more than half of the European bee species there are no available data to estimate their extinction risk using the IUCN criteria (Nieto et al., 2014). The lack of historical data on species occurrence makes virtually impossible to track local extinctions happened in the past also hindering our ability to understand the present faunal composition and predicting the future ones (Bartomeus and Dicks, 2019; van Tongeren *et al.*, 2023; Cornalba *et al.*, 2024; Ghisbain *et al.*, 2024). Incrementing the knowledge about pollinator species diversity in poorly studied area is thus a crucial step to promote evidence-based, targeted conservation actions and policies (deMaynadier et al., 2024). Due to logistical reasons -such as limited funds and time- pollinator surveys and conservation actions need to be optimised by promoting the protection of as many species as possible while minimising the efforts. The prioritisation is essential for biological conservation studies being a foundational element of the definition of "biodiversity hotspots" (Myers et al., 2000). While most of the effort is usually focused on assessing the most obvious layer of biodiversity, i.e. presence and

abundance of species, it is worth remembering that biodiversity is not only composed by a list of species, their abundance, uniqueness or changes in space and time (McGill et al., 2015) but it encompasses a broader range of diversity, including genetic diversity (at individual, sub-populational and populational levels), functional traits diversity, interspecific interactions biodiversity and habitat diversity (Russell & Kueffer, 2019; Thompson, 1997).

1.2 Islands: extremely valuable biodiversity at high risk

Islands provide a great and fascinating opportunity to study and protect insect biodiversity (Warren et al., 2015; Whittaker et al., 2017) in addition to representing priority targets for conservation due to their high levels of endemism (Kier et al., 2009; New, 2008). Indeed, the high richness of endemic taxa, the historical and current phenomena of colonisation and extinction, the *in situ* speciation and the occurrence of relict species (or genetic lineages) usually characterise island biodiversity creating unique faunal compositions (Cronk, 1997; Dapporto et al., 2017; Hausdorf & Hennig, 2005; McCulloch & Waters, 2019). Islands' contribution to global biodiversity is thus disproportionately high per unit area although in many cases their richness tend to be lower than similar size mainland areas due impoverishment and disharmony (Russell & Kueffer, 2019; Whittaker & Fernandez-Palacios, 2007). Due to their small size and confined habitats, islands are more vulnerable to external stressors and disturbances such as climate change, habitat destruction and introduction of non native species (Graham et al., 2017; Russell & Kueffer, 2019). Small and isolated populations tend to be at a higher risk of extinction (Purvis et al., 2000) than big and connected ones. As a consequence, many insular pollinator populations are threatened, as documented by the many extinctions recorded in Pacific Islands (Cox & Elmqvist, 2000), primarily due to the introduction of non-native pollinators and plants. In fact, the robustness of pollination networks (plant-pollinator assemblies) on islands is lower than on continental lands (Liu et al., 2021) highlighting the increased susceptibility of these environments to the introduction of non native pollinators (Dupont et al., 2004; Goulson & Hanley, 2004; Kato et al., 1999).

Although biodiversity protection should be pursued not only considering the economic and ecosystemic services it can provide but also -and mostly- for its own sake (Gowdy, 1997) some prioritisations among certain populations are needed. Each island has unique features such as size, isolation, habitats, species and genetic lineages, accounting for an irreplaceable and thus extremely valuable fraction of global biodiversity. Extinctions occurring on islands could hardly be compensated by a new colonisation as the arrival of new individuals depends on the isolation of the island from the nearest source population and on its dispersal ability. Moreover, if the causes of the previous extinctions persist and are not removed or mitigated, the new colonisation will very likely result in a failure.

To address the pollinator losses in such precious and fragile environments and to promote evidence-based conservation actions is fundamental to go beyond the application of monitoring protocols and creation of checklists. These can only provide correlative data of an eventual decline without deepening its causes. For these reason, extensive field research and threat-focused experimental frameworks are needed to effectively protect pollinators on small islands (Weisser et al., 2023).

In this optic, for my PhD project I focused on the poorly studied Giannutri Island (within the Tuscan Archipelago, Italy) and pursued several lines of research: I investigate the pollinator fauna of the island (Chapter 2 and 3); I studied in detail, morphologically and genetically (DNA-barcoding), one Tuscan Archipelago endemic taxa occurring on the island (Chapter 4). I applied and validated a method for the estimation of the population size of the two most abundant wild bee species (Figure 1B,C)(*Anthophora dispar* Lepetelier, 1841 and *Bombus terrestris* (Linnaeus 1758)) in Chapter 5; and finally I experimentally studied one of the main threat for the wild bees of the island, the exploitative competition with honey bees (Chapter 6).

1.3 Giannutri Island within the Tuscan Archipelago

The Tuscan Archipelago, in Italy, is protected under the Tuscan Archipelago National Park and is composed by seven islands: Elba, Giglio, Capraia, Montecristo, Pianosa, Giannutri and Gorgona (reported according to the

decreasing island area order). They have different geomorphological histories, environmental peculiarities and human disturbance levels (Dapporto et al., 2017). The Tuscan Archipelago insect biodiversity has been investigated for several taxa (i.e. acquatic beetles on Elba, Capraia, Giglio, Montecristo (Rocchi et al., 2017), Cerambycoidea on all islands (Ceccolini et al., 2012), Curculionoidea on all islands (Forbicioni, Abbazzi, et al., 2019), Pompilidae on all islands except for Giannutri (Dapporto et al., 2006), Chrysididae on all islands (Dapporto et al., 2006; Fattorini, 2009) and Papilionoidea on all the islands (Dapporto et al., 2017; Dapporto & Cini, 2007)) and a recent meta-analysis of these studies revealed that the main drivers of species richness are island area, distance from mainland and habitat diversity (Ruzzier et al., 2021). During the sea regression of the Pleistocene at least Elba, Pianosa and Giannutri were connected to the mainland (Bossio et al., 2000) creating a mosaic of different past and present geographical influences. The faunal composition of the Tuscan Archipelago is influenced by its past mainland connections (Fattorini, 2009) and by its actual geography due to its position between Sardinia and Corsica at West and the mainland Italy at East (Dapporto & Cini, 2007). This results in a unique composition of species and endemism making it an insular hotspot for butterfly diversity (Dapporto et al., 2017). The main pollinator fauna diversity of the Tuscan Archipelago, namely bees (Hymenoptera: Apoidea: Anthophila), hoverflies (Diptera: Syrphidae) and butterflies (Lepidoptera: Papilionoidea) is still severely understudied, hindering the possibility of effective and targeted conservation studies. In particular, the distribution of wild bees within the Tuscan Archipelago has been studied on few islands and for few groups (Filippi & Strumia, 2019; Forbicioni, Filippi, et al., 2019; Rasmont & Quaranta, 1997; Vicidomini, 2003). Giannutri has been ignored by these studies and there were no knowledge of the bee species of the island until 2021, when a first list of species, based on a single season of sampling, was produced (Cini et al., 2022). I participated to the field work and the writing of this study which was published at the beginning of my PhD period and it thus represents a starting point of this thesis (it will be reported and further described in the next sections). The knowledge of the hoverflies is even more scarce as there are no dedicated studies for the entire Archipelago. Lepidoptera are the most studied pollinators

in the archipelago (Balletto et al. 2007; Dapporto & Strumia 2008; Dapporto et al. 2017). However, a dedicated checklist for Giannutri Island is lacking and the existing information are limited to sporadic reports (Balletto et al. 2007; Ruffo & Stoch 2007; Dapporto & Cini 2007; Dapporto et al. 2017). Overall, Giannutri appear to be the less studied island of the Tuscan Archipelago.

Giannutri island is the southernmost, easternmost and the second smallest island of the Tuscan Archipelago with an area of 2.6 km². It is located at about 11.5 km from the mainland Tuscany (Mount Argentario) and approximately 15 km from Giglio Island (the nearest island). It is a limestone island with a maximum elevation of 89.4 m above sea level. It is characterised by a typical Mediterranean vegetation with a mosaic of high maquis, low maquis and *Juniperus*-thickets with a predominance of *Teucrium fruticans* L., 1753, *Salvia rosmarinus* Spenn., 1835, *Euphorbia dendroides* L., 1753 and *Juniperus turbinata* Guss., and patches of woodland of *Quercus ilex* L., 1753 and *Phillyrea latifolia* L., 1753 (Foggi et al., 2011)(Figure 1A). In the recent decades the high maquis and the *Juniperus*-thickets have increased their distribution on the island at the expense of the low maquis (Foggi et al., 2015). Despite being a private island, which is likely one of the reasons of the under sampling (Dapporto et al., 2006; Dapporto & Cini, 2007), it is entirely protected within the Tuscan Archipelago National Park with the southern part being an integral reserve. It is part of the Natura 2000 network, recognized as a Site of Community Importance (SCI) under the Habitat Directive and a Special Protection Area (SPA) under the Birds Directive.

Figure 1. A) Typical Mediterranean maquis on Giannutri Island, dominated by *Teucrium fruticans*, *Salvia rosmarinus* and *Euphorbia dendroides*. B) One of the target species of the studies described in this thesis: *Bombus terrestris*. On the left a female foraging on *S. rosmarinus* while on the right a female foraging on *Borago officinalis*. C) The other target species of the studies described in this thesis: *Anthophora dispar.* On the left a female foraging on *Teucrium fruticans* while on the right a male foraging on the same plant species.

1.4 Threats to the pollinators of Giannutri

Giannutri Island has a very low human disturbance level as most of the buildings are concentrated in a small village with a few other houses scattered in the central part of the island. There are no agricultural activities and from autumn to spring there are about 10 inhabitants on the island (inhabitants personal communication). As a consequence, the anthropogenic impact on the island appears to be limited. However, the pollinator community of Giannutri may be subjected to three main threats: climate change, the temporally and spatially localised application of insecticide, and the local introduction of 18 hives of *A. mellifera* for selection purposes. Climate change is widely recognised as one of the main drivers of pollinator decline (Castro et al., 2023; Potts et al., 2010; Zattara & Aizen, 2021). The Mediterranean region is an hotspot of climate

changes with several predictions envisaging for the next decades an increase in temperatures and drought periods (Cos et al., 2022; Dai, 2013; Drobinski et al., 2020; Giorgi & Lionello, 2008). Climate change can reduce flower nectar and pollen production (Rosenberger et al., 2024; Takkis et al., 2015, 2018; Waser & Price, 2016) and can thus affect wild bees foraging efficiency, mortality and/or mating performances (Hemberger et al., 2023; Martinet et al., 2021; Melone et al., 2024). The climate change threat cannot be locally removed from Giannutri Island (as it involves global dynamics) and the only feasible action is to mitigate its detrimental effects. This can be achieved by promoting native vegetation and pollinator community survival and by removing other "manageable" threats that may make the local pollinator populations even more susceptible to climate change.

The application of the insecticide (Flytrin 6.14, a water solution of permethrin, tetramethrin and piperonyl butoxide) to control the mosquito (Diptera: Culicidae) population of the island may represent an additional threat to the local pollinators. These substances are known to increase mortality on wild bees in agricultural landscapes where they are subjected to chronic exposures (Hagler et al., 1989). The treatment with the insecticide on Giannutri island is limited to the urbanised area which encompasses a restricted portion of the island and it is sprayed only on a few days during summer, when most of the wild bees species are absent (as adults) or aestivating (see Chapter 5 and 6 for information on the phenology of *An. dispar* and *B. terrestris*). These factors can mitigate the potential negative effects of the insecticide on the wild bees. However, future targeted studies are needed to ascertain the potential impact of these treatment on the wild pollinator fauna of Giannutri Island.

The presence of apicultural activities, with the National Park authorisation, during the period December-June, since 2018, has recently raised concerns leading also to this PhD project. Indeed, the exploitative competition between honey bees (*Apis mellifera* L. 1753) and wild bees has emerged as a threat to the latter (Iwasaki & Hogendoorn, 2022; Mallinger et al., 2017; Paini, 2004; Wojcik et al., 2018). Due to their density and foraging behaviour, honey bees can consume a great amount of resources reducing thus the nectar and pollen

available for other pollinators (Dupont et al., 2004; MacInnis & Forrest, 2019; Page & Williams, 2023; Torné-Noguera et al., 2016). Accordingly, wild bees can be competitively excluded from access to high-quality resources by a high density of honey bees (Angelella et al., 2021; Lindström et al., 2016; Ropars et al., 2020; Wignall et al., 2020). The foraging behaviour, and consequently the energetic budget, of wild bees can be altered by *A. mellifera* through changes in flower visitation rate (Herbertsson et al., 2016; Lázaro et al., 2021; Su et al., 2022; Walther-Hellwig et al., 2006; Weaver et al., 2022), time and ability to exploit flowers (Gross, 2001; Henry & Rodet, 2018; Thomson, 2004) or by shifts in their dietary choices (Magrach et al., 2017; Valido et al., 2019). As a result of intense competition, honey bees can reduce the reproductive output of both solitary (Paini and Roberts, 2005; Hudewenz and Klein, 2015) and social wild bees (Thomson, 2004; Elbgami et al., 2014), as well as their adult body size (Goulson and Sparrow, 2009). The first prerequisite for exploitative competition between managed honey bees and wild bees is the resource overlap: the higher the proportion of shared resources the higher the potential intensity of exploitative competition. The extent of resource overlap, and consequently the competition strength, is context-dependent and is influenced by several factors. The functional traits of wild bees and plants play an important role in shaping the extent of resource overlap (Cappellari et al., 2022). Accordingly, wild bee species with a higher trait similarity with honey bees are more likely to forage on the same flowers as *A. mellifera*. Among the functional traits, the length of the tongue, the body size and behaviour as central-place foragers may determine a higher probability of resource overlap with managed honey bees (higher tendency and ability to consume the same flowers) (Cappellari et al., 2022; Walther-Hellwig et al., 2006). Moreover, also the functional and taxonomic diversity of plants represents a key factor in determining the probability of resource overlap. A lower plant diversity may lead to a flowering assemblage more homogeneous with similar accessibility and attractivity features. This could force different pollinators to exploit the same resources (Cappellari et al., 2022). In addition, *A. mellifera*, by switching its feeding mechanisms, can efficiently visit a wide spectrum of flowering species enhancing the potential for resource overlap between wild bees (Wei et al., 2023). Accordingly, homogeneous

landscapes, which tend to host a lower environmental diversity and flowering resources, are known to exacerbate the intensity and impacts of exploitative competition (Herbertsson et al., 2016). For these reasons, the wild bee communities of urban areas (MacInnis & Forrest, 2019; Renner et al., 2021) and islands (Dupont et al., 2004; Kato et al., 1999; Lázaro et al., 2021) are particularly susceptible to the presence of highly competitive pollinators.

One of the main limitations of the studies identifying the exploitative competition as a driver of fitness reduction is that most of them provide only correlative data lacking of direct and manipulative approaches (Iwasaki & Hogendoorn, 2022; Wojcik et al., 2018). In fact, as delineated by Paini (2004), the demonstration of the negative impacts of beekeeping requires documenting i) resource overlap between honey bees and wild bees, ii) wild bee reduced visitation rate, iii) reduced resource harvesting by wild bees, and iv) at least one between reduction in survival, fecundity and/or population size. Providing evidence of all these steps is extremely challenging as *A. mellifera* is an ubiquitous species and disentangling its effects from environmental effects is possible only in exceptional circumstances through the adoption of specific experimental approaches. Also documenting reduced survival and fertility is challenging, as they involve invasive techniques and are not always feasible in natural environments, especially in protected areas (Hudewenz & Klein, 2013; Wojcik et al., 2018). Documenting reduction in population size requires to monitor target populations for several years in addition to experimental frameworks to ascertain the role of *A. mellifera* in the observed population abundance dynamics. Giannutri island, due to its isolation and confined environments, the absence of wild or other managed colonies of *A. mellifera* and the possibility to close the hives on selected days (in accordance and with the help of the beekeepers) to measure the effect of the presence or absence of honey bees on wild bees, represented a perfect open air laboratory to experimentally study the effect of trophic competition on wild bees. The presence of 18 hives with a density of approximately 7 hives/km^2, almost the double of the average 4.2 colonies/km² density for Europe (Chauzat et al., 2013), represents a potential threat that has been thoroughly studied by my PhD project.

1.5 Thesis outlines

I faced the objectives of this thesis trough five main research lines, following the logical steps needed to investigate the pollinator diversity of an unstudied area and to assess a local threat. Each point represents a chapter of the thesis and it is reported as a scientific paper (one of them has been published, two have been accepter for publication and 2 are submitted)

- **Chapter 2. Simple and informative: applying a basic Anthophila monitoring scheme in a simplified insular ecosystem.** The work presented in this chapter has been already published (Cini et al., 2022). I participated to the field work and most of the writing prior my PhD project as I was working as early-stage researcher in the research group. During my PhD, I completed the final stages of writing and revisions of this paper, leading to its publication. The text has been formatted in concordance with style of this thesis but the content of the paper has not been modified. This chapter is of crucial importance as it represents the first standardised investigation of the Anthophila (bees) community of the island with the monitoring transect method designed by Italian Institute for Environmental Protection and Research (ISPRA) to monitor biodiversity in National Park in Italy (Bonelli et al., 2020; D'Antoni et al., 2020). It also acted as a pilot study to design the experimental framework used to test the effects of competition.
- **Chapter 3. Bees, hoverflies and butterflies of Giannutri Island (Tuscan Archipelago, Italy), with the first record of *Eumerus narcissi* Smith, 1928 (Syrphidae) for Italy (Hymenoptera: Apoidea: Anthophila, Diptera: Syrphidae, Lepidoptera: Papilionoidea).** This paper has been accepted for publication in *Journal of Insect Biodiversity*. It provides a comprehensive checklist of bees, hoverflies and butterflies of the island incrementing the knowledge about these pollinators. It is based on the preliminary data collected during few days of field work of the previous chapter and on the three years of monitoring of the island during my PhD project, with the addition of some literature data. Through a four-year sampling we documented the presence of 6 new species for Giannutri

and 1 new species for the Tuscan Archipelago for bees, 14 new hoverflies for Giannutri, 3 new for the Tuscan Archipelago and 1 new for Italy and 10 new records of butterflies for Giannutri.

- **Chapter 4. Colour pattern and DNA-barcoding reveal a wide distribution of the insular endemic bumble bee Bombus xanthopus in the Tuscan Archipelago.** In this paper, that has been submitted to *The European Zoological Journal*, we investigated one of the valuable taxa described in the previous chapter, the endemic *Bombus xanthopus* (Kriechbaumer, 1870). This specie is endemic to Corsica and the Tuscan Archipelago and was previously known for the Island of Elba and Capraia in which it hybridises with *Bombus terrestris* producing individuals of intermediate colour pattern. We investigated the distribution of this endemic species in the Tuscan Archipelago along with the occurrence of intermediate coloured specimens (potentially hybrids) and we documented their presence also for Gorgona, Pianosa, Montecristo and Giannutri. We also analysed the DNA-barcoding pattern of these individuals to better characterise this endemism and unveil potential hybridisation or introgression patterns.
- **Chapter 5. Estimating wild bee population size with validated distance sampling.** In this paper, submitted to *Conservation Biology*, we applied and validated a monitoring method rarely used with insects, the Distance sampling. The qualitative characterisation of the pollinator fauna of Giannutri needs to be followed by a quantitative assessment of its principal components. Indeed, while the walking transect monitoring method provides data on the number of individuals counted over the years, it does not allow to estimate the size of insect populations. The distance sampling method we validate instead keeps into account the detectability at different distances of the individuals counted during a transect walk and thus makes it possible to estimate their density and thus the total number in the area. The presence of a known number of hives (18) on a confined ecosystems allowed us to validate this method by comparing the estimated population size of *A. mellifera* with its reference size (obtained through an independent method). The reliability revealed for *A. mellifera* allowed us

also to estimate the population size of the two most abundant wild bee species of the island: *Anthophora dispar* and *Bombus terrestris*. Our validation establishes this method as a powerful, easy and fast appliable tool for conservation studies, promoting its long-term applicability across diverse regions to advance understanding and effectively assess wild bee populations.

- **Chapter 6. Island-wide removal of honey bees reveals exploitative trophic competition with strongly declining wild bee populations.** This paper has been accepted for publication in *Current Biology*. After quantitively assessing the local population of wild bees it is important to experimentally evaluate the most important (and manageable) threat they are facing. The effects of honey bee closure on i) the pollinator visitation-networks, ii) nectar and pollen availability and iii) on the foraging activity and iv) behaviour of the target species of the study (the same assessed in the previous chapter: *An. dispar* and *B. terrestris*) was experimentally evaluated for three years. In addition, for four years (starting from the data collected and discussed in the first chapter) I monitored the abundance of the target wild bees using it as a proxy for their fitness over time. The results of this study, which adopted an innovative and seldom used experimental approach unveil the negative effect of honey bees in this small island incrementing the global knowledge on this timely topic In addition, it provides useful information for evidence-based conservation actions on a fragile and extremely valuable ecosystem.

All these chapters are formatted as stand-alone papers written for publication. As a consequence, there is an inevitable degree of repetition among them. Each chapter is provided with a stand-alone references section and an independent numbering of figure and tables.

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Chapter 2. Simple and informative: applying a basic Anthophila monitoring scheme in a simplified insular ecosystem

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2.1 Abstract

The decline of pollinators and the consequent decay of pollination services call for the establishment of monitoring schemes for several groups of pollinators. For Anthophila (Hymenoptera), the design of monitoring schemes is still under development. The main difficulties lie in combining a reliable but field-feasible taxonomic identification with the collection of informative data about the consistency and functional role of pollinator populations. Here we report on the application of the Italian monitoring scheme for pollinators recently defined by ISPRA and the University of Turin in agreement with the European Pollinators Monitoring Scheme on the small island of Giannutri (Tuscany), a simplified insular ecosystem with a virtually unknown pollinator community. This island has recently experienced a drastic change in its bee community, as since 2018 honey bee (*Apis mellifera* L.) hives are regularly moved every year to the island for breeding purposes. In the spring 2021 we established six 250 m long fixed transects and performed a total of 48 surveys (8 for each transect), recording more than 2300 observations of 9 Anthophila bee taxa and the flowers they visited. By using generalised additive mixed models, we showed that the monitoring protocol has a good potential for monitoring Anthophila, as we could verify several expected relationships between Anthophila abundance and abiotic factors (season, hour of the day, distance from the apiary) and biotic factors (abundance of flower resources). More importantly, we verified that A. mellifera represents by far the most frequent Anthophila taxon. Our data do not show evidence for spatial partition between *A. mellifera* and the other most frequent taxa (*Bombus terrestris* L. and *Anthophora spp*.). The visit network based on transect observations also showed that these taxa largely overlapped in terms of visits to flower resources. Overall, our data showed that the monitoring protocol allows gathering informative data about Anthophila taxa abundance, interactions and flower-visits. Moreover, the spatial and flower-visit overlap suggest potential for competition between honey bees and wild pollinators, with a potential consequent resource depletion for the latter. While this hypothesis could only be assessed by a long-term monitoring and ad hoc honey bee removal

experiments, our data show that this basic monitoring protocol produces rapid and valuable information about Anthophila community and dynamics.

Key words: Apoidea, Anthophila, pollination, pollinators, walking transects, honey bee, bumblebee, Anthophora.

2.2 Introduction

Animal-mediated pollination is crucial to ecosystem functioning and it is a key to global crop productivity and access to food (Potts et al., 2016), with an estimate of around 90% of wild flowering plants and 75% of food crops which rely on animal pollination (Halvorson et al., 2021; Ollerton, 2017). Bees (Anthophila) play a major role in providing pollination services (Ollerton, 2017), with honey bees (*Apis mellifera* L. in particular), bumblebees and many wild bees having dominant positions in the plant-pollinator networks all over the world (Carré et al., 2009; Carreck & Neumann, 2010; Klein et al., 2017; Ollerton, 2017). As many other insect taxa, a significant fraction of Apoidea are experiencing a drastic decrease, at local, regional and global scales, in both diversity and abundance (Ollerton, 2017; Sánchez-Bayo & Wyckhuys, 2019), which often lead to local or global extinctions (Kosior et al., 2007; Martins & Melo, 2010).

The understanding of what we are losing, where it is happening, and the causes of these declines is instrumental to mitigate this widespread and fast-paced loss. This is especially true at local scales and in small, fragile environments, where limited resources sustain reduced pollinator populations, which are thus particularly prone to resource depletion and local extinction (Cox & Elmqvist, 2000; Liu et al., 2021). Establishing a long-term monitoring system is the first step to provide information on trends of species diversity, abundance and biotic interactions over large temporal and spatial scales, retaining enough resolution on both scales in order to be useful to catch the local dynamics (Lebuhn et al., 2013; O'Connor et al., 2019; Ollerton, 2017; Westphal et al., 2008). Based on these data, *ad hoc* experimental studies can then be

implemented to identify and mitigate the drivers of the observed declines in bee communities.

There are many existing monitoring methods so far developed, such as pan traps, observation plots and walking transects (for methodological reviews and comparisons see for example Breeze et al., 2021; Garratt et al., 2019; O'Connor et al., 2019). These methods have different efficacy for monitoring the many aspects of pollinator biodiversity. For example, pan traps provide species resolution data independently from the taxonomic expertise of the operator deploying the traps, but they might be biased in attractiveness to different species and might miss information about the relationships with flowering vegetation. Observational methods, such as observation plots or transects, strongly depend on observer expertise (both for bee detection and species recognition) but have the advantage of allowing the characterisation of plant-pollinator interactions and the identification of which insect species (or taxa, more widely) are delivering pollination service to crops and/or wildflowers (Gibbs et al., 2017; Giovanetti et al., 2021; Kleijn et al., 2015). Walking transects can be seen as a good compromise, as they provide reliable information on species community (P. Dennis et al., 2009; Nielsen et al., 2011; Westphal et al., 2008), despite less than pan traps. They also provide information on plant-pollinator interactions (O'Connor et al., 2019; Giovanetti et al., 2021), even if are less reliable than observation plots. Notwithstanding the wealth of research into monitoring methods for bee pollinators, the adoption of national scale monitoring schemes using repeatable and standardised survey methods (Dicks et al., 2016) is still lacking in most countries (see (Potts et al., 2021; Powney et al., 2019) for few exceptions). As a result, contrary to what is happening for other pollinators such as butterflies (e.g. the Butterfly Monitoring Scheme project, eBMS), we sorely lack standardised, long-term and large-scale data for bees (Lebuhn et al., 2013), cohesively collected across a large spatial scale, which could inform national and international policy needs (O'Connor et al., 2019). This paper examines the implementation of the monitoring scheme based on fixed walking transects recently proposed in Italy by the Institute for Environmental Protection and Research (ISPRA), University of Turin in collaboration with other institutions

(http://www.parcocirceo.it/albOnline/2020/PNCIRdocu- mento53301-allegato3.pdf), according to the European Pollinator Monitoring Scheme (EU-PoMS) (Potts et al., 2021).

Italy hosts about 1000 Anthophila bee species (Pagliano, 1995) and it is one of the European countries which most depend from insect (and particularly bee) pollination services for its agricultural production (Lautenbach et al., 2012; Leonhardt et al., 2013). In order to implement the 2018 European Pollinators Initiative (https://ec.europa.eu/environment/nature/conservation/species/pollinators/policy_en.htm) and to collect data about the presence and abundance of pollinators, their threats and their possible decline, the Italian Ministry of the Ecological Transition has funded monitoring projects in all the Italian National Parks. Within this project, ISPRA, in collaboration with the University of Turin and other institutions, recently proposed a monitoring scheme comprising two different protocols for Papilionoidea and Anthophila to be adopted at the country level (Bonelli et al., 2020; D'Antoni et al., 2020). The protocol for Anthophila (hereafter called "the monitoring protocol") is based on standard-fixed walking transects of 250 metres (explained in detail in the material and method section) and it has the main advantages of being simple, replicable, and to adapt to the observer taxonomic expertise. Indeed, identification at the species level is required for honey bees and bumblebees as adopted in other European monitoring schemes (Potts et al., 2021, pages 73-74). As a further source of information, any other observed Anthophila individual has been recorded with the highest possible taxonomic resolution given the observer expertise. While the difficulty in identifying Anthophila in the field can clearly come with some limitations in the information gathered (in particular the limited taxonomic resolution) this method has a great potential to be widely adopted as the standardised monitoring scheme in the country, and, at least for bumblebees, to be completely in line with most existing monitoring schemes. Finally, this method can represent a complementary tool to more detailed studies with higher taxonomic resolution.

With the aim of boosting the adoption of a simplified standardised bee monitoring scheme in Italy, we here perform a first assessment of the

informative value of the monitoring protocol in a simplified ecosystem within a National Park, in order to assess the pros and cons of this monitoring scheme. For several reasons, we focused on the simplified ecosystem on the small island of Giannutri, which is part of the Tuscan Archipelago National Park. Firstly, a simplified insular ecosystem harbouring a limited set of pollinator and plant species represents a useful model where to test specific predictions that might be difficult to highlight in complex and well-connected ecosystems. Secondly, insular ecosystems are of huge ecological, biogeographical and conservation interest (Whittaker et al., 2017), often hosting a disproportionate fraction of biodiversity (including many endemic taxa) which, due to isolation and limited island size, is often more at risk than continental one (Manes et al., 2021). Indeed, the Tuscan Archipelago hosts a rich and peculiar invertebrate fauna with many endemic lineages, as a result of both historical and contemporary ecological as well as geographic determinants (Barbato et al., 2018; Dapporto et al., 2017; Dapporto & Cini, 2007; Fattorini, 2009; Ruzzier et al., 2021). Moreover, Giannutri island has recently experienced a drastic change in its bee community, as since 2018 several honey bee hives are regularly moved every year to the island from winter to the end of the spring season for breeding purposes. Managed honey bees can have negative effects on native bee fauna (Mallinger et al., 2017), especially in small and isolated ecosystems, as recently shown in the Aegean Archipelago (Lázaro et al., 2021). Giannutri island represents a promising case study where to assess the monitoring protocol in a context of potential competitive dynamics at the very beginning of the managed-wild bees interaction process. Finally, the knowledge of bee fauna and its pollinating activity is virtually absent, which allows us to test to what extent the monitoring protocol provides significant information about potential plant-pollinators relationships.

In particular, we here aim at: (1) verifying that the monitoring protocol allows to recover known relationships between ecological features and pollinator abundance, by capitalising on the wide knowledge about many aspects of pollinators ecology. If the monitoring protocol is informative, predictions based on known extant relationships should be met by our data; (2) identifying the pattern of spatial and functional overlap among local pollinators, which could

suggest potential insect-insect interaction dynamics, such as competition for floral resources, a known process driving pollinator guild spatial partitioning (e.g Jeavons et al., 2020; Wojcik et al., 2018) and finally (3) inferring the most important floral resources for the pollinator guild in this insular ecosystem.

2.3 Materials and method

The island

The study was carried out on Giannutri island (42°15'14"N 11°06'13"E), the southernmost and the easternmost island of the Tuscan Archipelago in the Tyrrhenian Sea, Italy (Figure 1a). It is a calcareous island placed at 11.5 kilometres from the coast (Mount Argentario), it has a coastal perimeter of 11 km, a length of about 3 km, a width of about 500 m, an area of 2.6 km^2 and a maximum altitude of 89.4 m (Poggio di Capel Rosso). The entire island is within the Tuscan Archipelago National Park, with the southern part being protected as an integral reserve. The island is also part of Natura 2000 (site code IT51A0024) as SIC (Site of Community Interest) and ZPS (Zone of Special Protection) for birds.

A diachronic analysis in the last decades (1950-2008) highlighted an increase in surface of vegetation types with greater physiognomic development (high Mediterranean maquis) to the detriment of areas with a lower level of dynamism (open meadows). The current scrubland vegetation is dominated by *Teucrium fruticans* L., *Rosmarinus officinalis* L., *Juniperus turbinata* (Gussone), *Euphorbia dendroides* L., *Erica multiflora* L. and *Pistacia lentiscus* L., in particular the coastal vegetation is dominated by *Senecio cineraria* (Chater) (Foggi et al., 2011). A single apiary is present on the island (figure 1b), consisting of 12 to 18 hives (according to the year, 18 hives in 2021) placed for the first time in 2018 in agreement with the National Park. Every year all hives are brought to the island around December and removed around mid-June.

The monitoring protocol

We performed simplified standardised transect walks following the guidelines of ISPRA (Bonelli et al., 2020; D'Antoni et al., 2020); the protocol is available

at the following link: http://www.parcocirceo.it/albOnline/2020/PNCIRdocumento53301-allegato3.pdf). These guidelines define the standard monitoring protocol for pollinators within National Parks and protected areas, which can fulfil several purposes such as evaluating the impact of phytosanitary products on agrosystems or monitoring the pollinator trends in natural and semi-natural environments (Bonelli et al., 2020; D'Antoni et al., 2020; Giovanetti et al., 2021). The sampling design is based on Westphal et al. (2008) and Nielsen et al. (2011): transects are identified as fixed walks 250 m long and 4 m wide. Each transect is divided into 10 subunits, 25 m long, to be surveyed in 5 minutes (for a total of 50 minutes). The monitoring protocol specifies to record, during the walk, the number of Anthophila bee taxa together with the flower they are visiting. Differently to the standard monitoring protocol, in order to keep the information of all observed individuals, we scored the Anthophila bees recording two different activities: flying or visiting a flower. According to the protocol, the Anthophila bee species were identified at the species level both for the European honey bee (*A. mellifera*, AM acronym) and for the genus *Bombus* (directly on the field or in the laboratory, B acronym), while all the other Anthophila bee species were classified as "Other Anthophila bee species" (AA in the Italian acronym). However, the genus and species of the Anthophila bee species can be included in the notes when identification is possible. While performing the transects we used two approaches: a) collection of specimens with a net and storage in tubes with ethyl acetate; b) recording of bees, identified at genus or species level without capture. We also performed surveys on the island besides the transects in order to collect additional specimens. All captured bees have been identified with the aid of a taxonomist (see acknowledgements) and are stored in Marco Bonifacino and Simone Flaminio collections. We did not collect every bee we observed along the transects in agreement with the indications of the Tuscan Archipelago National Park, to limit our impact on bee populations of such a small island.

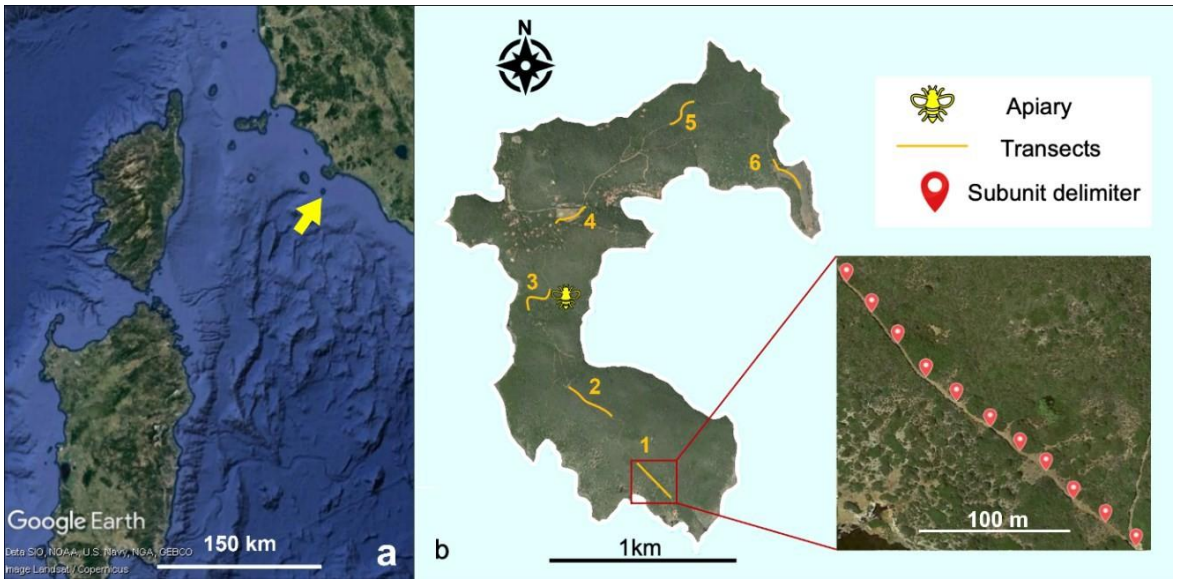

Figure 1. The location of Giannutri island (yellow arrow) in the context of the Tyrrhenian Sea **(a)**; Giannutri island with the position of the six transects and the apiary, the inset shows the details of a transect with the eleven subunit delimiters **(b)**.

According to the monitoring protocol, the flower visited by each observed insect is also recorded, at least at genus level. For each transect, the field-tables proposed by ISPRA and the University of Turin (see technical annex in D'Antoni et al., 2020) must be compiled by entering information on biotic and abiotic factors. The recorder scores a percentage of the nectariferous floral coverage representing potential resources for bees in each subunit for three vegetational levels: grassland, shrub and arboreal. The percentages are then transformed in coverage abundance classes: 0 = 0-10%; 1 = 11-30%; 2 = 31-50%; 3 = 51-100%. For the grassland level, the average height (cm) is also measured. Four parameters were recorded at the beginning and at the end of the transect: time (standard time), temperature (°C), strength of the wind (according to the range 0-5 of Beaufort scale) and cloud coverage (converted in percentage classes: 0 = 0%, 1 = 1-10%, 2 = 11-30%, 3 = 30-50%, 4 = 50-70%, 5 = 71-100%). For wind, temperature and cloud coverage the mean value is also entered.

In our study we established six transects along the island, extending from one extremity to the other: four of them (1, 2, 3, 5) are characterised by Mediterranean maquis, the remaining two (4, 6) by grassy habitats, with a

portion of transect 4 developing through an anthropised area (Figure 1b, supplemental material geographical Key-hole Markup Language). We carried out a total of three field sessions: the first took place from the 23rd to the 27th of March 2021 and it was performed by five operators (AC, FB, MB, LD, VS), the second occurred on the 14th of April 2021, performed by two operators (AC, FB) and the third was carried out by AC, GS, and LP from the 8th to the 9th of May 2021. Overall, seven operators performed the monitoring for a total of 48 samplings, 8 replications for each transect.

About 85% of the records of AA belonged to the easily identifiable *Anthophora* genus, while the remaining part could be attributed to a mix of at least 5 taxa. We thus focused our analysis on the most abundant taxa which could be unequivocally identified in the field: *A. mellifera*, *Bombus terrestris* L. and *Anthophora* spp. The only bumblebee species found on the island was *B. terrestris*. We exclude the two similar species occurring in Italy, *Bombus cryptarum* (F.) and *Bombus lucorum* (L.), on the basis of morphological analysis of collected specimens (including queens and males) and their distribution (both species are reported in upland areas of mainland Italy, (Intoppa et al., 1995)). Also, we did not record hybrids with *Bombus xanthopus* Kriechbaumer, the only species belonging to *Bombus* subgenus recorded for the Tuscan Archipelago (Forbicioni et al., 2019; Rasmont & Quaranta, 1997). Morphological examination in the laboratory indicated the presence of two *Anthophora* species, *Anthophora senescens* (Lepeletier) and *Anthophora dispar* (Lepeletier). As it was not possible to distinguish them while performing the transects, we treated these two species as a single taxon in our analysis. The results which include also the other AA species are reported in the supplementary material (supplemental material table S1, figure S1).

As cloud coverage and height of grassland level showed a very limited variance in our sampling, we did not consider these factors when deriving predictions (see below), in order to avoid lack of statistical power. Also, we did not include the effect of temperature in our predictions as it showed a very high significant correlation (multiple regression, $r^2 = 0.754$, $P < 0.001$) with a combination of day of sampling (estimate = 0.103, t = 34.941, $P < 0.001$) and time (estimate = 0.391, t = 14.44, $P < 0.001$).

Research aims

Aims 1. Verifying that the monitoring protocol allows to recover known relationships between ecological features and Anthophila abundance

We capitalise on the wide knowledge about pollinator ecology to derive specific predictions that, if the monitoring protocol is reliable, should be met by our data. We predict an influence of several time and ecological correlates on the abundance of different groups of pollinators (hour of the day, day of the year, flower resource abundance and, the distance from the nest for honey bees).

Firstly, nectariferous floral coverage, determining availability of flower resources, should clearly positively affect incidence of the three taxa. Secondly, in warm Mediterranean areas the flight period of most solitary Anthophila is narrowed to early spring (Potts et al., 2005). As we sampled from March to May, we predicted to observe a decrease in the abundance of *Anthophora* bees from the first dates of monitoring to the last ones. This pattern is not expected in the two colonial species *A. mellifera* and *B. terrestris*, which show more extended phenologies, although with differences in colony dynamics.

A. mellifera has multiannual colonies, while *B. terrestris* has annual ones, with at least two generations reported in Mediterranean areas (Rasmont et al., 2008), thus both species are expected to be observed all year round. Thirdly, the hour of the day is known to affect pollinator foraging activity, depending on many factors such as local climate, vegetational composition and species life history (Baldock et al., 2011; Herrera, 1990; Vaudo et al., 2014). Finally, the abundance of pollinators is clearly dependent on the distance from their nests. While for wild pollinators a clear relationship might be hard to find, as nesting sites are unknown and possibly dispersed, an influence of distance from the nest should be highlighted in colonial central foragers such as the managed *A. mellifera*. Knowing the exact location of the single apiary present on Giannutri, we predicted a negative effect of distance from the apiary on *A. mellifera* abundance.

Wind strength is known to affect flying insects, thus influencing the spatio-temporal heterogeneity of pollinator visitation (Crall et al., 2020). On one side wind may reduce manoeuvrability and impose energetic costs to flying insects,

on the other side wind disperses chemical cues that mediate interactions between insects and plants (Crall et al., 2017; Ravi et al., 2013). The many and contrasting effects of wind on pollinators make it difficult to unequivocally predict its effect, so we included wind in the model without any specific predictions.
Most monitoring schemes do not distinguish between individuals visiting flowers (likely flower-visiting, even if we cannot exclude the presence of male bees looking for mating opportunities) and flying individuals, providing a single count for both activities. However, we can expect the above relationships to be dependent on the different pollinator activity (flying, flower-visiting) performed by pollinators. For example, resource abundance is expected to affect more flower-visiting individuals than flying individuals ones, as pollinators can be frequent also in low flower areas, being involved in movements among sites or exploration flights. We thus performed the analyses separately for "visiting a flower" and "flying" to assess the importance of the above indicated factor on each of the two groups.

Aim 2. Assess potential spatial partitioning, i.e. the relationship between abundance of the three different groups

Spatial overlap among local pollinators is the first information needed to assess interaction dynamics, such as competition for floral resources, a known process driving pollinator guild spatial partitioning (Jeavons et al., 2020). The beehives are moved each year from late winter to summer to Giannutri and might potentially have a strong impact on native wild pollinators. While a proper assessment would require experimental manipulation of bee presence and assessment of fitness effects, walking transect monitoring scheme could provide a first important information. We thus assessed the effect of *A. mellifera* on the abundance of the two main wild pollinator taxa, *Anthophora* spp. and *B. terrestris*.

Aim 3. Identify the most important plant resources for pollinators on Giannutri

Pollinator conservation actions need to be based on evidence about pollinator ecological requirements, especially the use of floral resources. While a comprehensive knowledge can be only achieved with detailed *ad hoc* studies,

which require flower based observations and direct estimation of pollen transfer, walking transect monitoring scheme has been considered a rather efficient method for understanding plant-pollinator relationships, especially in simplified ecosystems with low pollinator richness (Hegland et al., 2010; Westphal et al., 2008). Indeed, transect data provide a rapid assessment of the main floral resources used by different pollinators, and a first estimation of flower resource partitioning among them. We thus recorded the plant taxa whose flowers were visited by the different bee taxa and drew flower-visitor networks to highlight the most used flower resources and provide a first assessment of the overlap in flower resource use among different bee taxa.

Data analysis

All analyses were performed using R version 4.0.5 using "fossil" (Vavrek, 2011), "mgcv" (Wood & Wood, 2015), and "bipartite" (Dormann et al., 2008) packages.

Aims 1 and 2. Anthophila abundance, relationships with ecological features and the potential interaction with A. mellifera

We assessed the influence of biotic and abiotic factors (see above) on the abundance of the three bee taxa with Generalised Additive Mixed Models (GAMM). We carried out separated models for each species and for the two different activities: flying and visiting a flower. As unit of analysis we considered the single replicate of each subunit of each transect, in order to account for the different ecological features within and among the transects. The count data of individuals recorded in each subunit have been modelled using the "Poisson" family. Spatial autocorrelation was considered in the model by including a covariance structure with distances based on midpoints of each subunit. We included the following predictors as smoothed terms: starting time of each transect subunit (hereafter "hour"), wind strength (hereafter "wind"), total abundance of floral coverage (hereafter "flowers"), obtained by summing, for each subunit, the scores for the three vegetation levels, day of year of the sampling (hereafter "day"), distance from the apiary (hereafter "dist"), and, for

bumblebees (B) and AA, the recorded abundance of *A. mellifera* (hereafter "AMtot"). "Dist" and "AMtot" were used to investigate a potential interaction between the wild Anthophila and *A. mellifera.* Operator identity was included as a random factor.

Aim 3. Identifying the most important plant resources for the pollinators on Giannutri

In order to provide a rapid assessment of the main floral resources visited by the different Anthophila and to detect potential resource partitioning among them, we constructed bipartite flower-visitor networks. First, we built an overall network by pooling all flower visit data from all sampling periods, then we computed individual networks for each of the three sampling periods (March, April and May) in order to detect seasonal changes in flower-visitor networks. As this paper is focused on the three main Anthophila taxa detected (*A. mellifera*, *B. terrestris*, *Anthophora* spp.), we report here networks built only using these taxa. Networks including the other bee species (not identified and thus pooled into a single group: other AA) are included in the supplemental material (table S1, figure S1).

For each network we also computed the main network and species-level indices considered to provide ecologically-relevant information about the structure and functioning of pollination networks (Prendergast & Ollerton, 2021). At the network-level we computed: network size (the sum of nodes, i.e. number of taxa of pollinators and plants), connectance (the proportion of possible links actually recorded); interaction evenness (which quantifies how balanced the distribution of interactions is across species, based on Shannon's diversity); H2 (a specialisation index, which ranges from 0-highly generalized to 1-highly specialised); nestedness (i.e. the extent to which specialised species interact with a subset of the species that also the generalists interact with; it ranges from 0 to 100, with increasing values representing an increase in nestedness); niche overlap which indicates the average similarity in interaction patterns between species of the same level (values near 0 indicate no common use of niches, while 1 indicates perfect niche overlap); robustness (which indicates the robustness of species of a given level to removal of species of the

other level; e.g. when computed for the pollinator level indicates whether, if many plant species are lost, most of the pollinators will still survive -high R-, or if even a small fraction of the plants in the network are removed many secondary extinctions of pollinators will occur -low R-, vice versa for the plant level).

At the species-level we computed: normalised degree (the sums of the links per species, scaled by the number of possible interaction targets); pollination service index (which attempts to quantify the pollination services of a given flower-visitor to all plants in the network); pushpull index (which measures dependence asymmetry and ranges from −1 to +1, with positive values indicating that a plant has a higher dependence on the flower-visitors, and negative values indicating that a flower-visitor is, on average, more dependent upon the plants); Bluthgen's d' (which measures the specialisation of each species and ranges from 0 (no specialisation) to 1 (full specialisation) based on frequency of visits). While a proper characterisation of the pollination networks requires an extensive sampling across multiple years, detailed assessment of visit duration, visitor behaviours and eventually direct measures of pollinator effectiveness (King et al., 2013), we here aim at assessing whether it is possible to gather a first understanding of flower-visitor networks using the information collected through the monitoring protocol. Index computation and network visualisation were performed using the package "bipartite".

2.4 Results

During our surveys we recorded 9 Anthophila taxa: *A. mellifera*, *A. dispar*, *A. senescens*, *B. terrestris*, *Nomada goodeniana* (Kirby), *Nomada succincta* Panzer, *Xylocopa* sp., *Lasioglossum transitorium* (Schenk) and *Andrena nigroaenea* (Kirby).

Aim 1 and Aim 2

The six GAMM analyses carried out separately for the three taxa showed that bee occurrence was correlated with several abiotic and biotic variables measured during the monitoring, in overall agreement with our predictions.

First, nectariferous floral coverage positively affected the occurrence of the three taxa (Table 1). Both flower visiting and flying honey bees and bumblebees were linearly and positively correlated with local flower abundance (honey bees: Table 1a, 1b; Figure 2a, 2f; bumblebees: Table 1c, 1d; Figure 2a, 2f), showing an average increase of about one individual ranging from flower abundance class 0 to class 5. Flower abundance also had a linear and positive correlation with *Anthophora* spp. occurrence, but the relationship was non-linear (in "flower visiting individuals'', Table 1e; Figure 2a) and linear (in "flying individuals", Table 1f; figure 2f).

Occurrence of the three taxa was also affected by the day of sampling, which showed different effects according to the taxon, differentiating in particular honey bees from both bumblebees and *Anthophora* spp. For honey bees visiting flowers, sampling day showed a significant and non-linear positive correlation, with fewer individuals at the beginning of the season (Julian day 84-85) (table 1a; figure 2b), while sampling day did not affect the occurrence of flying bees (table 1b; figure 2g). For both bumblebees and *Anthophora* spp. and in both flower-visiting and flying individuals, on the contrary, sampling day showed a non-linear negative correlation with a maximum in April and a decline towards late spring for "flying" activity (bumblebees: table 1c, 1d; figure 2b, 2g; *Anthophora* spp.: table 1e, 1f; figure 2b, 2g).

Table 1. Smoothing variables and their effect on the frequencies of the tree Anthophila groups derived by GAMM analysis. The significant variables are in bold. The biotic and abiotic variables are in white background while interplay variables are highlighted in grey.

a	*A. mellifera* visiting flowers			**c**	*B. terrestris* visiting flowers			**e**	*Anthophora* spp. visiting flowers		
	edf	F	p		edf	F	p		edf	F	p
s(flowers)	1.000	53.48	**<0.001**	**s(flowers)**	1.000	12.218	**<0.001**	**s(flowers)**	1.874	18.01	**<0.001**
s(day)	1.986	75.84	**<0.001**	**s(day)**	1.964	21.613	**<0.001**	**s(day)**	1.939	23.057	**<0.001**
s(hour)	1.684	30.87	**<0.001**	**s(hour)**	1.95	26.46	**<0.001**	**s(hour)**	1.941	8.411	**<0.001**
s(wind)	1.978	110.12	**<0.001**	s(wind)	1.000	1.668	0.197	s(wind)	1.000	0.043	0.837
s(dist)	1.996	128.19	**<0.001**	s(dist)	1.695	3.009	0.125	s(dist)	1.534	2.617	0.183
R-sq.(adj) = 0.305				**s(AMtot)**	1.000	57.161	**<0.001**	**s(AMtot)**	1.000	13.342	**<0.001**
				R-sq.(adj) = 0.17				R-sq.(adj) = 0.331			
b	*A. mellifera* flying			**d**	*B. terrestris* flying			**f**	*ophora* spp. flying		
	edf	F	p		edf	F	p		edf	F	p
s(flowers)	1.000	7.877	**0.005**	**s(flowers)**	1.000	3.932	**0.048**	**s(flowers)**	1.000	26.836	**<0.001**
s(day)	1.000	2.547	0.111	**s(day)**	1.899	17.892	**<0.001**	**s(day)**	1.907	16.648	**<0.001**
s(hour)	1.000	7.267	**0.007**	s(hour)	1.724	2.272	0.218	s(hour)	1.000	0.01	0.919
s(wind)	1.666	2.475	0.211	**s(wind)**	1.000	5.551	**0.019**	s(wind)	1.000	0.563	0.453
s(dist)	1.888	5.345	**0.018**	**s(dist)**	1.912	12.642	**<0.001**	**s(dist)**	1.871	5.881	**0.006**
R-sq.(adj) = 0.0363				**s(AMtot)**	1.000	4.824	**0.029**	s(AMtot)	1.000	0.233	0.629
				R-sq.(adj) = 0.245				R-sq.(adj) = 0.266			

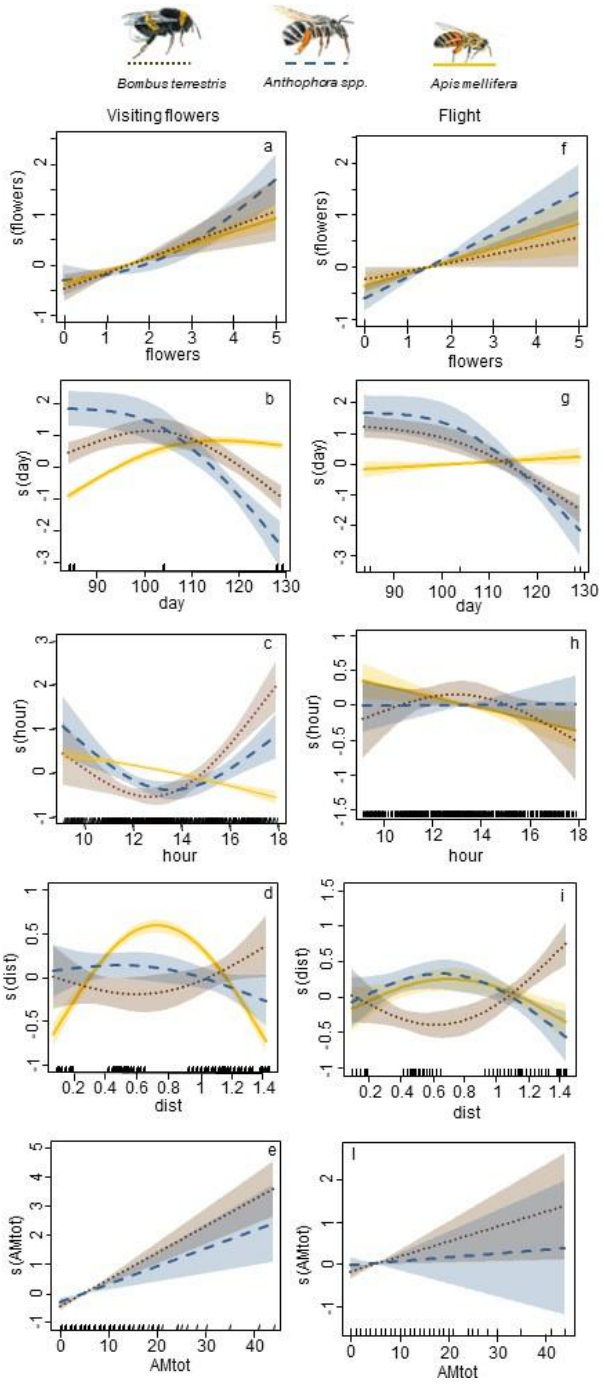

Figure 2. Smoothing curves of the abiotic and biotic variables, obtained through GAMM analysis and plotted together for the three bee taxa and divided by activity: a-e for "visiting flowers" and f-l for "flying". Yellow lines represent *A. mellifera* relations, brown lines represent *B. terrestris* relations and blue lines represent *Anthophora* spp. relations.

The hour of sampling significantly affected bee occurrence as well, even if with different patterns among the three taxa. Honey bee abundance was negatively affected, even if slightly, by the hour of sampling, with both flower-visiting and flying individuals decreasing at later hours of the day. The

relationship was nonlinear for flower-visiting individuals (Table 1a; Figure 2c) while linear for flying ones (Table 1b; Figure 2h). In bumblebees, on the contrary, the sampling hour showed a curvilinear relation with an opposite trend in the two activities: in flower-visiting individuals occurrence had a maximum value in late afternoon (6:00 pm) and a minimum in the warmer hours of the day (12:00 pm 2:00 pm) (Table 1c; Figure 2c), while in "flying" the maximum of the curve corresponded to this central moment of the day (Table 1d; Figure 2h). Occurrence of flower-visiting *Anthophora* spp. matched the pattern of bumblebees, with a curvilinear pattern with a maximum occurrence in the coolest hours of the day, early in the morning or late in the evening, and a minimum presence between 12:00 pm and 2:00 pm (Table 1e; Figure 2c). Occurrence of flying *Anthophora* spp. individuals was, on the contrary, not significantly affected by the hour of sampling, as shown by the horizontal trend, which means that the presence of flying wild bees was independent from the hour of the day (Table 1f; Figure 2h).

The distance from the apiary significantly affected bee occurrence as well, again with different patterns among taxa. In honey bees, a significant, bell-shaped relationship between distance from the apiary and occurrence revealed a maximum incidence of *A. mellifera* at a distance of 600-800 m from the apiary, for both flower-visiting and flying individuals (Table 1a, 1b; Figure 2d, 2i). The two transects located at this distance pass through an area of Mediterranean shrubs and in the area close to the village (Figure 1b), making it difficult to link this high incidence of bees with the occurrence of a peculiar environment. Bumblebees showed an opposite pattern, with a reversed bell-shaped relationship which shows a minimum occurrence at a distance of 600-800 m from the apiary. This relationship was significant for flying bumblebees while it was not for flower-visiting ones (Table 1c, 1d; Figure 2d, 2i). In *Anthophora* spp. the distance from the apiary showed a similar pattern for both flower-visiting and flying individuals: a curvilinear pattern with maximum values in the range of 700-800 m, even if this variable was not significant for flower-visiting individuals (Table 1e, 1f; Figure 2d, 2i).

Finally, our data did not provide any evidence of spatial segregation, but, rather, they showed that bumblebee and *Anthophora* spp. abundance was

overall positively correlated with honey bee presence. In both flying and flower visiting bumblebees the occurrence had a linear and positive correlation with *A. mellifera* abundance (AMtot, Figure 2e, 2l). A significant positive relationship was found for flower-visiting *Anthophora* spp. as well, with individuals increasing with the abundance of *A. mellifera* (Table 1e; Figure 2e). On the contrary, no significant relationship was found between *A. mellifera* abundance and flying *Anthophora* spp. (Table 1f; Figure 2l).

Aim 3

Flower-visitor network size (considering the visits of the three bee taxa across the whole sampling period, from March to May, pooled into a single visit network) included 20 nodes (the three bee taxa and 17 plant taxa). The network showed a high connectance (i.e. the realised proportion of possible links, c = 0.706), and moderate nestedness (NODF = 66.042), which suggests a relevant amount of overlap in visited plant taxa among the three pollinator groups. This is confirmed also by the niche overlap index (0.801), the low network specialisation (0.158) and a moderate interaction evenness (0.487) (table 2). The network appears rather robust, i.e. the extinction curve would only decrease mildly until almost all species of a given level are lost, with a moderate robustness for pollinators (0.657) (Table 2).

The network was dominated by *A. mellifera*. Honey bees were involved in 81.4% of the 2332 bee-flower interactions recorded (figure 3). Honey bees also visited a drastically greater number of plant species than both *Anthophora* spp. (16) and bumblebees (9) (table 3). The dominant role of *A. mellifera* is supported by PSI (pollination service index), higher for *A. mellifera* (0.849) than *Anthophora* spp. and bumblebees (respectively 0.170 and 0.212). While bumblebees visited a smaller number of plant species than honey bees and *Anthophora* spp., they tended to distribute their visits more equitably among the plant species (d', bumblebees: 0.236, for honey bees: 0.127, *Anthophora* spp.: 0.115).

When analysing the bee flower-visitor networks of the three different periods, the overall network characteristics remained stable, with honey bees dominating the network (Table 2). Some tendencies however emerged: the

network became smaller and less generalised, mainly due to a shrink in the importance of both *Anthophora* spp. and bumblebees, clearly paralleled by an increase in the dominance of *A. mellifera* from March to May (figure 3b and table 3). At the network level, this is confirmed by a decrease in network size and connectance and an increase in network specialisation (Table 2). At the species level this is supported by the increase and decrease in pollination service index for, respectively, *A. mellifera* and *Antophora* spp. from March to May (Table 3).

Table 2. Network-level indices providing ecologically relevant information about the structure and functioning of flower-visitor networks along the three spring months; definition of each index in material and methods section.

Measures	Total	March	April	May
Network size	20	13	13	10
Connectance	0.706	0.733	0.633	0.524
Interaction evenness	0.487	0.670	0.465	0.322
H2	0.158	0.137	0.102	0.237
Nestedness	66.042	59.821	75.000	50.000
Niche overlap	0.801	0.730	0.860	0.678
Robustness	0.657	0.676	0.654	0.531

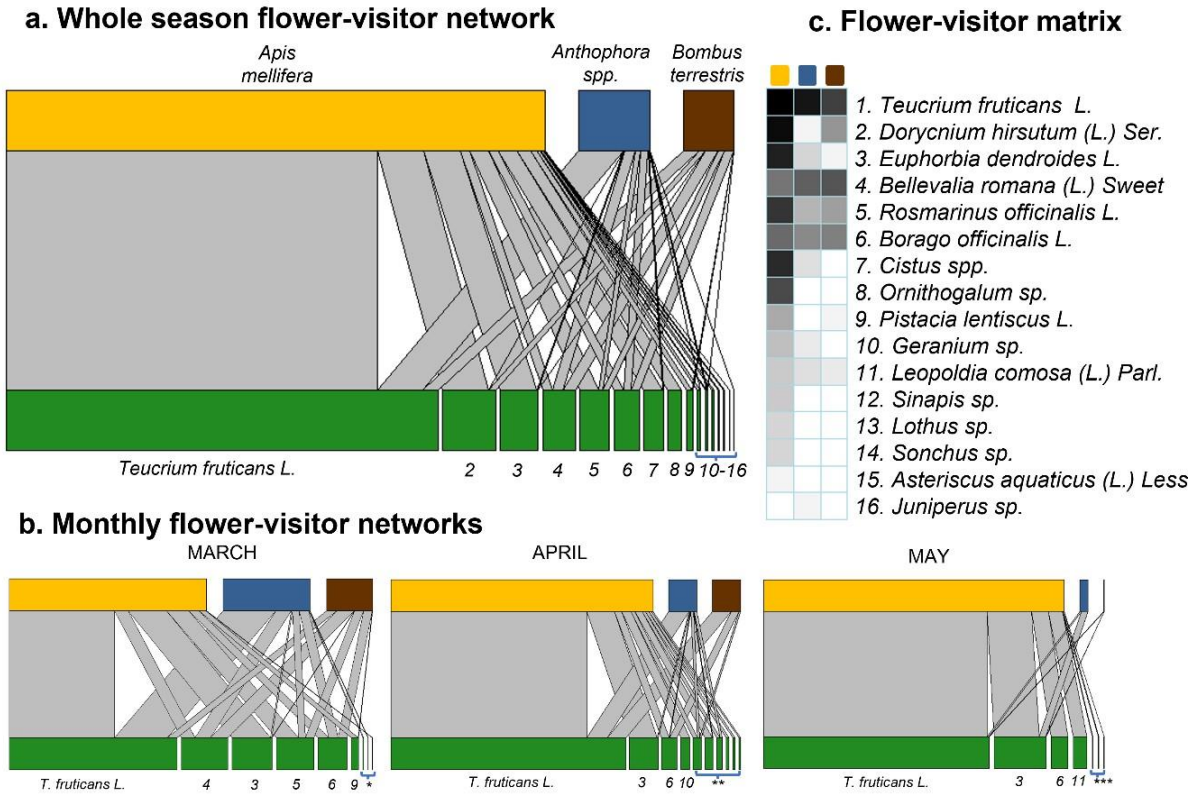

Figure 3. Flower-visitor networks on Giannutri island show the predominance of honey bees on both *Anthophora* spp. and *B. terrestris*, both considering the whole season **(a)** and each month separately **(b)**. The overall network shows a moderate nestedness **(c)** suggesting a relevant amount of overlap in visited plant taxa among the three pollinator groups; * indicates *Geranium* sp., *Lothus* sp. and *Juniperus* sp.; ** indicates *Leopoldia comosa*, *Rosmarinus officinalis*, *Cistus* sp., *Sonchus* sp., *Pistacia lentiscus* and *Sinapis* sp; *** indicates *Lotus* sp., *Sinapis* sp., *Asteriscus acquaticus* (all in order from left to right in the network).

Table 3. Individual-level indices providing ecologically-relevant information about the role of each taxon in the flower-visitor network along the three spring months; definition of each index in material and methods section.

Sampling period	Total			March			April			May		
Species	AM	ANT	B	AM	ANT	B	AM	ANT	B	AM	ANT	B
Normalised degree	0.941	0.647	0.529	0.800	0.700	0.700	1.000	0.600	0.300	1.000	0.286	0.286
Pollination service index	0.849	0.170	0.212	0.662	0.316	0.263	0.841	0.134	0.191	0.973	0.003	0.104
Push pull index	0.730	0.14	0.08	0.53	0.31	0.07	0.72	−0.00	−0.0	0.83	−0.49	−0.4

		0	7	3	5	6	5	1	82	7	6	32
Specialisation (Bluthgen's d')	0.127	0.11 5	0.23 6	0.11 0	0.10 2	0.21 0	0.05 6	0.072	0.16 9	0.04	0	0.273

2.5 Discussion

The analysis of the Anthophila community on Giannutri island revealed a strong influence of both abiotic and biotic variables on the abundance of the three main taxa, as in accordance with most of our predictions. Notably, we found no evidence for spatial and flower-resource segregations between managed *A. mellifera* and the two other wild bee taxa, *B. terrestris* and *Anthophora* spp. Indeed, the three taxa showed a great overlap in visited flower resources: *Anthophora* spp. and bumblebees showed higher abundance where also *A. mellifera* were noticeably abundant. This is not surprising since the three taxa show similar functional features (mid-large size and mid-long ligula) thus increasing the possibility for the use of similar resources. We also depicted the first flower-visitor network for the three pollinator taxa on Giannutri island, thus highlighting the most important floral resources that sustain the Anthophila community during spring. Crucially, this work provides a first assessment of the informative value of the monitoring protocol. Indeed, in this simplified insular ecosystem, the main predicted relationships between ecological features and pollinator abundance could be recorded using this monitoring protocol.

Anthophila abundance, relationships with ecological features and the potential influence of A. mellifera

As a first evidence, the abundance of the three taxa was positively influenced by the nectariferous floral coverage. While this relationship is rather predictable, not all methods are similarly able to recover it (O'Connor et al., 2019). The hour of the day influenced foraging activity in the three taxa as well. We found that *Anthophora* spp. and *B. terrestris* show a daily variation in abundance with peaks

in early morning and late afternoon and a decrease around midday. *A*. *mellifera* shows a different trend, with a linear decrease during the day, starting from a peak early in the morning, as already demonstrated (Abou-Shaara, 2014).

While honey bees can fly several kilometres away from their apiary in order to forage (Couvillon et al., 2014; Ratnieks, 2007) and could easily cover the entire surface of Giannutri island (maximum distance from the apiary about 1.5 km), at the same time many studies confirmed their preference to forage close to the beehive in order to reduce energetic costs and time-investment (Couvillon et al., 2014, 2015). This pattern is partially mirrored by our data, which depicted a bell-shaped abundance curve, with the highest abundance of *A. mellifera* at a distance about 600 metres from the apiary. As it is suggested by Thomson (2004), spatial overlap among pollinators, such as the negative correlation between honey bees and native bee abundance, could be seen as indirect evidence of competition. Concerning Giannutri, the data do not provide evidence of spatial segregation, since the abundance of honey bees is positively correlated with the abundance of the other two Anthophila taxa. Moreover, the large majority of the Anthophila we recorded belong to mid-large species with a mid to long ligula (*Apis*, *Bombus*, *Anthophora*), which increases the possibility for resource use overlap as revealed by network analysis. However, more reliable assessment of the honey bee vs local wild bees competition would require more accurate and dedicated methods, such as the observation of behavioural interaction on flower resources thanks to observation plots, hopefully coupled with the experimental introduction and removal of beehives (Shavit et al., 2009; Thomson, 2004).

Finally, the occurrence of the three taxa confidently mirrors the phenology of the investigated species. Despite our sampling effort covered just the late winter and spring months, a decrease in the abundance of *Anthophora* spp. in the last month of sampling (May) was evident, consistently with its phenology (Ballantyne et al., 2017). Also, as expected in a human-managed social species, *A. mellifera* abundance did not decrease in late spring (Ornai & Keasar, 2020). *B. terrestris* showed a decrease in its abundance from March to May. This might appear counter-intuitive for a social species, and it is in contrast with previous studies overviewed by Rasmont et al., (2008), where *B. terrestris* is found active all year with peaks in May and October. However, the decrease could be

potentially explained by a reduced survival of colonies in this wild social species (compared to the human-managed *A. mellifera*), due to the variation in food availability that could affect, and slow down, the colony cycle and by the process of aestivation, well known in insular population of bumblebees (Gurel et al., 2008). In this respect, the absence of spatial partition with honey bees and the consequent potential for competition for food resources could have exacerbated this phenomenon.

The most important plant resources for the pollinators on Giannutri

The data gathered through the monitoring protocol provided a first indication of the degree of overlap in visits to flower resources among the three taxa, as well as it allowed a rapid assessment of the main floral resources visited by the different Anthophila bees on the island. The flower-visitor network on Giannutri is overall well connected, robust and poorly specialised. The three taxa indeed showed a relevant overlap in the flower resources they visited, thus producing a rather nested network with no evidence of resource partitioning. *A. mellifera* visited the highest number of different plant taxa, while *Anthophora* spp. and *B. terrestris* visited less plant taxa (figure 3). However, the flower visit profile is rather similar between *A. mellifera* and *Anthophora* spp. when looking at the distribution of visits among plant taxa, as both strongly relied on one single species (*T. fruticans*) for almost half of their visits and then allocated their visits to the remaining flower species in a more equitable way. Contrastingly, bumblebees look less specialised on particular flower resources. Overall, the network is clearly dominated by the very abundant *A. mellifera*, on the visitor side, and by *T. fruticans*, on the plant side. The visit network is rather stable along the season, showing as a main tendency only the marked increase in dominance by *A. mellifera*, likely due to the decrease in abundance of the two other taxa.

Our results suggest no clear partitioning among the three taxa in flower visiting. The evidence presented here suggests that at the current stage there is no evidence for a spatial exclusion of a given taxon as a consequence of the presence of other taxa. Long term monitoring data would be instrumental to assess the possible decline in the abundance of a given bee taxon, thus highlighting possible negative effects of the coexistence on the island. However,

without direct manipulation of the abundance of given pollinator taxa (i.e. exclusion studies such as (Wignall et al., 2020) it would be rather difficult to assess the direction and the magnitude of the interaction among taxa.

Concerning the importance of different flower resources, our study highlighted the importance of *T. fruticans* as a main visited flower all along the spring season for all the three investigated taxa, and highlighted that other flowering species have a more time-limited influence on the visiting network, likely because of their narrower flowering seasons, (such as *E. dendroides* and *Bellevalia romana* L. in March and April, or *Dorycnium hirsutum* L. and *Cistus* sp. in later spring). Here again we highlight that the monitoring protocol provides a rapid way to gather information about the most important resources, but does not allow to infer pollinator flower preferences, as the abundance of flower resource taxa is not taken into account. A proper understanding of the nature and consequences of the interaction on both partners (plants and pollinators) will necessarily require more indepth studies (such as observation plot, measurements of pollination transfer and fitness effects), especially considering the different relative abundance and energy-reward of the flower resources.

Advantages and disadvantages of the monitoring protocol

The main advantages of the monitoring protocol are its simplicity as well as the large amount of ecological information gathered. These transects are fast to be set up and could be performed by non-expert people with a minimum of training, as the protocol does not necessarily require extensive knowledge in recognising Anthophila species. Given the rising engagement of the public in pollinator-correlated citizen science initiatives (Domroese & Johnson, 2017; Koffler et al., 2021), the use of the monitoring protocol could provide an unprecedented contribution to the Anthophila community monitoring (Fontaine et al., 2021; Mason & Arathi, 2019), similarly to the case of the eBMS project (Dennis et al., 2017). Indeed, we have shown here that even a single season sampling can provide data to recover the main interactions among different Anthophila species and the relationships between their abundance and environmental variables. Therefore, the monitoring data provided by the monitoring protocol transects look promisingly informative to detect alterations of bee pollinators abundance and

potential negative interactions between managed honey bees and wild Anthophila with a relatively simple monitoring protocol. Moreover, the monitoring protocol also allowed us to identify the most and the least influencing ecological variables for each taxon, a basic knowledge to establish conservation actions.

However, it must be noted that Giannutri is a very simplified ecosystem and the small dimension of the island and its relatively isolated position in the Tuscan Archipelago has determined the development of a very simplified ecosystem with a moderately low species richness (Whittaker & Fernandez-Palacios, 2007). Future studies should thus test the informative value of the monitoring protocol in more complex ecosystems, where the Anthophila communities are richer. Moreover, the easiness of the method, due to the categorisation of Anthophila bees in few groups (*A. mellifera*, bumblebees at the species level and all other Anthophila grouped together), could also be its Achille's heel. Due to the complex taxonomy and functional diversity of wild bees, merging most Anthophila into a few or a single category would likely result in losing any possibility to link overall bee incidence with environmental settings. The use of lower taxonomic categories, such as morphospecies based on the main genera and the coupling of the transect method with other techniques that guarantee a higher taxonomic resolution (such as pan traps) could solve this potential main weakness.

2.6 Conclusions

Despite the temporal limitation of this study (one season, March May 2021), the information obtained with the basic version of the Italian Anthophila monitoring scheme provided interesting results. We found that the relations with abiotic and biotic variables and pollinator abundance reflected literature-based predictions. The monitoring data pointed out that currently there is no evidence for a spatial segregation between managed honey bees and wild bees. The flower-visitor network showed a great overlap between *A. mellifera*, *B. terrestris* and *Anthophora* spp. resources preferences suggesting that neither a resource-segregation is occurring. These results demonstrate the informative value of the monitoring protocol: it provides reliable monitoring data (allowing to clearly distinguish between managed honey bees and wild bee abundance trends) and at the same

time it provides important ecological information for pollinators communities conservation (such as the pivotal role of *T. fruticans* during the spring for Anthophila species on Giannutri island). In conclusion, the monitoring protocol meets the needs of a national and standardised, even though very simplified, monitoring scheme for pollinators and, due to its flexibility, it also could be used as a starting point for more in-depth studies, other than directly monitoring Anthophila pollinators abundance, for which taxonomic expertise remains crucial. Indeed, the long-term implementation of the monitoring protocol represents a first step to highlight species-species interactions, pollinator community dynamics and thus paving the way to a better understanding, and conservation, of this highly threatened group of pollinators, especially in such fragmented, isolated and small-size insular Mediterranean ecosystems.

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The authors declare no conflict of interest.

The datasets generated and analysed during the current study are available from the corresponding author on reasonable request.

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Chapter 3. Bees, hoverflies and butterflies of Giannutri Island (Tuscan Archipelago, Italy), with the first record of *Eumerus narcissi* Smith, 1928 (Syrphidae) for Italy (Hymenoptera: Apoidea: Anthophila, Diptera: Syrphidae, Lepidoptera: Papilionoidea)

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3.1 Abstract

Local pollinator checklists are crucial for biodiversity conservation representing essential foundations for successive eco- logical and evolutionary studies. The Tuscan Archipelago, protected within a National Park in Italy, hosts a largely undiscovered pollinator diversity, especially on the small Giannutri Island. This study focuses on bees (Hymenoptera: Apoidea: Anthophila), hoverflies (Diptera: Syrphidae) and butterflies (Lepidoptera: Papilionoidea). Bees on Giannutri are poorly documented, with no comprehensive checklist available, while hoverflies remain understudied across the Archipelago. Although butterfly data are more extensive, they consist mainly of sporadic records. To unveil the pollinator fauna of Giannutri, we sampled the island over four years (2021–2024), recording: 14 bee species (eight new to the island and one new to the Archipelago), 14 hoverfly species (all new to the island, three new to the Archipelago and one new to Italy) and 23 butterfly species (10 new to the island). The presence of *Eumerus narcissi* Smith, 1928 is of pivotal importance representing a new species for the Italian fauna. Among bees, the presence of *Nomada sheppardana* (Kirby, 1802) is reported for the first time in the Archipelago, while the new record of *Bombus xanthopu*s (Kriechbaumer, 1870) extends the distribution of this sub- endemic species to a fourth island, following Corsica, Capraia, and Elba. The species richness recorded on Giannutri Island aligned with the expectations based on size and isolation in comparison with the other islands of the Archipelago. Giannutri emerges as a noteworthy component of the Tuscan Archipelago and Mediterranean biodiversity highlighting the importance of checklists in conservation studies.

Keywords: hoverflies, bees, butterflies, distribution, pollinator conservation, insular, endemism, species-area relationship

3.2 Introduction

Insect abundance and diversity are undergoing drastic global declines (Eggleton 2020; Montgomery *et al.* 2020; Wagner *et al.* 2021), with an estimated 40% of species at risk of extinction in the coming decades (Sánchez-Bayo & Wyckhuys 2019, 2021). Lepidoptera and Hymenoptera are among the most studied insect taxa for which population declines are more documented (Sánchez-Bayo & Wyckhuys 2019). The collapse of wild pollinators, crucially involved in both agricultural and natural ecosystems, is well-documented (Potts *et al.* 2010; Ollerton *et al.* 2011; Vanbergen & The Insect Pollinators Initiative 2013; Hristov *et al.* 2020; Zattara & Aizen 2021; Nath *et al.* 2023). However, data on population abundance and species presence remain scattered across time and space (Sánchez-Bayo & Wyckhuys 2019, 2021), resulting in significant local knowledge gaps (Montgomery *et al.* 2020).

Pollinator distributions in many areas remain poorly understood due to a lack of historical data and recent surveys. Historical records are particularly valuable for identifying past local extinctions (Bartomeus & Dicks 2019; Mathiasson & Rehan 2019; European Commission *et al.* 2023; van Tongeren *et al.* 2023; Cornalba *et al.* 2024). Mapping biodiversity is therefore pivotal for understanding current species distributions and enabling future monitoring efforts. Local faunal checklists serve as essential tools for advancing knowledge of species distributions (Nieto *et al.* 2014; European Commission *et al.* 2023; van Tongeren *et al.* 2023).

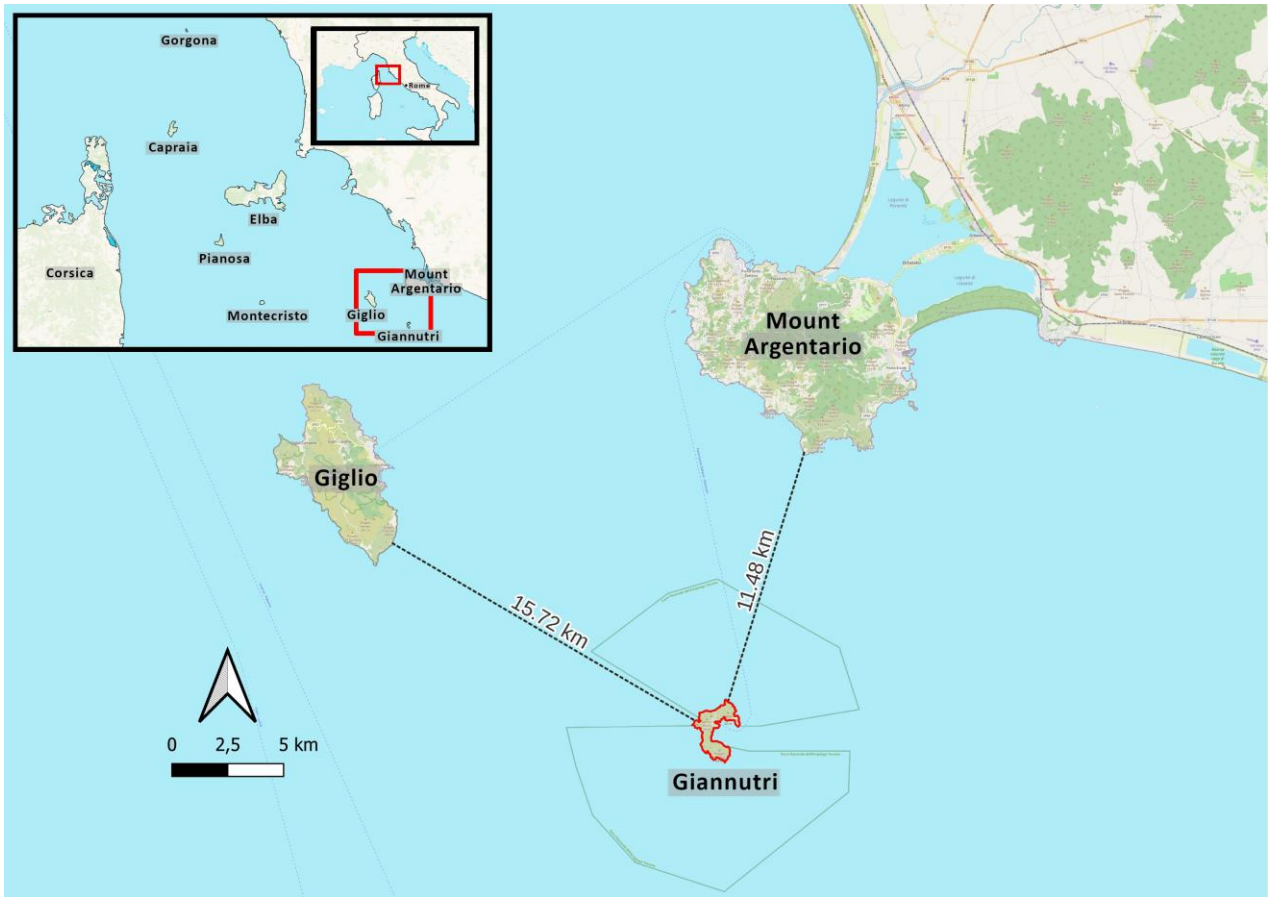

Figure 1. The position of Giannutri island. The smallest inset reports the position of the Tuscan Archipelago in Italy (red-bordered square). The middle inset shows the Tuscan Archipelago position between Corsica and mainland Tuscany, with all the seven islands reported, the red-bordered square indicates the position of Giannutri. Giannutri is bordered in red and its distances from Giglio Island and Mount Argentario are reported in km on the map. The green lines around Giannutri represent the border of the protected area of the National Park.

In this study, we focused on some diurnal pollinators of Giannutri, the southernmost island of the Tuscan Archipelago, comprising seven islands between the Tuscan mainland (Central-North Italy) and Corsica. These islands vary widely in size, isolation, human disturbance level, and the environments they host (Dapporto *et al.* 2017). Scientific knowledge of the archipelago faunal biodiversity of insects is fragmented, with data available for only a few islands and specific taxa (Ruzzier *et al.* 2021). Giannutri Island (hereafter Giannutri) is likely the least studied island in

this respect. Spanning approximately 2.6 km², it is situated near the border of Tuscany and Lazio, about 11.5 km from the mainland (Mount Argentario) and 15 km from the nearest island (Giglio) (Fig. 1). Despite being privately owned, Giannutri is entirely encompassed within the National Park, with sections designated as integral reserves and others as oriented general reserves.

The island's geology features carbonate rocks from the Triassic *Calcare Cavernoso* formation, characterised by brecciation and faulting systems with prominent coastal caves (Aringoli *et al.* 2009). Its vegetation includes annual grasslands, low and high scrub with a prevalence of *Salvia rosmarinus* Spenn., *Teucrium fruticans* L., *Euphorbia dendroides* L. and *Pistacia lentiscus* L., *Juniperus turbinata* Guss. (juniper thickets), *Quercus ilex* L. woods, and coastal communities (Foggi *et al.* 2011). Recently the island experienced an expansion of juniper thickets and high maquis at the expense of low maquis (Foggi *et al.* 2015).

We compiled a comprehensive checklist of three key pollinator groups: bees (Hymenoptera, Apoidea, Anthophila), hoverflies (Diptera, Syrphidae) and butterflies (Lepidoptera, Papilionoidea) (Ollerton 2017). Among these, Lepidoptera is the most studied taxon in the archipelago, with data available on both resident and adventive species (Balletto *et al.* 2007; Dapporto & Strumia 2008; Dapporto *et al.* 2017). However, a dedicated checklist for Giannutri is lacking, with existing information limited to sporadic reports (Balletto *et al.* 2007; Dapporto & Cini 2007; Dapporto *et al.* 2017). While studies on Hymenoptera exist for other islands in the archipelago (Rasmont & Quaranta 1997; Generani *et al.* 2001; Vicidomini 2003; Filippi & Strumia 2019; Forbicioni *et al.* 2019; Strumia 2019), for Giannutri there is only a list, based on a single season sampling provided by Cini *et al.* (2022). Hoverflies are the least studied group: the few records of hoverflies for the entire archipelago are one century old on Capraia (Razzauti 1917) and Giglio (Bezzi 1926) islands, whilst there are no data available for Giannutri. Updating data on these taxa is therefore essential to establish a comprehensive biodiversity snapshot of Giannutri's pollinators within the framework of the Tuscan Archipelago National Park.

3.3 Material and Methods

We collected occurrence data of Apoidea, Syrphidae and Papilionoidea on Giannutri through intensive surveys from March 2021 to September 2024 based on opportunistic sampling of the island and on walking transects. In 2021 we established six transects according to the Italian Institute for Environmental Protection and Research (ISPRA) guidelines (Cini *et al.* 2022) in order to monitor the abundance of Apoidea and Papilionoidea, replicating the transects monthly. During each visit to the island, we also collected and recorded hoverflies even though they were not the target species of the monitoring transects. In each year since 2022, the sampling effort was more intense between mid-February to the first week of April, since in this period we monitored Giannutri every day, within a wider study aimed at highlighting possible trophic competitive interaction between locally introduced hives of *Apis mellifera* Linnaeus, 1758 and wild pollinators.

Bees and hoverflies were collected with entomological nets, euthanised with ethyl acetate and later identified in the laboratory. Butterflies were identified directly in the field, but some specimens were collected each year. Most morphological identifications of bees and hoverflies were carried out by MB using standard keys and the relevant specialised literature (Lieftinck 1980; Rasmont 1995; Amiet 2004; Amiet *et al.* 2007; Amiet 2010; Smit 2018; Rasmont *et al.* 2021; Bot & van de Meutter 2023). The bee specimens are stored both in MB private collection and in the ZENlab collection (Biology Department of the University of Florence), while the hoverfly specimens are stored in MB collection and the butterfly specimens in the ZENlab and the Roger Vila collection (Institut de Biologia Evolutiva, Barcelona).

In addition to our sampling activities (opportunistic and standardised), we searched occurrence data of the target taxa for Giannutri from the following sources: 1) literature and the Italian CkMap project (Balletto *et al.* 2007); 2) iNaturalist observations validated by us, including only records for which the identification was possible from the uploaded picture; 3) butterfly specimens stored in the Roger Vila collection; 4) update of Italian checklist of Syrphidae

(Sommaggio *et al.* unpublished data).

For each pollinator group, we listed the species recorded on Giannutri following the taxonomy adopted by Wiemers *et al.* (2018) and updated by Dapporto *et al.* (2022) for butterflies and the recent checklists by Ghisbain *et al.* (2023) and Reverté *et al.* 2023) for bees and hoverflies. For each species, we indicated the recording year and the data source. We also specified the collection date of relevant specimens during our surveys (i.e. the first report for the island). Most specimens were collected in 2021 and 2022, as they were the first years of samplings. The wild bees collected in 2021 were already identified and reported by Cini *et al*. (2022). Accordingly, we specified the months of occurrence of each species, based on our surveys (especially for the most abundant and visible bees and butterflies) instead of listing several consecutive days of collection or records.

3.4 Results

Apoidea

A total of 14 bee species have been recorded on Giannutri, listed in Table 1. Six of them were already reported by Cini *et al*. (2022). One species, *Nomada sheppardana,* (Kirby, 1802) is new to the Tuscan Archipelago.

Table 1. *Bee species recorded on Giannutri with the year of observation specified. Source of data: • = our samplings; + = iNaturalist data (in the case of bees, all the reports on iNaturalist correspond to observation collected during our surveys). The asterisk (*) indicates a hybrid specimen (see Bombus xanthopus (Kriechbaumer, 1870) description).*

	Family	Species	2021	2022	2023	2024
1	Andrenidae	*Andrena nigroaenea* (Kirby, 1802)	•	•	+	•+
2	Apidae	*Anthophora dispar* Lepeletier, 1841	•+	•	•+	•
3	Apidae	*Apis mellifera* Linnaeus, 1758	•	•+	•	•
4	Apidae	*Bombus terrestris* (Linnaeus, 1758)	•+	•	•+	•
5	Apidae	*Bombus xanthopus* (Kriechbaumer, 1870)		•	•*	
6	Apidae	*Melecta italica* Radoszkowski, 1876	•	•+	•+	•+
7	Apidae	*Nomada sheppardana* (Kirby, 1802)	•	•		
8	Apidae	*Nomada succincta* Panzer, 1798	•		•+	
9	Apidae	*Xylocopa violacea* (Linnaeus, 1758)	•	•	•+	•

10	Halictidae	*Lasioglossum nitidulum* (Fabricius, 1804)				•
11	Halictidae	*Lasioglossum transitorium* (Schenck, 1870)	•	•	•	•+
12	Megachilidae	*Heriades crenulata* Nylander, 1856	•		•	•
13	Megachilidae	*Megachile argentata* (Fabricius, 1793)				•
14	Megachilidae	*Osmia latreillei* (Spinola, 1806)		•	•+	•+

Andrena nigroaenea (Kirby, 1802)

Recorded in most Italian regions, including Tuscany (Comba 2019). In the Tuscan Archipelago it is reported for Capraia, Elba, Pianosa and Giglio islands (Generani *et al.* 2001; Forbicioni *et al.* 2019). The first records for the island concern the specimens collected in 2021, reported by Cini *et al*. (2022). Recorded on the island primarily and abundantly in February, March and April with some sporadic records also in May.

Anthophora dispar Lepeletier, 1841

Recorded in most Italian regions, including Tuscany (Comba 2019), in the Tuscan Archipelago it is reported for Elba and Giglio islands (Generani *et al*. 2001; Forbicioni *et al*. 2019). The first records for the island were documented by Cini *et al*. (2022) in 2021. Recorded on the island in February, March and April with some sporadic records in May 2021 and one specimen observed in November 2021. It is the most abundant wild bee on the island and is numerically overcome only by the managed honey bees.

Apis mellifera Linnaeus, 1758

Distributed in all Italian regions, both with native and managed populations (Pagliano 1995). In the Tuscan Archipelago, it is reported for Gorgona, Capraia, Elba, Giglio (Generani *et al*. 2001; Forbicioni *et al*. 2019) and Pianosa Island (Zavattari 1905; Generani *et al.* 2001). *A. mellifera* belonging to the subspecies *ligustica* Spinola, 1806, has been locally introduced to Giannutri with beehives since 2018, with no naturalised colonies on the island (Cini *et al.* 2022). It is present on the island each year from December to June.

Bombus terrestris (Linnaeus, 1758)

Recorded in most Italian regions, including Tuscany (Comba 2019). In the Tuscan Archipelago, it is reported for Gorgona, Capraia, Elba, Pianosa, Montecristo and Giglio islands (Rasmont & Quaranta 1997; Generani *et al.* 2001; Forbicioni *et al.* 2019). The first records for Giannutri date back to 2021 (Cini *et al*. 2022) with observations primarily from February to May and fewer records from June to September, likely reflecting a typical Mediterranean anticipated phenology (Gurel *et al.* 2008). It is the second most abundant wild bee species on the island.

Bombus xanthopus (Kriechbaumer, 1870)

Species endemic of the Corsican-Tuscan island complex, formerly reported on Corsica, Capraia and Elba islands (Generani *et al.* 2001; Forbicioni *et al.* 2019; Rasmont *et al.* 2021). Considered by some authors as a subspecies of *B. terrestris* (Bertsch & Schweer 2012; Williams et al. 2012), though the specific status has been suggested on the basis of genetic and biochemical markers (Lecocq *et al.* 2015). Our records are the first for Giannutri, where we observed queens, workers and males in 2022: three ♀ (25.II.2022); one ♂, three ♀ (9.III.2022/29.III.2022). Potential hybrids with *B. terrestris*, showing varied intermediate colour patterns (Boni *et al.* 2023), are reported for Elba and Capraia islands (Rasmont & Adamski 1996; Rasmont & Quaranta 1997; Boni *et al.* 2023) and a locality on the Tuscan coast (Quaranta & Felicioli 2012). We collected a specimen with this pattern in May 2023: one ♀ (31.V.2023).

Melecta italica Radoszkowski, 1876

Recorded in most Italian regions, including Tuscany (Comba 2019). In the Tuscan Archipelago, it is reported for Elba Island by Forbicioni *et al*. (2019). Our records are the first for Giannutri. Like other *Melecta* it is a brood parasite of *Anthophora* species *(Lieftinck 1980)*, and it likely exploits *A. dispar* as a host on Giannutri, as we observed it entering the nests of this species. We recorded *M. italica* primarily in February and March, with some records also in April.

Nomada sheppardana (Kirby, 1802)

Recorded in northern and central Italy, including Tuscany and Sicily (Comba 2019). Our records are the first for the Tuscan Archipelago. It is a brood parasite of *Lasioglossum* species*,* likely using *L. transitorium* as a host species on Giannutri. Recorded in March, in 2021 and 2022: one ♂, one ♀ (26.III.2021), one ♂ (22.III.2022).

Nomada succincta Panzer, 1798

Recorded in many Italian regions, including Tuscany (Comba 2019). In the Tuscan Archipelago, it is reported for Elba Island by Forbicioni *et al*. (2019). Our records are the first for Giannutri. Here it is likely a brood parasite of *Andrena nigroaenea,* one of its known hosts together with *Andrena nitida* (Müller, 1776) (Smit 2018). Two specimens collected in 2021 (♂, 26.III.2021) were misidentified and reported as the similar *Nomada goodeniana* (Kirby, 1802) in Cini *et al*. (2022).

Xylocopa violacea (Linnaeus, 1758)

Recorded in most Italian regions, including Tuscany (Comba 2019). In the Tuscan Archipelago, it is reported for Gorgona, Capraia, Elba, Pianosa, Montecristo and Giglio islands (Generani *et al.* 2001; Vicidomini 2003; Forbicioni *et al.* 2019). Reported as *Xylocopa* sp. by Cini *et al.* (2022) as it was only observed in flight, subsequent collections (two ♂, 10.III.2023) and in-hand examinations confirmed the presence of *X. violacea* on the island, with records from March to May.

Lasioglossum nitidulum (Fabricius, 1804)

Recorded in most Italian regions (Comba 2019), in the Tuscan Archipelago it is reported for Elba and Giglio islands (Generani *et al.* 2001; Forbicioni *et al.* 2019). Our records are the first for Giannutri, where we recorded it in September 2024 (one ♀, 30.IX.2024).

Lasioglossum transitorium (Schenck, 1870)

Recorded in many Italian regions, including Tuscany (Comba 2019). In the Tuscan Archipelago, it is reported for Capraia and Elba islands (Generani *et al.* 2001; Forbicioni *et al.* 2019). First reported in 2021 on Giannutri (Cini *et al.* 2022), it has been observed there from February to September.

Heriades crenulata Nylander, 1856

Recorded in most Italian regions, including Tuscany (Comba 2019). In the Tuscan Archipelago, it is reported for Elba, Pianosa and Montecristo islands (Generani *et al.* 2001; Filippi & Strumia 2019; Forbicioni *et al.* 2019). Our records are the first for Giannutri, where we recorded it in September 2021 (6 ♀, 15.IX.2021), June 2023 (one ♀, 28.VI.2023) and September 2024 (♀, 30.IX.2024).

Megachile argentata (Fabricius, 1793)

Recorded in all Italian regions, including Tuscany (Comba 2019). In the Tuscan Archipelago it is reported for Elba and Giglio islands (Generani *et al.* 2001; Forbicioni *et al.* 2019). Our records are the first for Giannutri, where we recorded it in September 2024 (one ♀, 30.IX.2024).

Osmia latreillei (Spinola, 1806)

Recorded in most Italian regions, including Tuscany (Comba 2019). In the Tuscan Archipelago, it is reported for Capraia, Elba and Giglio islands (Generani *et al.* 2001; Forbicioni *et al.* 2019). Our records are the first for Giannutri, where several specimens were detected in March and April.

Syrphidae

A total of 14 hoverfly species have been recorded on Giannutri, listed in Table 2. None of them were reported before our sampling activities on Giannutri. One species, *Eumerus narcissi* (Smith, 1928) is new to Italy and *Callicera rufa* (Schummel, 1842) is new for Tuscany. Two species (*Melanostoma mellinum* (Linnaeus, 1758), *Scaeva dignota* (Rondani, 1857)) were not previously recorded for the Tuscan Archipelago (Razzauti 1917; Bezzi 1926; iNaturalist records).

Table 2. *Hoverflies species recorded on Giannutri with specified the year of observation. Source of data: • = our samplings; + = iNaturalist data.*

	Family	Species	2021	2022	2023	2024
1	Syrphidae	*Callicera rufa* Schummel, 1842			•	
2	Syrphidae	*Chrysotoxum intermedium* Meigen, 1822	•		•+	+
3	Syrphidae	*Episyrphus balteatus* (De Geer, 1776)	•	•	•+	+
4	Syrphidae	*Eristalinus aeneus* (Scopoli, 1763)			•	
5	Syrphidae	*Eristalis similis* (Fallén, 1817)		•	+	
6	Syrphidae	*Eristalis tenax* (Linnaeus, 1758)	•	•	•	
7	Syrphidae	*Eumerus narcissi* Smith, 1928	•		•	
8	Syrphidae	*Eupeodes corollae* (Fabricius, 1794)	•	•	•	+
9	Syrphidae	*Melanostoma mellinum* (Linnaeus, 1758)			•	
10	Syrphidae	*Meliscaeva auricollis* (Meigen, 1822)		•	•	+
11	Syrphidae	*Scaeva dignota* (Rondani, 1857)			•	
12	Syrphidae	*Scaeva pyrastri* (Linnaeus, 1758)		•	•+	
13	Syrphidae	*Sphaerophoria scripta* (Linnaeus, 1758)			•	
14	Syrphidae	*Xanthandrus comtus* (Harris, 1780)	•	•		

Callicera rufa Schummel, 1842

This species has been recorded only in Lazio region in Central Italy (Paparatti 1997). Our record is the first for the Tuscan Archipelago and Tuscany region. It occurs in conifer forests, with arboreal adults and larvae living in wood rot-holes (Speight 2024). On Giannutri we collected this species in a *Pinus halepensis* Mill. grove in March (one ♂, 10.III.2023).

Chrysotoxum intermedium Meigen, 1822

A great confusion is present in the *Chrysotoxum intermedium* species group, mainly from a taxonomic point of view. The *C. intermedium* described by Meigen is probably different from *C. intermedium* sensu other authors mainly from

South Europe. The specimens found in Giannutri belong to the latter species in accordance with *C. lunulatum* Brullé, 1833. However, until taxonomic problems are solved, we prefer to maintain here the name *C. intermedium*. The species, under the name *C. italicum* Rondani 1845, has been recorded for Giglio Island by Bezzi (1926). On Giannutri we recorded it in March.

Episyrphus balteatus (De Geer, 1776)

Known for all Italian regions (Sommaggio 2006; Burgio *et al.* 2015). In the Tuscan Archipelago it is reported for Capraia (Razzauti 1917) and Elba islands (two iNaturalist records). Our records are the first for Giannutri. We recorded it in February and March.

Eristalinus aeneus (Scopoli, 1763)

Reported across most Italian regions (Sommaggio 2006). Our records are the first for Giannutri and the Tuscan
Archipelago. We recorded a single specimen in April (one ♀, 3.IV.2023).

Eristalis similis (Fallén, 1817)

Reported across most Italian regions (Burgio *et al.* 2015). The species has been recorded under the name *E. pratorum* by Bezzi (1926) on Giglio Island. Our records are the first for Giannutri, where we collected a single specimen in February (one ♂, 25.II.2022).

Eristalis tenax (Linnaeus, 1758)

This is one of the most common hoverflies, reported across most Italian regions. In the Tuscan Archipelago, it has been reported for Giglio (Bezzi 1926) and Elba islands (one observation on iNaturalist). Our records are the first for Giannutri, where we detected the species in February and March.

Eumerus narcissi Smith, 1928

The species has been reported for southern France and Corsica (Speight *et al.* 2013; Lebard *et al.* 2019), as well as for Great Britain (Thomas *et al.* 2023). The specimens we collected (four ♂, 26.III.2021) represent the first records for Italy (Fig. 2A). Male genitalia (Fig. 2B) and other morphological features allow the separation of *E. narcissi* from related species (Speight *et al.* 2013; Speight *et al.* 2021).

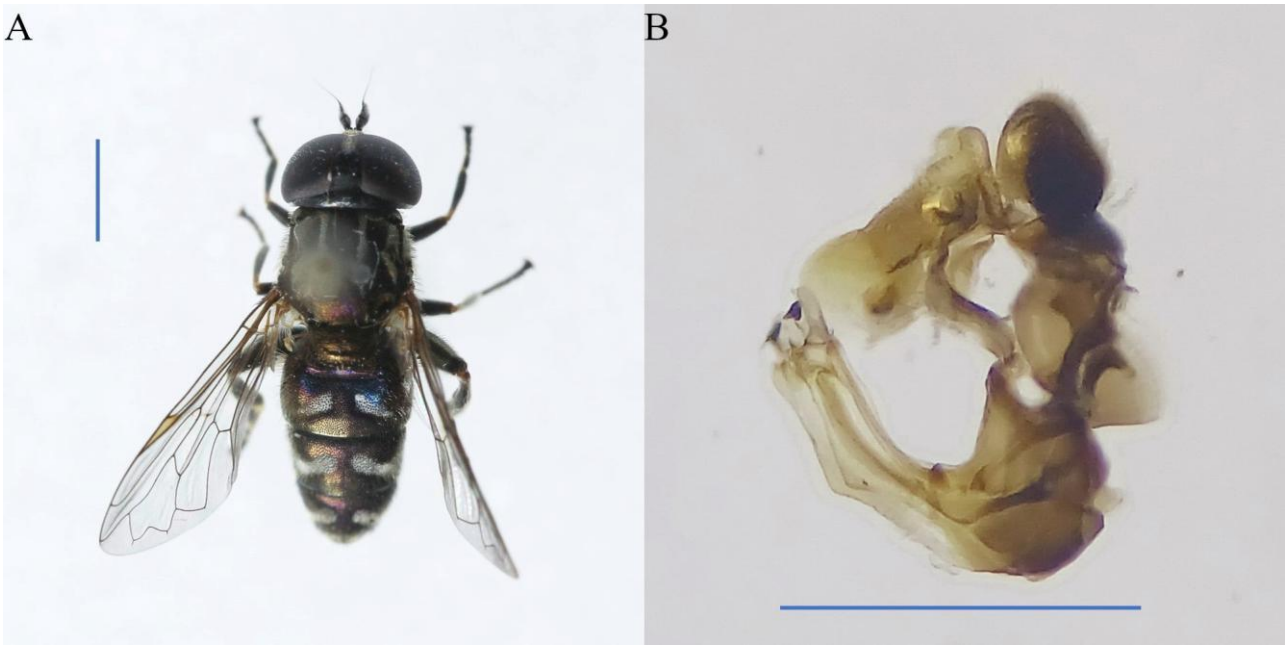

Figure 2. ***A,*** *Male specimen of* Eumerus narcissi *collected on Giannutri.* ***B,*** *genitalia of the specimen in A. Blue bars correspond to one millimetre in both A. and B.*

Eupeodes corollae (Fabricius, 1794)

A common species, largely used also for mass rearing and release in agroecosystems (Burgio *et al.* 2024). Known for all Italian regions, with records for Pianosa and Giglio islands (Bezzi 1926; Sommaggio 2006). Our record is the first for Giannutri. Recorded in March (2 ♂, 10.III.2023).

Melanostoma mellinum (Linnaeus, 1758)

Despite being a very common species, largely distributed in all Europe (Reverté

et al. 2023; Speight 2024), our records
are the first for Giannutri and the Tuscan Archipelago. We recorded a single individual in March (1 ♀, 10.III.2023).

Meliscaeva auricollis (Meigen, 1822)

Reported across most Italian regions (Burgio *et al.* 2015) with a record for Giglio Island in 1926 (Bezzi 1926). Our records are the first for Giannutri, where we recorded it in March.

Scaeva dignota (Rondani, 1857)

Known for all Italian regions, including Tuscany (Sommaggio 2006; Burgio *et al.* 2015). Our records are the first for
Giannutri and the Tuscan Archipelago. Recorded in March for a single individual (one ♂, 10.III.2023).

Scaeva pyrastri (Linnaeus, 1758)

Known for all Italian regions, with a record for Giglio Island (Bezzi 1926). Our records are the first for Giannutri.
Recorded in February (one ♀, 25.II.2022), March (one iNaturalist observation) and April (one ♀, 4.IV.2023).

Sphaerophoria scripta (Linnaeus, 1758)

Known for all Italian regions, with a record for Giglio Island (Bezzi 1926). Our records are the first for Giannutri.
Recorded in March (1 ♀, 10.III.2023) and April (two ♂, 4.IV.2023).

Xanthandrus comtus (Harris, 1780)

Reported across most Italian regions (Burgio *et al.* 2015). Our records are the first for Giannutri and the Tuscan Archipelago. Recorded in February and March.

Papilionoidea

A total of 23 butterfly species have been recorded on Giannutri, listed in Table 3, with 10 new records for the island.

Gonepteryx rhamni (Linnaeus, 1758)

Known for all Italian regions, with several records for the Tuscan Archipelago at the beginning of the 20th century (Biermann & Hesch 1982; Balletto *et al.* 2007), it became sporadic in the last decades being found on Giglio Island in 2003 (Favilli *et al.* 2011) and on Elba Island in 2008 (Balletto *et al.* 2007). Our record, in April, of only one individual (3.IV.2023), is the first for Giannutri.

Gonepteryx cleopatra (Linnaeus, 1767)

Reported for most of the Italian regions, in the Tuscan Archipelago it has been recorded on Gorgona island since 2015 (Dapporto *et al.* 2017), on Capraia Island since 1914 (Razzauti 1917), on Elba Island since 1908 (Biermann & Hesch 1982; Balletto *et al.* 2007), on Pianosa Island since 2011 (Dapporto & Cini 2007), on Montecristo Island on 2019 (Dapporto, pers. obs.) and on Giglio Island since 1980 (Biermann & Hesch 1982). On Giannutri it was first recorded by Dapporto & Cini (2007) and has been observed in February, March, June and September.

Colias crocea (Geoffroy, 1785)

Known for all the Italian regions, it is reported for all the Tuscan Archipelago Islands. It has been known for Gorgona Island since 1999 (Dapporto 2002), for Capraia Island since 1968 (Gross 1970), for Elba Island since 1908 (Biermann & Hesch 1982; Balletto *et al.* 2007), for Pianosa Island since 1998 (Dapporto *et al.* 1999), for Montecristo Island since 1983 (Raineri 1986) and for Giglio Island since 1980 (Biermann & Hesch 1982). On Giannutri it was first recorded by Dapporto & Cini (2007). Recorded on the island from February to November.

Pontia edusa (Fabricius, 1777)

Known for all the Italian regions. In the Tuscan Archipelago, it has been reported for Elba Island since 1908 (Biermann & Hesch 1982; Balletto *et al.* 2007), for Pianosa Island since 1998 (Dapporto *et al.* 1999) and for Giglio Island since 1908 (Rocci & Turati 1925). For Capraia, the sibling *P. daplice* could also be present, but specimens were only recorded in 1968-1970 (Gross 1970). On Giannutri it was first recorded by Dapporto & Cini (2007). Observed in March, May, September and October.

Table 3. *Butterfly species recorded on Giannutri. Source of data: •= our sampling activities, + = iNaturalist data, § = literature data, ^ = Roger Vila collection data.*

	Family	Species	1994	2005	2007	2008	2012	2014	2021	2022	2023	2024
1	Pieridae	*Gonepteryx rhamni* (Linnaeus, 1758)									•	
2	Pieridae	*Gonepteryx cleopatra* (Linnaeus, 1767)		§				§^			•+	•
3	Pieridae	*Colias crocea* (Geoffroy, 1785)		§					•	•	•	•
4	Pieridae	*Pontia edusa* (Fabricius, 1777)		§					•		•	•
5	Pieridae	*Pieris brassicae* (Linnaeus, 1758)							•	•+	•+	•
6	Pieridae	*Pieris rapae* (Linnaeus, 1758)		§				§^	•	•+	•	•
7	Pieridae	*Pieris napi* (Linnaeus, 1758)						§^	•			
8	Pieridae	*Lycaena phlaeas* (Linnaeus, [1760])						§			•	
9	Lycaenidae	*Leptotes pirithous* (Linnaeus, 1767)		§				§^	•		•	•+
10	Lycaenidae	*Lampides boeticus* (Linnaeus, 1767)							•		•	•
11	Lycaenidae	*Cacyreus marshalli* Butler, 1898							•	•+	•+	•
12	Lycaenidae	*Celastrina argiolus* (Linnaeus, 1758)								•	•	•
13	Lycaenidae	*Aricia agestis* ([Denis & Schiffermüller], 1775)		§					•	•	•+	•
14	Lycaenidae	*Polyommatus icarus* (Rottemburg, 1775)							•			•
15	Lycaenidae	*Argynnis paphia* (Linnaeus, 1758)										•

16	Nymphalidae	*Vanessa cardui* (Linnaeus, 1758)		§			^	•	•	•	•
17	Nymphalidae	*Vanessa atalanta* (Linnaeus, 1758)		§		+		•+	•+	•+	•
18	Nymphalidae	*Aglais io* (Linnaeus, 1758)								•	
19	Nymphalidae	*Nymphalis polychloros* (Linnaeus, 1758)								•	•
20	Nymphalidae	*Charaxes jasius* (Linnaeus, 1767)		§			§^	•	•	•+	•
21	Nymphalidae	*Coenonympha corinna* (Hübner, [1804])	§		§		§^	•	•	•	•
22	Nymphalidae	*Pararge aegeria* (Linnaeus, 1758)					§^	•	•+		•+
23	Nymphalidae	*Lasiommata megera* (Linnaeus, 1767)						•	•	•+	•

Pieris brassicae (Linnaeus, 1758)

Known for all the Italian regions. It is reported for Gorgona Island since 1999 (Dapporto 2002), for Capraia Island since 1915 (Razzauti 1917), for Elba Island since 1908 (Biermann & Hesch 1982; Balletto *et al.* 2007), for Pianosa Island since 2008 (Dapporto *et al.* 2017), for Montecristo Island since 2001 (Dapporto *et al.* 2017) and for Giglio Island since 2007 (Dapporto *et al.* 2017). Our records are the first for Giannutri. Recorded on the island from February to November.

Pieris rapae (Linnaeus, 1758)

Known for all the Italian regions. It is reported for all the Tuscan Archipelago Islands and in particular for Gorgona Island since 1999 (Dapporto 2002), for Capraia Island since 1915 (Razzauti 1917), for Elba Island since 1908 (Biermann & Hesch 1982; Balletto *et al.* 2007), for Pianosa Island since 1998 (Dapporto *et al.* 1999), for Montecristo Island since 1983 (Raineri 1986) and for Giglio Island since 1908 (Rocci & Turati 1925). On Giannutri it was first recorded by Dapporto & Cini (2007). Recorded from February to June and in October.

Pieris napi (Linnaeus, 1758).

Known for all the Italian regions except for Sardinia. It has been reported for

Gorgona Island in 1999 (Dapporto 2002) and Elba Island since 1908 (Biermann & Hesch 1982; Balletto *et al.* 2007). The first record for Giannutri is from 2014 (Dapporto *et al.* 2017). We recorded this species in November 2021 (two ♂, 18.ix.2021).

Lycaena phlaeas (Linnaeus, [1760])

Known for all the Italian regions. In the Tuscan Archipelago, it has been recorded on Gorgona Island in 2021 (our research group observation), on Capraia Island in 1979 (Biermann & Hesch 1982), on Elba Island since 1908 (Biermann & Hesch 1982; Balletto *et al.* 2007), on Montecristo Island since 2014 (Dapporto *et al.* 2017) and on Giglio Island since 1908 (Rocci & Turati 1925). The first record for Giannutri referred to a specimen collected in 2014 and stored in the Roger Vila collection. Recorded in March.

Leptotes pirithous (Linnaeus, 1767)

Known for most of the Italian regions. Reported for all the Tuscan Archipelago islands, in particular on Gorgona Island since 1999 (Dapporto 2002), on Capraia Island since 1914 (Razzauti 1917), on Elba Island since 1908 (Biermann & Hesch 1982; Balletto *et al.* 2007), on Pianosa Island since 2019 (our research group observation), on Montecristo Island since 2012 (Dapporto *et al.* 2017) and on Giglio Island since 2010 (Dapporto *et al.* 2017). On Giannutri was first recorded by Dapporto & Cini (2007). Recorded from February to May and from September to November.

Lampides boeticus (Linnaeus, 1767)

Known for all the Italian regions. Reported for all the Tuscan Archipelago islands and in particular on Gorgona Island since 1999 (Dapporto 2002), on Capraia Island since 1968 (revised by Balletto *et al*. 2007), on Elba Island since 1908 (Biermann & Hesch 1982; Balletto *et al.* 2007), on Pianosa Island since 2014 (our research group observation), on Montecristo Island since 2012 (Dapporto

et al. 2017) and on Giglio Island since 1980 (Biermann & Hesch 1982). Our record is the first for Giannutri. Recorded in February, May, September and November.

Cacyreus marshalli Butler, 1898

Native to South Africa, first reported in Italy in 1996 (Trematerra *et al.* 1997). Recorded on Gorgona Island (Dapporto 2003), Capraia Island (Dapporto 2003), Elba Island (Biermann 2003) and Giglio Island (Biermann 2003). Our records are the first for Giannutri. Recorded in March, April, June, October and November.

Celastrina argiolus (Linnaeus, 1758)

Known for all the Italian regions, with records in all the Tuscan Archipelago islands. It has been reported for Gorgona Island since 1999 (Dapporto 2002), for Capraia Island since 1968 (Biermann & Hesch 1982; Balletto *et al.* 2007), for Elba Island since 1908 (Biermann & Hesch 1982; Balletto *et al.* 2007), for Pianosa Island since 2011 (Dapporto *et al.* 2017), for Montecristo Island since 2014 (Dapporto *et al.* 2017) and for Giglio Island since 1908 (revised by (Biermann & Hesch 1982; Balletto *et al.* 2007). Our records are the first for Giannutri. Recorded in March and October.

Aricia agestis ([Denis & Schiffermüller], 1775)

Known for all the Italian regions except for Sardinia. Recorded in all the Tuscan Archipelago islands except for Montecristo. In particular, it has been recorded on Gorgona Island in 2005 (Dapporto & Cini 2007), on Capraia Island in 2013 (Dapporto *et al.* 2017), on Elba Island since 1908 (Biermann & Hesch 1982; Balletto *et al.* 2007), on Pianosa Island since 1998 (Dapporto *et al.* 1999) and on Giglio Island since 1908 (Rocci & Turati 1925) with no records since 1979 (Biermann & Hesch 1982). The first record on Giannutri is by Dapporto & Cini (2007). Recorded from February to May.

Polyommatus icarus (Rottemburg, 1775)

Known for all the Italian regions except for Sardinia and Sicily. In the Tuscan Archipelago, it was reported for Capraia, Elba, Pianosa and Giglio islands. In particular, on Capraia Island it was first recorded in 1979 (Biermann & Hesch 1982), on Elba Island since (Biermann & Hesch 1982), on Pianosa Island since 1998 (Dapporto *et al.* 1999) and on Giglio Island since 1908 with no records since 1979 (Biermann & Hesch 1982; Balletto *et al.* 2007). Our records are the first for Giannutri. Recorded in April, May, September and October.

Argynnis paphia (Linnaeus, 1758)

Known for all the Italian regions. It has been recorded on Elba Island since 1908 (Biermann & Hesch 1982; Balletto *et al.* 2007) where it is uncommon and on Giglio Island (Biermann 2003) where after the first sighting in 1908 it has never been observed again. Our record of a single male (30.IX.2024) is the first on Giannutri.

Vanessa cardui (Linnaeus, 1758)

Known for all the Italian regions, with records for all the Tuscan Archipelago islands. On Gorgona Island has been reported since 2015 (specimens stored in the Roger Vila collection), on Capraia Island has been known since 1915 (Razzauti 1917), on Elba Island is known since 1908 (Biermann & Hesch 1982; Balletto *et al.* 2007), on Pianosa Island is known since 1998 (Dapporto *et al.* 1999), on Montecristo Island is known since 1979 (Fanfani & Groppali 1979) and on Giglio Island was first reported in 1908 (Rocci & Turati 1925). The first records on Giannutri are from Dapporto & Cini (2007). Recorded from February to June and from September to November.

Vanessa atalanta (Linnaeus, 1758)

Known for all the Italian regions with records for all the Tuscan Archipelago islands. The first record for Gorgona Island is from 1999 (Dapporto 2002), for

Capraia Island is in 1915 (Razzauti 1917), on Elba Island is known since 1908 (Biermann & Hesch 1982; Balletto *et al.* 2007), on Pianosa Island is known since 1998 (Dapporto *et al.* 1999), on Montecristo Island is known since 1983 (Raineri 1986) and on Giglio Island since 1908 (Biermann & Hesch 1982; Balletto *et al.* 2007). The first records on Giannutri are reported by Dapporto & Cini (2007). Recorded from February to November.

Aglais io (Linnaeus, 1758)

Reported for most Italian regions and never reported for the Tuscan Archipelago except for single records on Elba Island (Biermann 2003) and on Giglio Island (Biermann 1998; Biermann 2003). Our record, of a single individual (3.IV.2023) is the first for Giannutri.

Nymphalis polychloros (Linnaeus, 1758)

Known for all the Italian regions. In the Tuscan Archipelago, it is reported for Elba Island, where it was recorded in 1908 and 1916 (Biermann & Hesch 1982; Balletto *et al.* 2007) and later reconfirmed in 2019 (Dapporto, pers. obs.). It was also observed in 2023 on Giglio Island (Forbicioni, pers. comm.). Our records of a few individuals are the first on Giannutri. Recorded in February and March.

Charaxes jasius (Linnaeus, 1767)

Known for many coastal regions in Italy, with records in all Tuscan Archipelago islands. On Gorgona Island it has been recorded since 2004 (Dapporto & Cini 2007), on Capraia Island from 1996 (Jutzeler & de Bros 1997), on Elba Island since 1908 (Biermann & Hesch 1982; Balletto *et al.* 2007), on Pianosa Island since 2005 (Dapporto & Cini 2007), on Montecristo Island since 2021 (Dapporto, pers. obs.) and on Giglio Island since 1908 (Rocci & Turati 1925). On Giannutri it was first recorded in 2014 (Rocci & Turati 1925). Recorded from May to September.

Coenonympha corinna (Hübner, [1804])

Considered by some authors as an endemic species of Sardinia and Corsica, separated from C*oenonympha elbana* Staudinger, 1910 which is endemic to the Tuscan Archipelago and Tuscan mainland (Jutzeler 1997; Balletto *et al.* 2014). Data on mitochondrial DNA show differences between three main haplogroups (Sardinia, Corsica + Capraia, Elba + Giannutri + mainland) thus only partially confirming the taxonomic solution of two species. Moreover, morphometric analyses revealed no significant differences in the male genitalia of the two species (Dapporto & Strumia 2008) suggesting to consider the two entities as a single species. Accordingly, following the taxonomy by Wiemers *et al*. (2018) and (Dapporto *et al.* 2022), we attribute the Giannutri population to *C. corinna*. On Capraia Island it has been recorded since 1915 (Biermann & Hesch 1982; Balletto *et al.* 2007), on Elba Island since 1908 (Biermann & Hesch 1982; Balletto *et al.* 2007) and on Giglio Island since 1980 (Biermann & Hesch 1982) but in the last decades it has apparently disappeared from the island. On Giannutri it is reported from 1994 (Balletto *et al.* 2007). Recorded in May, June and September.

Pararge aegeria (Linnaeus, 1758)

Known for all the Italian regions, with records for all the Tuscan Archipelago islands. It has been recorded on Gorgona Island from 2015 (Dapporto *et al.* 2017), on Capraia Island from 2008 (Dapporto *et al.* 2017), on Elba Island from 1908 (Biermann & Hesch 1982; Balletto *et al.* 2007), on Pianosa Island from 2011 (Dapporto *et al.* 2017), on Montecristo Island from 1979 (Fanfani & Groppali 1979) and on Giglio Island from 1908 (Rocci & Turati 1925). On Giannutri it was first recorded in 2014 (Dapporto *et al.* 2017). Recorded from February to April and in November.

Lasiommata megera (Linnaeus, 1767)

Known for all the Italian regions except for Sardinia. In the Tuscan Archipelago is recorded for all the islands except for Capraia and Montecristo, where

Lasiommata paramegaera (Hübner, [1824]) is present. It has been reported on Gorgona Island since (Dapporto 2002), on Elba Island since 1908 (Biermann & Hesch 1982; Balletto *et al.* 2007), on Pianosa Island since 1998 (Dapporto *et al.* 1999) and on Giglio Island since 1908 (Rocci & Turati 1925). Our records are the first for Giannutri. Recorded from February to November.

3.5 Discussion

In this study, we reported several new taxa of diurnal pollinators for Giannutri, making it possible to compare the resulting richness of communities with those from the other islands of the Tuscan Archipelago. For bees, the richness of Giannutri aligns with the findings of Forbicioni *et al.* (2019), who observed a clear species-area relationship across the six other islands of the Tuscan Archipelago: 133 species on Elba Island (223.5 km²), 69 on Giglio Island (21.2 km²), 42 on Capraia Island (15.8 km²), 31 on Montecristo Island (10.4 km²), 22 on Pianosa Island (10.3 km²), and three on Gorgona Island (2.2 km²). Our data for the island of Giannutri (2.6 km² and 14 species) fit this pattern and suggest that Gorgona Island is likely undersampled. For butterflies, our surveys add 10 new species to the existing records for Giannutri, bringing the total to 23 species. However, if we exclude *G. rhamni*, *A. paphia*, *N. polychloros* and *A. io* (all observed only once, likely as dispersing individuals) the species found across multiple years of sampling total to 19. The butterfly species-area relationship is less clear than that observed for bees, with approximately 52 species recorded on Elba Island in the last 20 years, 27 on Giglio Island, 19 on Capraia Island, 14 on Montecristo Island, 22 on Pianosa Island, and 20 on Gorgona Island (Dapporto *et al.* 2017). Thus, the butterfly species recorded on Giannutri closely match those on the similarly sized Gorgona Island. In contrast, Capraia and Montecristo islands, despite their larger size, host the same or fewer butterfly species. On Capraia Island, this is likely due to the extinction of several species linked to the reduction of woody habitats (Dapporto *et al.* 2017), whereas the strong isolation of Montecristo island likely explains the absence of several taxa (Dapporto & Cini 2007; Fattorini 2009). For hoverflies (Syrphidae), we recorded 14 species on Giannutri, all previously unreported for the island: comparisons

with other islands are not possible as the hoverfly fauna of the Tuscan Archipelago remains largely unstudied.

Most bee species were previously recorded by Cini *et al.* (2022). However, we also recorded several new species (*B. xanthopus*, *L. nitidulum, M. italica*, *N. sheppardana*, *H. crenulata*, *M. argentata* and *O. latreillei*) and revised the misidentification of *N. goodeniana*. Among these, the presence of *B. xanthopus* is particularly noteworthy. This species was first recorded on Giannutri in 2022, while in 2021 only *B. terrestris* was observed (Cini *et al.* 2022). During intensive sampling in 2023, hybrids with *B. terrestris* were detected, whereas in 2024 no *B. xanthopus* or hybrids were found. It is possible that *B. xanthopus* colonised the island in 2022, leading to hybridisation with the local *B. terrestris* population in 2023, but went extinct in 2024. Alternatively, *B. xanthopus* may have disappeared due to genetic swamping, which occurs when outbreeding depression is not severe, leading to the replacement of one or both parental lineages, typically the less common one, by hybrids (Todesco *et al.* 2016). Given that the number of *B. xanthopus* individuals on the island in 2023 was significantly smaller than that of *B. terrestris*, it is plausible that genetic swamping resulted in hybrids that are almost indistinguishable from *B. terrestris* by 2024. The risk of *B. xanthopus* being replaced by *B. terrestris* through hybridization on the Tuscan islands was recently highlighted by Boni *et al.* (2023). It has to be noted that specimens were identified morphologically using recent detailed descriptions (Boni *et al.* 2023), but genetic analyses are needed to confirm the presence and status of this taxon on the island. All other bee species were recorded consistently in different years, except for *Nomada* spp., *H. crenulata* and *O. latreillei*. The absence of these species in some years is likely due to their specific ecological traits: being brood parasites, *Nomada* spp. are elusive and relatively rare (Cini *et al.* 2022), while *H. crenulata* and *O. latreillei* primarily fly during periods when our sampling effort was reduced. Notably, we recorded a population of *N. sheppardana*, the first report of this species in the Tuscan Archipelago, underlining the importance of Giannutri for bee biodiversity within the National Park.

Among hoverflies, the presence of *E. narcissi* is particularly remarkable as it represents the first record for Italy. This is a species described on the basis of specimens from North America, where it has been introduced with *Narcissus* bulbs, its host plants, from Europe (Speight *et al.* 2013). It was first recorded in Europe (Southern France) in 2010, then in Corsica (Lebard *et al.* 2019) as well as in the Scilly Islands and Cornwall (Thomas *et al.* 2023). Its distribution in Europe is still little known, also due to its similarity to related species (Speight *et al.* 2013). The main host plant seems to be *Narcissus tazetta* (Speight *et al.* 2013) which very likely represents the main trophic resource for larvae on Giannutri. This plant spontaneously grows on Giannutri in the few maquis clearings of the southern part of the island (Foggi *et al.* 2011 and authors (pers. obs.), which is protected as an integral reserve. The occurrence of *C. rufa* is also noteworthy. This species, characterized by saproxylic larvae and arboreal adults, is typically associated with mature and senescent forests (Burgio *et al.* 2015; Speight 2024). On Giannutri, *C. rufa* was found in a *P. halepensis* grove, a tree species not previously associated with this hoverfly. Other hoverflies recorded on the island include migratory species such as *E. balteatus*, *E. tenax*, and *S. pyrastri* (Jensen 2001; Speight 2024), which likely use the island as a stopover during their migratory routes.

For butterflies, notable findings include *G. rhamni*, *A. io*, *N. polychloros*, and *A. paphia*. These species have few records in the Tuscan Archipelago (Dapporto *et al.* 2017). For *N. polychloros* and *A. paphia*, we suspect that their primary host plants (*Salix* spp. and *Viola* spp., respectively) are absent from the island, as we observed no such plants, and there are no freshwater bodies. This suggests that these species represent individuals dispersing from the mainland, likely facilitated by stochastic meteorological events (e.g., strong winds), as evidenced by the simultaneous collection of *G. rhamni* and *A. io* specimens on a single day. Such dispersal events raise concerns about including species in an insular fauna based on single records, a practice that could inflate estimates of richness, particularly for well-studied islands.

Most of the remaining 19 butterfly species, confirmed in multiple years over the past 25 years, likely represent a stable core community. Of these, 13 are

widespread across the Archipelago and probably undergo recurrent immigration events to Giannutri (e.g., *C. crocea*, *V. cardui*, *L. pirithous*, *L. boeticus*, possibly *P. brassicae* and *V. atalanta*). The other six species (*P. edusa*, *A. agestis*, *P. icarus*, *P. aegeria*, *L. megera*, and *C. corinna*) appear to have resident populations on the island and are also common on the nearby Tuscan mainland. Unlike other islands such as Elba, Giglio, Gorgona and Montecristo, Giannutri lacks any insular subendemic butterfly taxa (e.g. *Hipparchia aristaeus* (Bonelli, 1826), *Hipparchia neomiris* (Godart, 1823), *Plebejus bellieri* (Oberthur, 1910), *Lasiommata paramegaera* (Hübner, [1824])). Two butterfly species (*C. jasius* and *C. corinna*) are included in the Tuscan red list and protected under a regional law (56/2000).

Our study significantly reduces knowledge gaps regarding pollinators on Giannutri and, on a larger scale, in the Tuscan Archipelago. Future studies should aim to determine the resident status of recorded species, especially hoverflies, many of which were observed only once. Integrating molecular tools and targeted experiments will help clarify species composition, colonisation dynamics (recurrent vs. sporadic), and their roles in pollination and ecosystem services. Such data would enhance biogeographical analyses and inform evidence-based conservation and monitoring strategies.

Although collected on a small island, these findings are significant as Giannutri is part of a National Park actively involved in pollinator surveys under Italy's Biodiversity Directive (2019). This directive, aligned with the European Biodiversity Strategy for 2030, underscores the importance of addressing pollinator declines as a conservation priority.

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Chapter 4. Colour pattern and DNA-barcoding reveal a wide distribution of the insular endemic bumble bee *Bombus xanthopus* in the Tuscan Archipelago

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4.1 Abstract

Insular ecosystems, due to their isolation from the mainland and limited area, are often endemism hotspots. However, the same factors that drive high endemicity also make islands vulnerable to anthropogenic pressure and thus to biodiversity loss. We focused on a taxon of emerging conservation interest, the insular endemic bumble bee *Bombus xanthopus*, in the Tuscan Archipelago. It was formerly known to occur in Corsica and in two of seven islands of the archipelago where it hybridises with the widely distributed *Bombus terrestris*. We conducted distributional, morphological and molecular analyses of *B. terrestris-xanthopus* populations across the seven islands to update the distribution of *B. xanthopus* and characterise its morphological and molecular distinctiveness from *B. terrestris*. We found that *B. xanthopus* and intermediate colour morphs occur more widely than previously reported, being present across the entire archipelago. Significant associations between typical morphs of both *B. xanthopus* and *B. terrestris* and COI haplogroups highlight the reliability of both markers in identifying the two taxa (more than 80% of individuals had concordant colour patterns and genetic markers). Despite evidence of hybridisation, our findings indicate that complete discordance between morphological and molecular markers is uncommon. Moreover, most intermediate individuals (12 out of 14) carried *B. xanthopus* haplotypes. The absence of *B. xanthopus* from mainland Tuscany suggests possible relictualism phenomena. This study provides a comprehensive update on the distribution and genetic structure of *B. xanthopus*. Our results show that targeted efforts are essential to protect this unique component of the Tuscan Archipelago pollinator community. As these islands represent a biodiversity hotspot, where evolutionary processes and conservation challenges intersect, we encourage national park authorities and policymakers to continue prioritising the protection of pollinators of the Archipelago in their conservation plans.

Key policy highlights

- The insular endemic *Bombus xanthopus*, an important taxon of the Tuscan Archipelago's biodiversity, has a broader distribution than previously thought
- *B. xanthopus* seems to hybridise with *B. terrestris* due to the existence of specimens with intermediate colour patterns
- *B. xanthopus* faces the threat of being locally replaced by *B. terrestris* and thus the archipelago's population needs to be thoroughly studied and monitored
- The introduction of commercially reared bumblebees should be carefully regulated on these islands to preserve the insular endemic *B. xanthopus*.

Keywords: Apoidea, Hymenoptera, hybrid, mitochondrial DNA, pollinator conservation

4.2 Introduction

Archipelagos are remarkable biodiversity hotspots and offer natural laboratories for evolution and ecology studies (Whittaker et al., 2017; Whittaker & Fernández-Palacios, 2007). However, the intrinsic characteristics that define island environments—such as isolation, a limited area, and natural fragmentation—make their populations highly vulnerable to environmental changes (Fernández-Palacios et al., 2021). The adoption of effective conservation practices to mitigate biodiversity loss firstly requires identifying the evolutionarily significant units to be protected and determining their distribution within the target archipelago (Casacci et al., 2014). Achieving this goal is often hindered by a limited understanding of colonisation dynamics and potential hybridisation among island populations together with unknown species genetic makeup and distribution patterns. Such challenges hinder the ability to set clear conservation priorities and determine their spatial allocation (Allendorf et al., 2001; Fitzpatrick et al., 2015).

The Tuscan Archipelago, positioned at the crossroad of two centres of endemism - the Italian Peninsula and Corsica island - hosts peculiar biodiversity patterns. Endemic species from these regions occupy different islands, with their distribution shaped by both deterministic and stochastic processes. This dynamic results in unique communities where endemisms coexist in combinations found nowhere else (Barbato, 2018; Dapporto et al., 2017; Dapporto & Cini, 2007; Fattorini, 2009, 2010). In certain cases, these species hybridise on some islands as demonstrated in butterflies, e.g. *Lasiommata megera* (Linnaeus, 1767) and *Lasiommata paramegaera* (Hübner, [1824]) (Platania et al., 2020).

Among bees, the bumble bee *Bombus xanthopus* Kriechbaumer, 1870 occurs in Corsica in Sardinia (Annessi et al., 2025) and in two islands of the Tuscan Archipelago (Elba and Capraia), where it is reported to hybridise with *B. terrestris* (Linnaeus, 1758) (Boni et al., 2023; Rasmont et al., 2008; Rasmont & Quaranta, 1997). Concerns have been raised about the conservation status of *B. xanthopus* due to the islands' colonisation by *B. terrestris,* with consequent

hybridisation events (Boni et al., 2023; Lecocq, Brasero, et al., 2015). The taxonomic status of *B. xanthopus* is still debated. Several lines of evidence support the identification of *B. xanthopus* as a subspecies of *B. terrestris*: the presence of individuals exhibiting intermediate morphology, consistent with natural hybridisation, the successful production of fertile hybrid offspring under laboratory conditions from both parental combinations, and little genetic differentiation (De Jonghe, 1986; Estoup et al., 1996; Rasmont et al., 2008). However, other data - such as a different composition of labial secretions, considered as good support for a split in species delimitation techniques, and differences in phenology and pathogen resistance - might support the classification of *B. xanthopus* as a distinct species (Lecocq, Brasero, et al., 2015; Lecocq, Coppée, et al., 2015; Williams, 2021). This likely represents a case of ongoing speciation favoured by island isolation. Regardless of its taxonomic status, *B. xanthopus* is undoubtedly one of the most distinctive taxa of the bee fauna in the Tuscan Archipelago requiring urgent conservation attention (Boni et al 2023).

In this study, we performed faunistic, morphological and molecular investigations on *B. terrestris* and *B. xanthopus* and their hybrids in the Tuscan Archipelago in order to: 1) update the distribution of the endemic *B. xanthopus,* 2) assess whether individuals displaying the *B. xanthopus* colour pattern share the same mitochondrial COI haplotypes across islands and with typical *B. xanthopu*s from Corsica (Williams, 2021), 3) assess to what extent individuals with intermediate colour patterns possess COI sequences belonging to *B. xanthopus* or *B. terrestris* haplogroups. These data provide important faunistic and biogeographic foundations for the conservation of this endemism that represents a distinctive element of the Tuscan Archipelago National Park.

4.3 Materials and Methods

Data collection

The seven islands of the Tuscan Archipelago (Fig. 1) were surveyed at least once per year from 2019 to 2024. During these fieldwork sessions, opportunistic

searches were also conducted to enhance the knowledge of the islands' pollinator communities. *B. terrestris* and *B. xanthopus* were among the most targeted taxa due to the endemic status of the latter. Specimens exhibiting intermediate characteristics (queens and workers) in terms of the chromatic pattern of the thorax, abdomen and legs, as well as specimens with a colour pattern typical of *B. terrestris* and *B. xanthopus*, were collected, euthanised using ethyl acetate, and transported to the laboratory, where they were stored at -20°C until preparation. During preparation, a single meso-thoracic leg (or a portion of it) was removed from each specimen for COI sequencing.

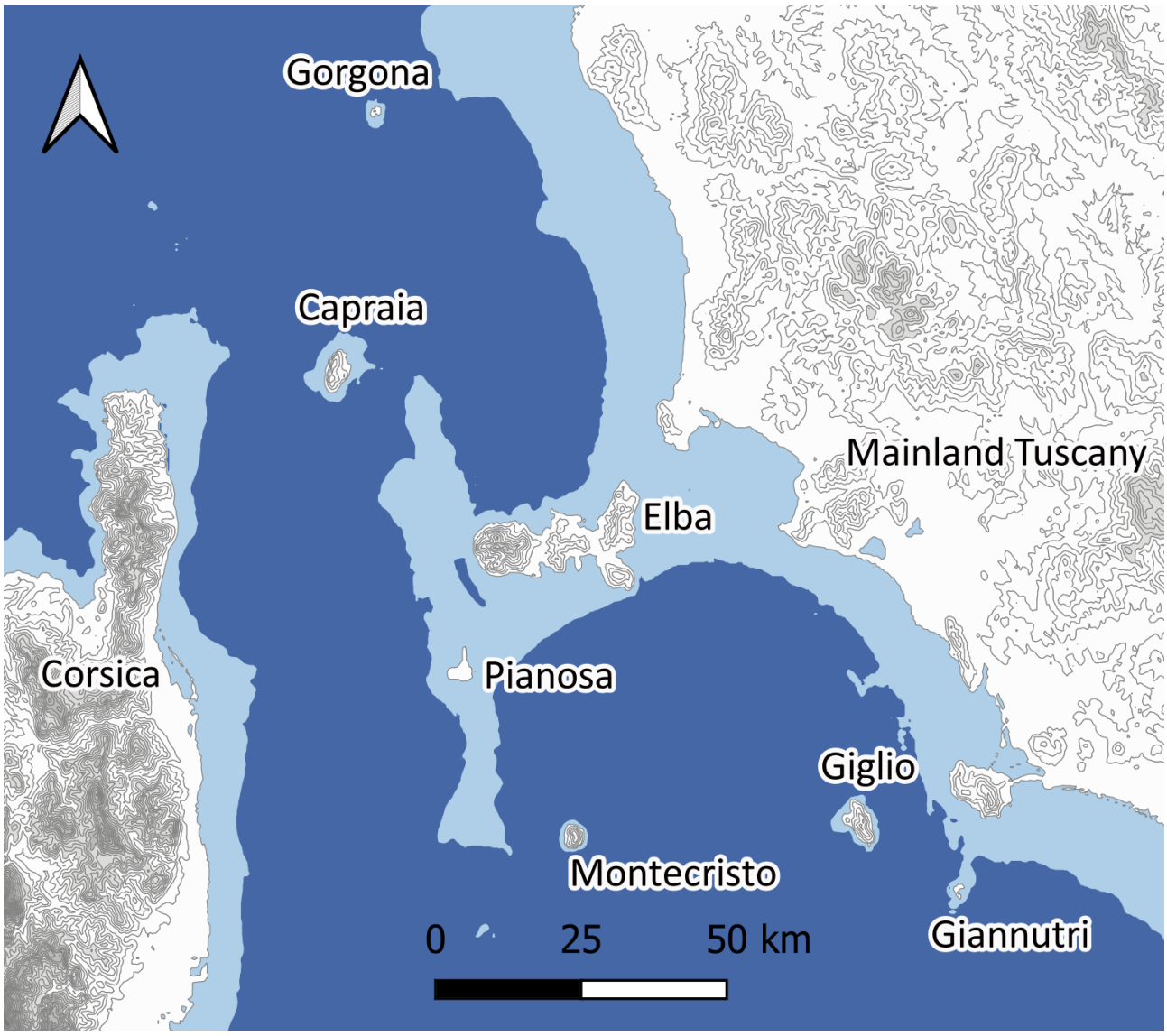

Figure 1. A map of the Tuscan Archipelago islands. The light blue area shows the 100 m isobath highlighting the Pleistocene connection between islands and mainland.

Morphological identification

B. terrestris specimens were identified based on their colour pattern following the description by Boni et al. (2023): black facial and vertex hair, a broad yellow collar extending slightly below the tegulae, with isolated and sparse black hairs at the collar margins; the remainder of the thorax covered in black hairs, legs with black cuticle covered in short black hairs, black corbicula bristles, tergites 1 and 3 black; tergite 2 yellow, tergites 4 and 5 ivory white, and tergite 6 covered with short black hair (Fig. 2 a,b). Typical *B. xanthopus* specimens were identified as having a black thorax with the collar absent or limited to a few yellow hairs, tergites 1, 2, 3 entirely black or with sparse yellow hairs, tergite 4 to tergite 6 reddish, legs with reddish cuticle and entirely reddish corbicula bristles (Fig. 2 e,f). Potential hybrids were identified as specimens exhibiting intermediate characteristics with special reference to the presence of a reddish leg cuticle and reddish corbicula bristles (Fig. 2 c,d).

Figure 2. Colour pattern variations in *B. xanthopus* and *B. terrestris* from the Tuscan Archipelago: a) a specimen from Giannutri island (BIBSA2762-22) showing concordant *B. terrestris* colour pattern and COI haplotype; b) a unique specimen (BIBSA3126-23) from Elba island showing a *B. terrestris* colour pattern but a *B. xanthopus* haplotype c) a morphologically intermediate specimen from Elba island (BIBSA3123-23) presenting a *B. xanthopus* haplotype and d) a morphologically intermediate specimen from Pianosa island (BIBSA3127-23) showing a *B. terrestris* haplotype; e) a specimen showing a typical *B. xanthopus* colour pattern and COI haplotype (BOLD ID BIBSA3121-23); f) a morphological *B. xanthopus* from Giannutri island (BIBSA2771-22) carrying a *B. terrestris* haplotype.

Molecular identification

COI sequencing was performed in the iBOL laboratories (Guelph, Canada) for 37 specimens following standard protocols for DNA barcoding (deWaard et al., 2008). For 16 specimens, dry legs were grinded with a sterile pestle, then 70 µl of lysis buffer (150 mM Tris-SCl, 100 mM NaCl, 65 mM DTT, 0.5 mM EDTA pH8, 450 mg/ml Proteinase K; modified from Cilia et al., 2022) was added and the sample incubated at 56°C for 2 hours in a shaker set to750 rpm. Proteinase K was then deactivated at 70°C for 10 minutes and the reaction cooled in the fridge for 10 minutes. The digested material was centrifuged at 13000 rpm for 1 minute and the supernatant was transferred to a clean Eppendorf and cleaned with 1.8 volumes of GeneMAGNET PCR Clean-Up beads (EURX) according to the manufacturer's instructions. Purified DNA was resuspended in 50 µl of nuclease-free, sterile ddH2O. We used the HCO1490 + LCO2198 (Flomer, 1994) primers with a standard PCR mix. The PCR profile for amplification was as follows: 5 min initial denaturation at 95°C, followed by 30 s denaturation at 95°C, 90 s annealing at 51°C, 60 s elongation at 72°C for 35 cycles and 10 min of final elongation at 72°C. PCR products were visualized through 1.5% agarose gel electrophoresis stained with Midori Green (Nippon Genetics) and purified with the Enzymatic Post-PCR immediate Clean-up kit (EPPiC Fast; A& A Biotechnology). Sequencing reactions were carried out in a total volume of 10 µl containing: 1.0 µl 5 × buffer, 0.5 µl BriliantDye® Terminator v3.1 (Life Technologies), 0.15 µl of primer (10 pmol/µl), 2.0 µl of the purified PCR product, and 6.35 µl of ddH2O. Sequencing settings were as follows: initial denaturation at 96°C for 1 min, followed by 25 cycles of denaturation at 96°C for 10 s, annealing at 55°C for 5 s, and elongation at 60°C for 4 min. Sequencing products were then purified with the ExTerminator kit (A&A Biotechnology) and suspended in 25 µl of formamide. Sequencing products were read with the ABI 3130xl sequencer in Genomed (Warsaw, Poland).

Previously sequenced and publicly available sequences of all *B. terrestris* subspecies and *B. xanthopus*, following the latest molecular classification (Williams, 2021), were retrieved from BOLD and curated to remove misidentified specimens, such as individuals mislabeled as other species without reported introgression (Dincă et al., 2021). Only specimens with a clear geographic indication and sequence length of at least 400 bases were included in the analyses, comprising two specimens of *B. xanthopus* from Corsica and Elba islands published in previous papers (Williams, 2021). We only kept sequences of natural populations, excluding data publicly available for commercially-reared colonies. A total of 439 sequences (386 retrieved from BOLD and 53 provided through this study) were used and are provided in the BOLD System dataset ECCEBMB. Three sequences were excluded from the haplotype network analysis due to low overall quality, but they were retained in the analyses of the association between colour pattern and haplogroup as their diagnostic sites were of sufficient quality.

A haplotype network was inferred using TCS 1.21 (Clement et al., 2000) with a 95% connection limit (11 steps) and visualised with TCS Beautifier (Múrias Dos Santos et al., 2016). In the resulting haplotype network, specimens from our dataset were coloured according to their colour pattern, whereas the remaining sequences were left white.

Comparisons between morphological and molecular identification

We compared morphological identification based on colour patterns with molecular identification based on COI sequences to evaluate the correspondence between colour patterns and the detected haplogroups. Specifically, we addressed the following questions:

1. To what extent is the typical colour pattern associated with the haplogroups of *B. terrestris* and *B. xanthopus* across the islands of the Tuscan Archipelago?

2. To what extent is the frequency of the intermediate colour pattern associated with each of the two haplogroups?

We evaluated these research questions by building a contingency table with the number of specimens for each island, presenting either *B. xanthopus*, *B. terrestris* or hybrid colour patterns (sensu Boni et al., 2023) and their genetic membership (the identification provided by DNA barcoding) and applied a Chi squared test to their frequencies.

4.4 Results

Previous studies on the distribution of *B. xanthopus* reported this taxon only on the islands of Capraia and Elba within the Tuscan Archipelago. We sampled 65 individuals from which we obtained 53 good-quality sequences. We did not obtain sequences from all the intermediate morphs from Giglio island (5 samples). Individuals displaying the typical *B. xanthopus* colour pattern or intermediate morphs are present on all Tuscan islands (typical *B. xanthopus* colour pattern on Capraia, Elba, Pianosa, Montecristo and Giannutri, whereas intermediate morphs are also present on Gorgona and Giglio) (Table 2, Fig. 4). On Elba Island we found only *B. xanthopus* haplotypes, while on Giglio island we found only *B. terrestris* haplotypes. Intermediate morphs exhibited a disrupted colour pattern with varying degrees of similarity to either *B. xanthopus* or *B. terrestris*, but all shared the distinctive feature of reddish legs.

A haplotype network based on 436 COI sequences — an analysis that, to our knowledge, has not been conducted before for the *B. terrestris-xanthopus* group — revealed that *B. xanthopus* falls within the genetic variability of *B. terrestris*. It forms a distinct clade consisting of four haplotypes, each separated by a single mutation, and shows a minimum distance of three mutations from the main *B. terrestris* haplotype across the 658 bp maximum length of our COI dataset (Fig. 3). The main *B. xanthopus* haplotype showed five diagnostic mutations compared to the main *B. terrestris* haplotype in the barcoding region. Diagnostic loci are indicated in Table 1. Some rare haplotypes found in Iberia are separated by only one mutation from *B. xanthopus* (Fig. 3).

The only available sequence from the Sardinian population, *Bombus terrestris sassaricus*, also displayed a distinct COI signature, with its closest relatives found within the haplogroup composed of specimens from the British Isles (Fig. 3).

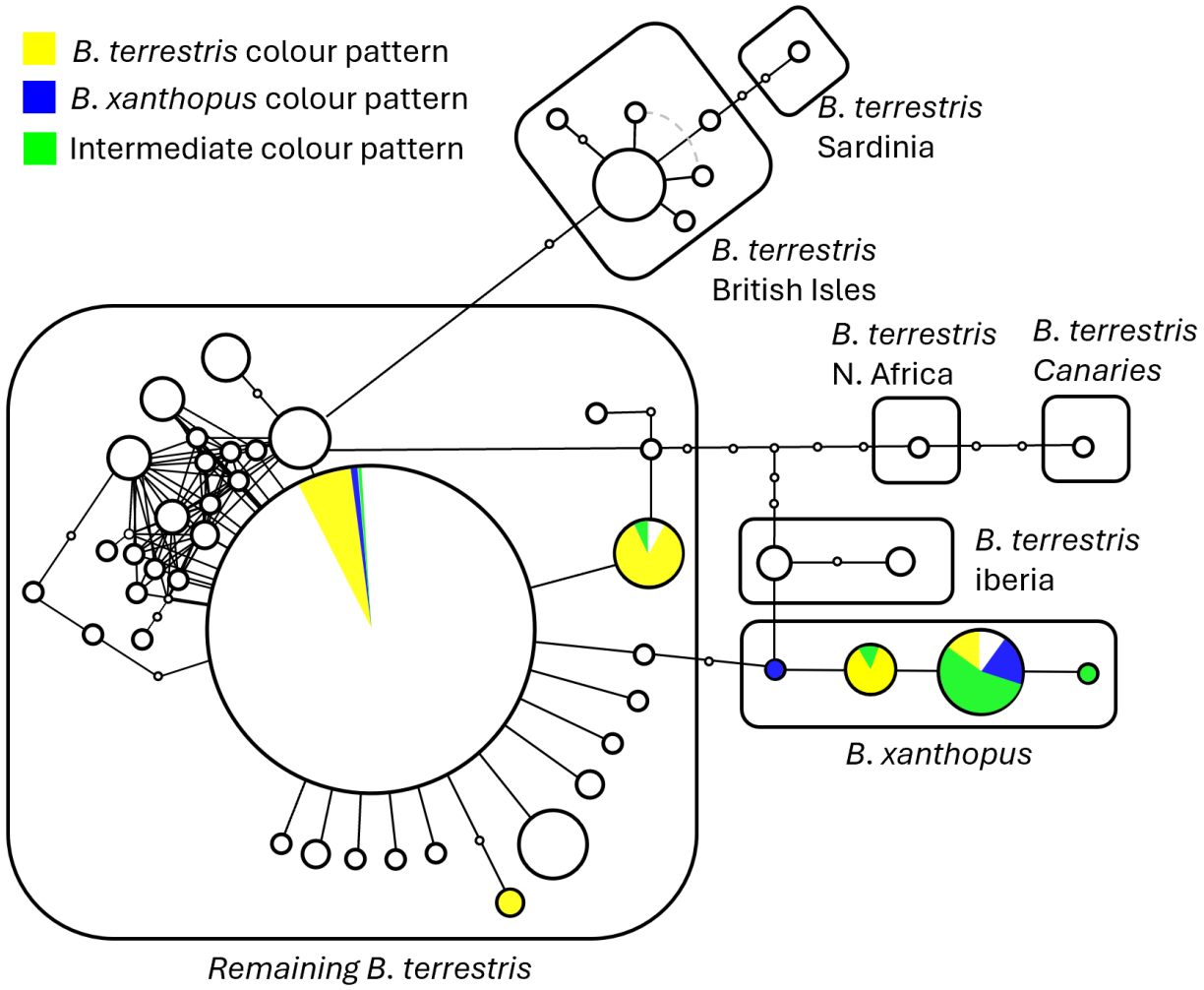

Figure 3. A TCS haplotype network of COI sequences available in literature (white) and those belonging to our samples from Tuscan islands (coloured). The *B. xanthopus* box represents the haplogroup to which a Corsican specimen available in literature belongs (indicated in white since the colour pattern is unknown) and to which most specimens with a *B. xanthopus* colour pattern (blue) and hybrid specimens (green) are associated. The specimens with typical *B. terrestris* colour patterns are indicated in yellow.

Figure 4. A schematic representation of the colour morphs found on each island (*B. terrestris*, *B. xanthopus* and intermediate morphs) and the fraction of *B. terrestris* haplotypes (black diagonal stripes pattern) and *B. xanthopus* haplotypes (black) collected on each island. The asterisk (*) on the intermediate morph on Giglio Island indicates that it was not possible to obtain COI sequences from these specimens.

We found a strong association between colour patterns and haplogroup, as revealed by the chi-square analyses. A chi-square test on the frequencies of the two haplogroups in typical individuals (Table 2, rows 1 and 2) revealed a significant association between colour pattern and haplogroups ($\chi^2 = 16.39$, df = 1, $P < 0.0001$). Specifically, 81.2% of *B. xanthopus* individuals identified by

colour pattern carried *B. xanthopus* haplogroups, while 88.0% of *B. terrestris* individuals carried *B. terrestris* haplogroups. Moreover, 14 out of 16 individuals with the intermediate colour pattern (Table 2, row 3) carried *B. xanthopus* haplogroups while the other two belonged to *B. terrestris* haplogroups. This pattern showed a significant association (expected frequency = 0.5, $\chi^2 = 9.0$, df = 1, $P = 0.0027$).

	160	197	220	536	562
B. terrestris	A	C	C	T	C
B. xanthopus	G	T	T	C	T

Table 1. Diagnostic loci of a typical 658 bp COI barcode sequence.

	B. xanthopus (COI)	*B. terrestris* (COI)
B. xanthopus (colour pattern)	Capraia: 3 Giannutri: 6 Pianosa: 1 Elba: 1	Giannutri: 1 Montecristo: 1
B. terrestris (colour pattern)	Elba: 1 Montecristo:2	Capraia: 2 Giannutri:10 Gorgona: 3 Montecristo: 1 Pianosa: 2 Giglio: 4
Intermediate (colour pattern)	Elba: 4 Gorgona: 3 Capraia: 2 Pianosa: 3 Giannutri: 1 Montecristo: 1	Pianosa: 1 Montecristo: 1

Table 2. A contingency table showing the number of specimens analysed for each island, their morphological (colour pattern typical of *B. xanthopus, B. terrestris* or intermediate) and molecular (COI membership) features. Rows indicate the colour pattern identity and columns the COI genetic membership.

4.5 Discussion

Field surveys across all seven islands of the Tuscan Archipelago revealed that the distribution of individuals displaying a colour pattern typical of *B. xanthopus* is broader than previously reported in literature (Boni et al., 2023; Intoppa et al., 1995; Rasmont & Quaranta, 1997). *B. xanthopus* morphs were collected on all islands, except Giglio and Gorgona, while *B. terrestris* and intermediate individuals occurred throughout the archipelago. Thus, *B. xanthopus* morphs were observed in sympatry with individuals showing a colour pattern typical of *B. terrestris.* The mitochondrial COI marker revealed that, across the various islands, *B. xanthopus* and intermediate colour morphs are strictly associated with haplotypes found in Corsica and Elba islands and indicated as diagnostic for this taxon in literature (Williams, 2021). Similarly, individuals with the *B. terrestris* colour pattern predominantly exhibit haplotypes typical of this taxon in the western Palearctic region (Williams, 2021). Both *B. terrestris*-associated and *B. xanthopus*-associated haplotypes have been found on six islands (being absent on Elba and Giglio, respectively). Whether this absence reflects the actual *Bombus* community of these islands or is simply due to a limited number of samples should be evaluated by future studies including a greater sampling effort, specifically on these two islands.

Because most of the intermediate individuals from other islands carried a *B. xanthopus* haplotype, it is likely that also intermediate individuals from Giglio island (from which we did not obtain good-quality sequences) exhibit a similar haplotype. Surprisingly, *B. xanthopus* morphs and haplotypes are not reported from mainland Tuscany, even though it is separated by only 6.5 km from Elba Island, and it was connected to some of the islands inhabited by *B. xanthopus* until the end of the last glacial cycle (Bossio et al., 2000).

Regardless of their still debated taxonomic status (whether *B. xanthopus* is a standalone species or a subspecies of *B. terrestris*) (Estoup et al., 1996; Lecocq, Coppée, et al., 2015; Rasmont et al., 2008; Williams, 2021), the

comparison of morphological and mitochondrial DNA markers reveals a strong association between these traits. Intermediate individuals, unlike the case of a single male specimen reported from mainland Tuscany in 1999 (Quaranta & Felicioli, 2012), occur in the presence of typical morphological forms (either *B. terrestris* or *B. xanthopus* or both) on all seven islands. Most of them (12 out of 14) show *B. xanthopus* haplotypes, indicating a possible phenomenon of asymmetric introgression (male *B. terrestris* x female *B. xanthopus* is likely more frequent than the opposite). Asymmetric introgression revealing discordant and directional genotypic and phenotypic mismatches suggests that, while hybridisation between these two taxa is frequent, some form of reproductive barrier involving reduced hybrid fitness did emerge during the evolution of these taxa. A detailed investigation of these phenomena lies beyond the scope of this study, as it would necessitate approaches such as genomic analyses, behavioural studies, and evaluations of F1 and F2 hybrid fitness. Specifically, while the analysis of COI and colour patterns can provide useful insights, it cannot conclusively demonstrate the presence of F2 individuals, as completely discordant specimens may still fall within the phenotypic range of hybrids. For similar reasons, we will not conclude whether *B. xanthopus* should be considered a subspecies or a distinct species from *B. terrestris*. However, we note that the number of COI mutations separating specimens with a *B. xanthopus* morphology from typical *B. terrestris* is very small (1–3 mutations over >600 bp). We can however affirm that the high concordance between morphological and molecular markers suggests that they can be used independently to identify the presence of either species on an island with a high degree of certainty. Moreover, the presence of specimens with either a *B. xanthopus* colour pattern or intermediate patterns is a strong indication for the occurrence of typical *B. xanthopus* haplotypes. Future studies should characterise more precisely their status on each island (i.e. resident or adventive) through long-term monitoring and population size estimates.

The updated distribution of *B. xanthopus* deserves further discussion since it can provide insight into its conservation. Our data indicate that *B. xanthopus* inhabits Corsica and most islands of the Tuscan Archipelago while

being virtually absent from mainland Tuscany. Comparative studies combining genetic and morphological data for butterflies in the Tuscan Archipelago yielded similar results, offering a potential interpretation for the observed pattern. The occurrence of the same genetic lineages on Corsica and the Tuscan Archipelago, and their absence from mainland Italy, has been documented for butterfly species such as *Maniola jurtina* (Linnaeus, 1758), *Plebejus bellieri* (Oberthür, 1910), *Callophrys rubi* (Linnaeus, 1758), and *Hipparchia aristaeus* (Bonelli, 1826) (Dapporto et al., 2017) and interpreted as a complex scenario of double filtering (geographical and ecological) and/or relictualism phenomena (Dapporto et al., 2017; Dapporto & Cini, 2007; Fattorini, 2009). In *L. megera* and *L. paramegaera*, the Capraia population displays a typical L. *paramegaera* morphology but has a mixed genetic makeup with *L. megera*, while the population from Montecristo shows intermediate morphology and typical *L. paramegaera* nuclear and mitochondrial markers (Platania et al., 2020). The most similar haplotypes to those of *B. xanthopus* have been found in several specimens from the Iberian Peninsula, whereas B*. terrestris sassaricus* from Sardinia shows affinities with the *B. terrestris audax* subspecies from the British Isles (Fig. 3) but in a recent paper (Annessi et al., 2025) it has been showed that *B. xanthopus* morphs also occur in Sardinia and *Bombus terrestris sassaricus* shows very similar COI sequences to *B. xanthopus* from Corsica.

The distribution of *B. terrestris/xanthopus* on the studied islands suggests that this taxa have likely experienced a recent series of genetic sweeps driven by haplotypes with adaptive advantages, after the commencement of the marine transgression at the end of the last glacial period (which occurred ~11,600 years ago) (Arif et al., 2021; Dapporto & Bruschini, 2012). In the butterfly *M. jurtina*, the morphological and genetic differentiation between Corsica, the Tuscan Archipelago and mainland Tuscany persists along the Italian peninsula to Sicily, including the Campanian Archipelago (Dapporto et al., 2014). This has been attributed to the existence of an ancestral population shared between islands and the Italian mainland, which had subsequently been replaced on the mainland by a new population, likely arriving from the east, through an adaptive sweep (Dapporto & Bruschini, 2012). The islands, owing to their, albeit modest,

isolation, remained relatively unaffected by this event, preserving their original populations, a process similar to what has been documented for mammals (Masini et al., 2008). Similar patterns are evident on a broader scale in other species. For instance, in butterflies such as *Coenonympha corinna*, *Polyommatus celina/icarus* and *Aricia agestis/cramera* (Dapporto et al., 2017; Vodă et al., 2015), differentiation is observed between Corsican and Sardinian populations, despite both islands having been connected to each other during relatively recent cold periods. Furthermore, *P. icarus* displays differentiated haplogroups in marginal and distant areas like the Hebrides, Crete, and the Sierra Nevada (Arif et al., 2021).

Our findings have thus important implications for the conservation of the endemic *B. xanthopus*. In the Tuscan Archipelago, the two bumble bee taxa may currently be in an unstable equilibrium, with the potential for *B. terrestris* to eventually replace *B. xanthopus* populations. Although this phenomenon should be considered a natural process, the coexistence of these two taxa may be promoted by the reduced propagule pressure of *B. terrestris* on islands. Our observations of the genetic lineage distribution reinforce the conclusions of Boni et al. (2023), who argue that the anthropogenic facilitation of *B. terrestris* dispersal poses a significant risk to small *B. xanthopus* populations in the Tuscan Archipelago. If this taxon is to persist, every effort must be made to preserve populations inhabiting these islands, by regulating the importation of commercial bumble bees and honey bees, which may compete with or transmit pathogens to local bumble bee populations (Graystock et al., 2013; Iwasaki & Hogendoorn, 2022; Lázaro et al., 2021; Mallinger et al., 2017). Once the original populations on these islands are lost, recolonisation will likely proceed from the nearby mainland, leading to the disappearance of the endemic *B. xanthopus*.

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Disclosure statement

The authors report there are no competing interests to declare

Data availability statement

The data that support the findings of this study (COI sequences already available and obtained from this study) are available in the BOLD System within the dataset DS-ECCBMB and in GenBank (Accession numbers XX00000 - NN).

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Chapter 5. Estimating wild bee population size with validated distance sampling.

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5.1 Abstract

Pollinators play a critical role in ecosystem services, yet their populations are declining globally highlighting the urgent need the accurate methods to monitoring their densities and abundances. Distance sampling (DS) is a promising, non-invasive method underused for estimating insect populations, but it has not yet been validated for this taxon. We addressed this methodological gap by validating DS estimates, comparing estimated number of foraging honey bees (*Apis mellifera*) with the known reference population of managed colonies, estimated using a modified Liebefeld method. Field surveys were conducted on Giannutri island (Italy) across 41 transects spanning five environmental units over 12 days. Our abundance estimates for honey bees foraging on the island were consistent with the proportion of individuals performing external tasks reported in literature. DS applied to the two most common wild pollinators on the island, *Anthophora dispar* and *Bombus terrestris*, revealed: 1) a strong consistency in abundance estimates across consecutive days; 2) significant effects of weather conditions on detected abundances; 3) temporal trends matching the known phenology of adults in both species; 4) marked differences in densities across environmental units consistent with non-biological traits. In conclusion, DS offers a practical, non-invasive method for obtaining reliable insect population data, addressing a critical need in the context of global pollinator declines. Its efficiency and adaptability make it particularly valuable in resource-limited settings where traditional methods are impractical.

Keywords: density estimates, population size, insular ecosystem, pollinators, insect monitoring

5.2 Introduction

Insect pollinators play a critical role in ecosystem services including pollination, nutrient cycling, and maintaining food web dynamics (Kevan & Baker, 1983; Winfree et al., 2007). However, their populations are undergoing alarming declines worldwide due to habitat loss, pesticide use, climate change and pollution (Abrol, 2012; Vanbergen et al., 2013). Accurate monitoring of insect population is critical to inform conservation strategies and address the causes of their decline (Geldmann et al., 2023; Warren et al., 2021). Estimating insect abundance remains a complex task in biodiversity conservation (Buckland & York, 2017; Prendergast & Ollerton, 2021) due to their the high mobility, large population sizes and temporal fluctuations typical of insect populations (Didham et al., 2020), which complicates the implementation of reliable and efficient estimation methods (Callaghan et al., 2024).

One widely used approach to track changes in relative abundance of insect populations over time is the Pollard transect (Pollard, 1977), involving systematic counts along fixed paths. While effective, particularly for butterflies (Parmesan et al., 1999; Warren et al., 2021), this method does not provide estimates of population size or density (Haddad et al., 2008) with confidence intervals. Moreover, it cannot account for variation in detectability of individuals that can vary significantly among species and/or environments (Kral et al., 2018; Pollard & Yates, 1993), limiting its utility for comprehensive assessments.

Estimating absolute population size is crucial for evaluating population trends over time (McNeil et al., 2019), determining minimum viable population sizes and assessing conservation status of endangered or potentially endangered insect species (IUCN, 2024; Callaghan et al., 2024). Accordingly, most insect species listed as threatened on the IUCN Red List fall under criteria B or D2 (small geographic range; very small geographic range + imminent threat) (Edgar, 2025). This gap in methodology highlights the need for robust approaches that allow accurate and reliable population size estimation for insects, particularly in fragile and understudied ecosystems.

Capture-mark-recapture (CMR) is a common and effective technique for estimating absolute animal abundance with explicit measures of precision such as standard error (SE) (Buckland & York, 2017; Henderson & Southwood, 2000 and Briggs et al., 2022 for bees). While effective in various contexts, CMR presents limitations. Despite efforts to minimise the cost associated with marking and recapturing (e.g. Prado et al., 2017), these methods remain invasive. Handling and marking insects can disrupt their natural behaviours and raise ethical concerns, especially for sensitive species (Soulsbury et al., 2020). For instance, even a manipulation of few minutes reduced fecundity and hatching success in the butterfly *Pararge aegeria* (Gibbs et al., 2010). Moreover, CMR requires intensive sampling: accurate estimates demand sampling over 25% of populations smaller than 250 individuals and 10% for larger ones (Briggs et al., 2022). For very large populations, this may exceed the adult phenology. These limitations make CMR difficult to apply in contexts such as protected areas, where handling can harm the species, or remote locations, such as islands or mountain peaks, where consistent sampling is logistically challenging (Banaszak, 1996; Henderson, 2002).

To address these challenges, distance sampling (DS) offers a feasible and non-invasive alternative for estimating population size, widely used in vertebrates (Buckland et al., 1993, 2001, 2015; Cassey & Mcardle, 1999). DS estimates population density by recording individuals observed along transects or from observation points and modelling how detection probability decreases with distance from the observer. This approach overcome the two main limitations of CMR: it avoids handling or marking individuals, minimizing disturbance, and it does not demand sampling high percentages of the population (Buckland et al., 2001; Thomas et al., 2010).

Studies applying DS to insects highlighted its potential to address imperfect detection, especially when detectability depends on traits like body size, behaviour, or environmental conditions. For instance, DS has been used to estimate bumble bee and ant nest densities, accounting for factors such as vegetation cover and nest size (Baccaro & Ferraz, 2013; McNeil et al., 2019).

Similarly, odonates densities have been estimated using detection model tailored to their behaviours and habitats (Darshetkar et al., 2023). Across studies on insects, DS consistently demonstrated that observation probability decreases with distance, underscoring the need to adjust specimen counts based on observer distance to avoid underestimating densities and abundances (McNeil et al., 2019; Baccaro & Ferraz, 2013; Darshetkar et al., 2023). For instance, DS was compared to Pollard transects for butterflies and was shown to provide higher density estimates, especially for smaller species that are harder to detect (Patterson et al., 2023). Similarly, for bumble bees, DS provided density estimates that better reflect habitat preferences based on species knowledge compared to results obtained by netting and fixed-width transects (McNeil et al., 2019).

Despite its potential to provide robust estimates over large spatial scales with minimal disturbance, DS remains underused in insect ecology, partly due to the lack of validation studies comparing its estimates with known population sizes. In vertebrates, studies validated DS against accurately censused populations, such as managed *Ovis canadensis* (desert bighorn sheep) in restricted areas (Harris et al., 2020). This approach is more challenging for insects, which typically wild and lack definite reference sizes.

To address this gap, we conducted a validation study on the Mediterranean island of Giannutri (Italy). This isolated ecosystem, with limited size, low species richness and restricted insect immigration/emigration dynamics, offers ideal conditions for controlled research (Cini et al., 2022). Since 2018, the Tuscan Archipelago National Park has allowed the introduction of a temporary single apiary of 18 manged *A. mellifera* hives from December to June. We experimentally manipulated hive activity by closing all or a fraction of the hives, allowing us to test whether DS estimates reflected changes in honey bee abundance. Colony size was accurately estimated through hive counts with professional beekeepers (Dainat et al., 2020). Since it is well established in the literature that approximately one-quarter to one-third of honey bee workers are foragers (Khoury et al., 2013; Van der Steen, 2015), although with different

efforts (Arias-Calluari et al., 2023; Eckert et al., 1994; Klein et al., 2019; Pernal & Currie, 2001), and that a large portion (though not all) of their time budget is spent outside the colony (Seeley, 1995), we hypothesize that the fraction of bees estimated outside and inside the colony during peak activity time should be around 1:5 - 1:8, depending on the proportion of time spent outside the colony. It is thus possible to use the ratio between the independent estimates of active foragers obtained through DS and the count of bees inside the hive, measured using well-established methods, to validate the accuracy of the DS method for honey bees.

This setup enabled us to validate DS estimates for honey bee foragers and indirectly assess its applicability to the most common wild pollinators of the island like the social *Bombus terrestris* and the solitary *Anthophora dispar*. Our study aimed at testing the following *a priori* hypotheses: 1) the population size estimates of foraging *A. mellifera* obtained with DS match those inferred by direct measurement of colony size; 2) the estimates of estimate the active individuals of *A. mellifera*, *An. dispar* and *B. terrestris* are relatively constant in consecutive days and they vary according to weather conditions and adult phenology; 3) density estimates for the three species vary across the island environmental units, as expected being linked to pollinator abundance linked to vegetation types (Roulston & Goodell, 2011).

By validating DS for insect populations and exploring its ability to capture density variation, this research addresses important gaps in pollinator monitoring. These findings advance conservation tools for studying pollinators in complex and rapidly changing ecosystems and contribute to mitigating declines in key insect populations (Abrol, 2012; Potts et al., 2010).

5.3 Material and Methods

Study area and target species

The research was conducted on Giannutri island (Tuscan Archipelago National Park) from March 1 to April 15, 2024, over 12 field survey days. This 2.6 km² island, with a maximum elevation of 89.4 m, features a Mediterranean landscape, with most buildings concentrated in a small village. The Mediterranean maquis is dominated by *Teucrium fruticans*, *Salvia rosmarinus*, *Euphorbia dendroides* and *Juniperus turbinata*, interspersed with woodlands of *Quercus ilex* and *Phillyrea latifolia* (Foggi et al., 2011).

The island hosts about 10 wild Anthophila species (Cini et al., 2022). Among these, two were selected as target species: *An. dispar* and *B. terrestris* (family Apidae), which account for over 95% of wild bee sightings in spring (Cini et al., 2022). *An. dispar* is a solitary bee widely distributed across Europe and parts of the Mediterranean. Females nest in the ground, digging tunnels to lay eggs and store pollen and nectar for their larvae. This species is primarily active in early spring (Cini et al., 2022; Orr, 2017). *B. terrestris* is a social bumble bee broadly widespread in the West Palaearctic, known for its ecological adaptability. It nests underground, often in abandoned rodent burrows, and remain active most of the year, with colony cycles adapted to Mediterranean climates (Gurel et al., 2008). This species often produces multiple generations annually (Rasmont et al., 2008). Due to the island's isolation from nearby areas (12km from mainland and 15km from Giglio island), both species exhibit closed population dynamics. Since 2018, the island has hosted a temporary apiary of 18 *A. mellifera* hives from December to June (Cini et al., 2022). Four years of transect sampling (Pollard method) from mid-February to mid-October revealed that *An. dispar* peaks in late winter, while *B. terrestris* peaks in early spring (Pasquali et al., 2025).

Identification of transects

Distance sampling is a widely used method for estimating animal or plant populations. It involves walking along transects or observing from fixed points, with precise measurement of the distance between the observed individuals and

either the transect centreline or observation point. The method relies on four key assumptions: i) animals are distributed independently of transects or points; ii) animals on the line or at the point are detected with certainty; iii) distance measurements are accurate; iv) animals are detected at their initial location. Additionally, while transects should ideally be positioned randomly, this assumption can be relaxed under specific conditions (Buckland et al. 1993).

We identified suitable transects to represent the island's diverse environmental units while accounting for physical constraints. Environmental stratification was necessary, as pollinator abundance likely varies across units. Using the vegetation map by Foggi et al. (2011) and a high-definition Corine Land Cover map (1:10.000 scale with a 10 m resolution), we identified five environmental units: Urban, Meadow, Tall Shrubland (Tall_S), Low Shrubland (Low_S) and Woodland (Wood). These were cross-referenced and refined through satellite imagery and field validation (Fig. 1). Transects were selected to ensure environmental homogeneity, avoiding boundaries with a 15m buffer from other environmental units to minimise edge effects. A minimum spacing of 50m between transects was maintained when possible. Accessibility and operator safety were prioritized, but placement was constrained by factors such as fragmented areas, dense vegetation, rugged terrain, and property access issues in urban zones. Moreover, the southern part of the island includes a fully protected area where we had to limit the number of transects to already existing trails to minimize disturbance. In some cases, the 15m buffer could not be maintained, particularly along narrow trails in five transects (two Urban, two Meadows, one Low_S). Considering these constraints, we identified the maximum number of transects in the larger areas (Low_S and Tall_S) and then selected a reasonable number of transects in the remaining environmental units according to their relative size, to achieve a similar representation.

The final design included 41 transects across accessible units: 3 Urban, 5 Meadow, 11 Tall_S, 14 Low_S, and 8 Wood. Each transect was 30m long and 10m wide, marked with red-and-white tape at 10m intervals. Locations were digitally mapped in QGIS (Version 3.34.8) and GPS coordinates were recorded.

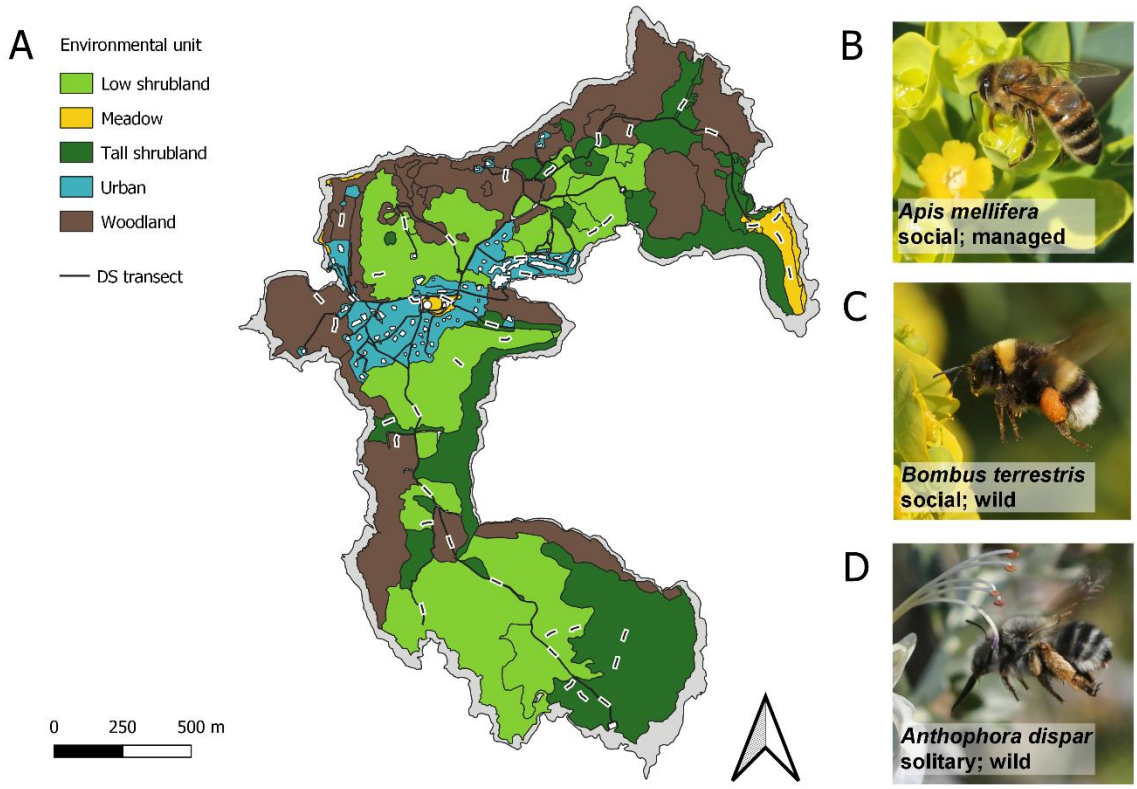

Figure 1 Giannutri island and the target species of the study. A) Map of Giannutri island showing the division of areas into environmental units and the location of transects used for distance sampling; B) *A. mellifera*; C) *B. terrestris*; D) *An. dispar.*

Operator Training and Sampling Procedure

Operators underwent two days of training to estimate distances to observed pollinators within five predefined bands (0–1m, 1–2m, 2–3m, 3–4m, 4–5m) until their estimates were consistent. The sampling team included four trained operators (MP, OB, CB, VS).

DS surveys were conducted between 10:00 AM and 4:00 PM, targeting peak pollinator activity. Observations focused on the two wild pollinators, *An. dispar* and *B. terrestris*, as well as *A. mellifera*, under three hive conditions: fully open (18 hives, six days), partially closed (9 hives, four days) and completely closed (no hives, two days). Temporal closure lasted between 6.00 AM to 5.00 PM.

During each transect, operators recorded sightings of the three species and assigned them to distance bands. The 41 transects were evenly distributed among operators each sampling day to ensure comparable environmental coverage. Transects were grouped into two or three subsets depending on the operator's availability and assignments rotated daily to minimise systematic bias. For each transect, operators recorded their name, start time (standard time), date, transect name, wind intensity (Beaufort scale) and cloud cover. Wind stronger than Beaufort 5 exceeding the maximum for pollinator sampling (O'Connor et al., 2019), prevented completion of some transects, but at least 32 were surveyed daily. Each 30m transect was walked steadily for five minutes (Buckland et al., 1993), with observations limited to the first 3m ahead and 5m on either side. This approach aimed to reduce the risk of multiple counts (Buckland et al., 2015). Observations were documented using a voice recorder.

Assessing Honey Bee Colony Size

Colony size estimates was conducted during the warmest hours of four days (February 21, March 16, March 25 and April 11) with the help of beekeepers, using a modified version of the Liebefeld method (Dainat et al., 2020). The traditional Liebefeld Method involves visually estimating, for each frame, the number of adult bees and the areas covered by open brood, capped brood, honey and pollen stores (Dainat et al., 2020). In this study, beekeepers estimated the number of frames fully occupied by bees on both sides after inspecting each hive. Photographs of both sides of the central frame, considered representative of bee abundance per frame, were taken and analysed using the open-source software *DotDotGoos*e (v.1.5.3; Ersts, 2019). This allowed for manual counting of visible individual bees. The number of bees counted on the central frame was then multiplied by the number of occupied frames to estimate the colony population. Daily honey bee population were calculated by summing the colony size of all open hives on each sampling day. To estimate the number of bees inside the colonies each day, we modelled colony growth using a linear

regression for each hive based on data from the four observed days, generating predicted values for the unobserved days.

Statistical Analysis

To estimate the population size of the three Apoidea species (*A. mellifera*, *An. dispar*, and *B. terrestris*) foraging on the island, we calculated density using the ds function from the Distance R package (Miller et al., 2019). Analyses were performed with a truncation distance of five meters (the maximum distance at which specimens were recorded), divided into one-meter intervals (0–1m, 1–2m, etc.). Different models were fitted to the data collected daily for each species. We applied three key functions: uniform, half-normal and hazard rate, with the latter two assessed with or without cosine adjustment using the default term selection implemented in the ds function (resulting in five models). Each of the three unadjusted model was tested twice, either including or excluding operator identity as a random factor (note: DS does not allow adjustments with random factors). A total of eight models were applied for each species per day. Models producing errors, warnings, or standard errors an order of magnitude higher than the estimate were excluded. From the remaining models for each species, we selected those with an AIC difference of less than two compared to the best model, and averaged the total estimates and standard errors, weighting contributions based on AIC values. All the analyses have been carried out in R environment and the script together with the complete dataset is available in the supporting information.

Aim 1: DS validation

We validated the efficacy of DS by comparing the estimated number of *A. mellifera* foraging on the island (calculated via DS) with the total number of bees within the colonies estimated using the modified Liebefeld Method. We calculated the ratio of the estimated foraging honey bees to the total colony population. If DS provided reliable estimates, this ratio should align with the proportion of

foraging workers reported in the literature. To evaluate whether daily ratio values were influenced by phenology or weather, we applied a GAMM analysis, using the gamm function from the mgcv R package (Wood & Wood, 2015). The model included Julian Day (smooth variable) to assess phenological changes, along with cloud cover (percentage) and wind (Beaufort scale) as predictors of weather influence. Temporal autocorrelation was accounted for with a covariance structure and daily estimates were weighted by the reciprocal of the squared standard error, adjusted to ensure weights summed to sample size.

gamm(ratio ~ s(day, k = 3) + clouds + wind, correlation = corAR1(form = ~ day), weights = weights_std)

We checked for the normality of residuals by using Shapiro test using the shapiro.test R function. The collinearity among variables has been preliminary tested using Pearson correlation and Variance Inflation Factor (VIF) using the vif function of the car package. In case of highly correlated variables (Pearson $p<0.7$ and VIF >5) we conducted separated GAMMs removing one of the two correlated predictors.

Aim 2: Estimate of population size with temporal trend and weather effect on wild bees

To test whether the estimated population size of *An. dispar* and *B. terrestris* varied over the 43-day study period, reflecting adult phenology and to examine the effect of cloud cover and/or wind on the number of active individuals, we applied GAMMs like those used for honey bees' ratios. Here the response variable was population size (weighted by standard errors) rather than the ratio.

Aim 3: Environmental trend of estimates

Finally, we compared the density estimates for each species across the five environmental units on different days using Friedmann tests (paired by survey day) with the friedman.test R function. For honey bees, only day with fully open hives were included. In cases of significant differences, we applied pairwise comparisons using the frdAllPairsExactTest function of the PMCMRplus Package (Seabold & Perktold, 2010) using the Nemenyi test with Benjamini-Hochberg correction for p-values ($P < 0.05$).

5.4 Results

Aim 1: DS validation

The number of foraging honey bees estimated on the island varied across the 10 days of observation, ranging from approximately 13,000 to 98,000 (Fig. 2A). Wind force and cloud cover were highly correlated (Pearson R = 0.887), resulting in VIF values of 5.89 and 5.12 in explaining the ratio between the estimated number of foraging bees obtained through distance sampling and the total number of bees estimated on the open hives. To address this, we conducted two separated GAMMs which showed no significant variation in the ratio over time (Table 1). Both wind force and cloud cover had significant effects in their respective models (GAMM, R-sq = 0.632 and 0.631, respectively; Table 1). The highest ratio (approximately 0.69) was recorded on a sunny day, while the lowest ratio (around 0.03) occurred under strong winds and denser cloud cover (Fig. 2). The two models estimated average ratios of 0.128 and 0.143 (overall average: 0.136), with values consistently decreasing by 1.28 times between consecutive days (± 1.17 S.D., n = 4 pairs). Both models showed no significant deviation of residuals from normality (Shapiro-Wilk normality test, wind force p = 0,914 and cloud coverage 0.730).

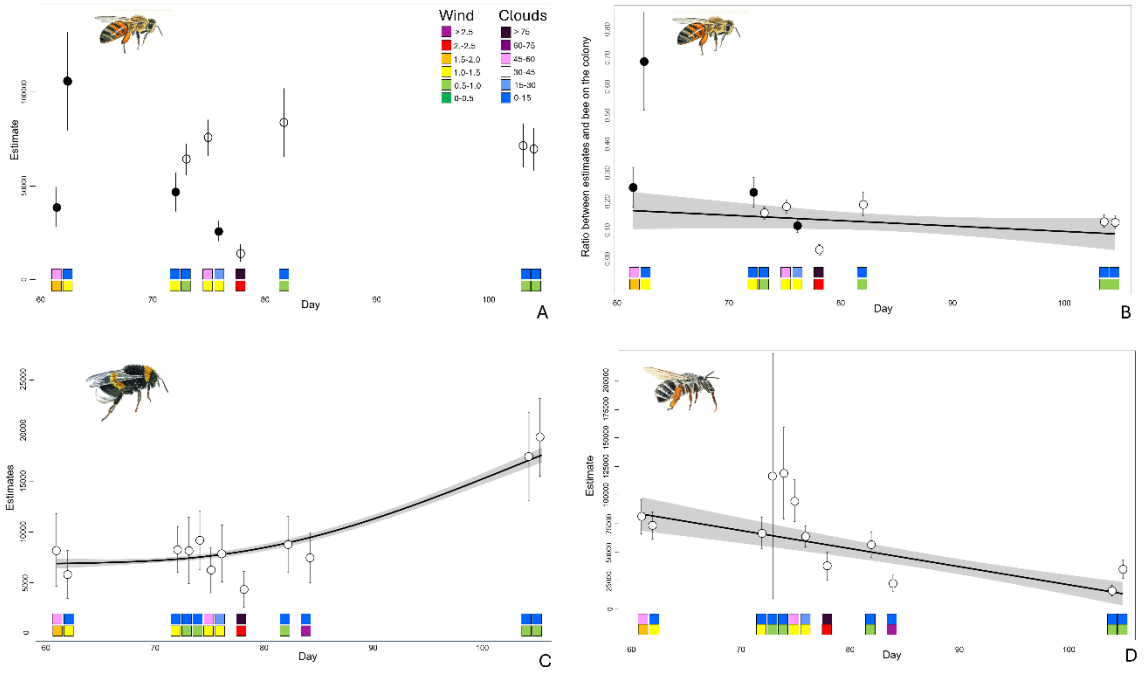

Figure. 2. A) Estimated number of *A. mellifera* foraging on the island in each Julian Day. Black dots represent estimates in days when half colonies were open and white dots represent days when all hives were open. Bars represent standard errors. Average weather conditions collected along the transects (wind strength in Beaufort scale and percentage of cloud cover) are reported in the coloured squares. B) Ratio between the estimates of *A. mellifera* individuals foraging on the island and those estimated inside the hives that were opened that day. The tendency line was obtained by GAMM, using wind force as weather predictor. Bars represent standard errors. C) Estimates for *B. terrestris* population on the island each day. D) Estimates for *A. dispar* population on the island each day.

Aim 2: Estimate of population size with temporal trend and weather effect on wild bees

For *B. terrestris* (Fig. 2C), the estimates of active individuals have been consistent among the first 25 days, with values ranging around 7,000 bees in March (days 60-84), increase to approximately 18,000 bees in April (days 104-105). The variables included in GAMM showed low collinearity (Pearson, -0.3< R <0.3 in all cases), resulting in VIF values < 1.2 for all variables. Accordingly,

there was a significant curvilinear relationship between estimates and time (GAMM, n = 12 days. R-sq = 0.84, Table 1). Both wind force and cloud cover had a significant negative effect on abundance (Table 1). Estimates were consistent between consecutive days, with an average ratio between the estimates of 1.08 ± 0.29 S.D. (n= 6 pairs of consecutive days). There was no significant deviation of residuals from normality (Shapiro-Wilk normality test, p = 0.759)

An. dispar showed the opposite temporal pattern (Fig. 2D) with a linear decline in number of estimated individuals from March to April with an initial estimate of about 85,000 individuals and a final one of about 15,000 (GAMM, n = 12 days, R-sq = 0.77; Table 1). Wind force showed a significant negative effect on abundance while cloud cover percentage did not (Table 1). The variables included in GAMM showed low collinearity (Pearson, -0.3< R <0.3 in all cases), resulting in VIF values < 1.2 for all variables. Estimates were consistent between consecutive days, with an average ratio between the estimates of 0.98 ± 0.38 S.D. (n= 6 pairs of consecutive days). There was no significant deviation of residuals from normality (Shapiro-Wilk normality test, p = 0.444).

A.mellifera ratio (2 separate models)		Estimate	S. E.	t	P
	clouds	-0.002	0.001	-5.014	0.002
		edf		F	P
	s(day)	1		4.587	0.069
		Estimate	S. E.	t	P
	wind	-0.083	0.018	-4.430	0.003
		edf		F	P

	s(day)	1		2.238	0.178
B. terrestris abundance		Estimate	S. E.	t	P
	clouds	-38.037	4.834	-7.869	<0.001
	wind	-665.262	156.623	-4.248	0.004
		edf		F	P
	s(day)	1.992		358.900	<0.001
A.dispar abundance		Estimate	S. E.	t	P
	clouds	-42.140	140.420	-0.300	0.772
	wind	-9299.100	2976.590	-3.124	0.014
		edf		F	P
	s(day)	1.000		42.190	<0.001

Table 1. GAMM results for *A. mellifera* ratio of bees estimated by DS to those estimated on hives (two models separated for wind force and cloud coverage due to high VIF values) and results for single models of *Bombus terrestris* and *Anthophora dispar* abundance counted by DS.

Aim 3: Environmental trend of estimates

The three species show different densities in the five environmental units (Friedman tests: *A. mellifera*, n = 30, chi-squared = 17.467, df = 4, p-value = 0.002; *B. terrestris*, n = 60, chi-squared = 26.527, df = 4, p-value < 0.001; *An. dispar*, n = 60, chi-squared = 31.013, df = 4, p-value < 0.001). Post hoc tests revealed that *B. terrestris* used the meadows more frequently than the other environments (Fig. 3B), while *A. dispar* and *A. mellifera* showed an overall similar

pattern with a preference for high and low shrubs (Fig. 3A,C). *A. mellifera*, however, showed an intermediate preference for meadows while for *An. dispar* meadows belonged to the group of significantly less used environments (Fig. 3A,C).

Among the models selected by AIC for each species the uniform chi-function showed the lowest number of selected models followed by hazard ratio and half-normal function (3,9,12 for *A. mellifera* respectively; 9,11, 20 for *B. terrestris* and 8, 15,17 for *An. dispar*).

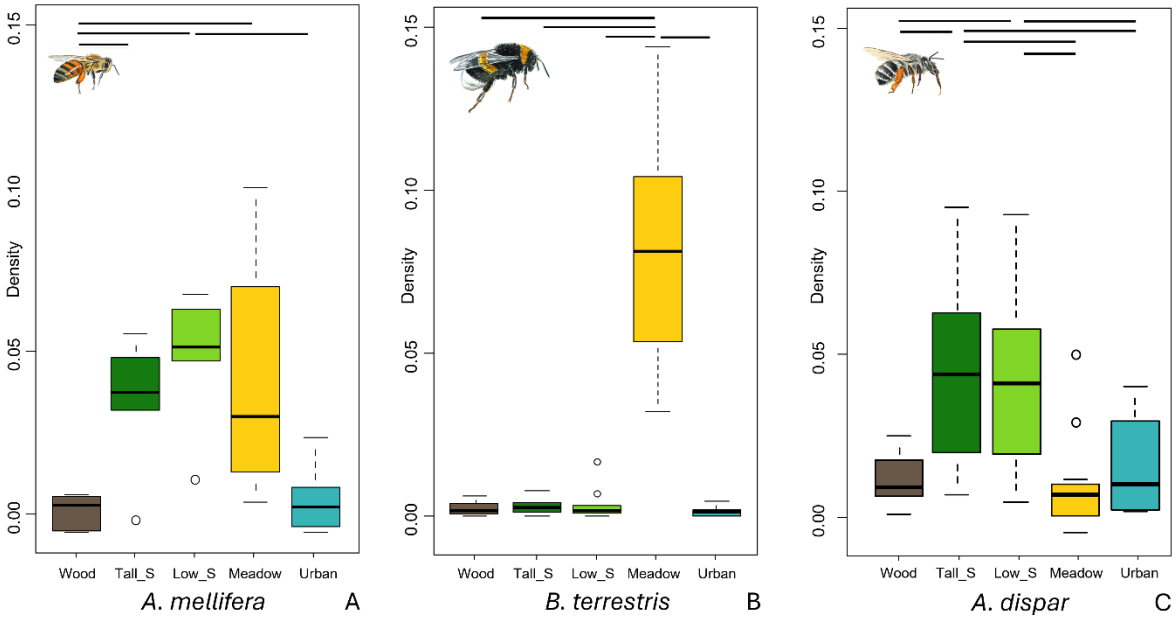

Figure 3. Density of the three target species recorded in different environmental units. The lines above the boxplots connect environments with significantly different densities. Environmental unit abbreviations: Wood indicates the woodland environment, Tall_S the tall shrubland, Low_S the low shrubland, Meadow the meadows, and Urban the urban environment. Results for: A) *A. mellifera*, B) *B. terrestris* and C) *An. dispar*.

5.5 Discussion

Distance sampling (DS) is a promising non-invasive tool for monitoring animal population, yet its application to insects remain limited (Baccaro & Ferraz, 2013; Darshetkar et al., 2023; McNeil et al., 2019) despite it overperforms largely used

methods like Pollard transects (Kral et al., 2018; Patterson et al., 2023). Our study reinforces this finding showing that incorporating decay of detectability with increasing distance explains more variance in observed counts than the uniform chi-function which assumes a constant detection rate as in Pollard transects. An impediment to adopting DS in insect studies is the lack of validation against known population sizes. This challenge is due to the typically large insect populations which activity patterns are highly influenced by phenology and weather conditions. We addressed these limitations by leveraging a controlled insular system where all honey bees were managed within a single apiary. This unique setup unable a direct comparison between DS-derived estimates of foraging bees and the number inferred from direct measurement of colony size.

In honey bee colonies, 25–30% of individuals typically engage in foraging (27.2%, Tenczar et al., 2014; 25–30%, Van der Steen, 2015; 27%, Klein et al., 2019) and are thus detectable on the field. However, not all foragers are active outside the hive simultaneously. A large proportion (40–90%) remains inside the hive, waiting for scout bees to provide information about food sources (Klein et al., 2019; Tenczar et al., 2014; Van der Steen, 2015). Additionally, a study on *Apis mellifera ligustica* (the same subspecies we observed) revealed that foragers spend an average of four hours per day on external tasks, which represents less than half of the daily foraging time window (Chen et al., 2012). Consequently, at any given time, fewer than half of the of foragers (<15% of the total colony) are engaged in resource collection. Our results align with these expectations as the ratio of DS-estimated foraging bees to hive counts consistently average 0.14 (approximately 12.3% of the colony members). This represents the validated application of DS in an insect population confirming its accuracy at least under our experimental settings.

The experimental manipulation of the number of active colonies further demonstrated DS sensitivity to population size variation. Closing half or all hives on specific days, resulted in proportional changes in DS estimates with the foraging-to-colony size ratio remaining consistent. Two notable exceptions occurred: 1) day 62, showing a very high fraction of foragers, likely reflecting

the response to the first sunny day after a week of rain, and 2) day 78, with less foraging bees than expected, attributable to particularly unfavourable weather conditions, as revealed by both significant negative effect of wind force and cloud cover.

Following this validation we confidently applied DS to estimate the densities of two other key pollinators on the island: the solitary *An. dispar* and the social *B. terrestris.* Estimates for both species were consistent across consecutive days, suggesting DS reliability even with a single day of sampling. At the same time, we found a relevant source of estimate variation among days due to the influence of weather conditions. *B. terrestris* was significantly affected by wind strength and cloud coverage, while only the former influenced *An. dispar*. These findings align with previous research, which identified sunny, low wind days as optimal for DS application on *Bombus spp.* (McNeil et al., 2019).

DS estimates also reflected the known phenology of the two wild bee species (Pasquali et al., 2025). The adults of the solitary and univoltine *An. dispar* occur on Giannutri from January to the end of April. Our experiment, started during the activity peak at the beginning of March, documented the expected linear decrease in *An. dispar* abundance with time. Conversely, the adults of the social species *B. terrestris* occur on the island all year round, with a peak at the end of April, a variation detected also by our DS approach.

Additionally DS revealed significant densities variations across environmental units, aligning with previous studies that emphasize the specialized habitat requirements of certain Apoidea species (Hennessy et al., 2021). *A. mellifera* and *An. dispar* were more evenly distributed across different environmental units, while *B. terrestris* was predominantly found in meadows. These results underscore the potential of DS to compare densities across habitats, also providing valuable insights for conservation. As a clear example, the importance for bumble bees of the small and secondary meadows present on Giannutri island can inform targeted management strategies to support pollinator populations.

Despite its advantages, DS also has limitations. First, it captures only active individuals, excluding those resting, nesting or performing other non-visible activities. This constraint particularly pronounced in social species like honey bees and bumble bees, leads to underestimates as most colony members are non-foragers. Furthermore, DS cannot provide life-history parameters, such as lifespan or dispersal, which are accessible through CMR (Briggs et al., 2022). Combining DS with complementary methods, such as genetic analyses to estimate effective population size, could further enhance its reliability and applicability (Morgan et al., 2024).

In conclusion, our study validated DS for insects by demonstrating strong concordance between DS estimates of foraging *A. mellifera* and colony size measurements. Reliable density estimates were also obtained for *An. dispar* and *B. terrestris*, with values remaining quite stable across consecutive days and reflecting the species' known phenology. Weather conditions, such as high cloud coverage and wind, significantly affected estimates for wild species, confirming the method's sensitivity to environmental factors. Density estimates confirmed strong environmental preference in *B. terrestris*, less marked preference in *A. dispar*, and broader generalism in *A. mellifera*. DS emerges as a scalable, resource-efficient tool for monitoring insect populations, offering precise density estimates while accounting for species detectability and environmental variation. Its non-invasive nature and adaptability make it particularly suitable for large-scale and resource-limited studies. We anticipate DS will play a pivotal role in informing conservation strategies aimed at mitigating insects' biodiversity loss (Prendergast & Ollerton, 2021).

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Chapter 6. Island-wide removal of honey bees reveals exploitative trophic competition with strongly declining wild bee populations

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6.1 Summary

High densities of managed honeybees (*Apis mellifera*) can threaten wild bees through exploitative competition, thus leading to population declines of the latter. Although reviews have outlined key steps to demonstrate these impacts—measuring resource overlap, changes in wild bee behaviour, and population trends—studies that comprehensively address these aspects are virtually absent. We were granted access to the entire protected island of Giannutri (2.6 km²) and to the apiary (18 hives) located there, during the early phase of co-existence between honeybees and wild bees. Using the island as an open-air laboratory, we experimentally manipulated honeybee pressure by closing the hives on selected days during the peak of the wild bee foraging period. In the plants most visited by pollinators even short-term honeybee removals (11 hours per day) increased nectar volume (~60%) and pollen availability (~30%). In the absence of honeybees, target wild bees (*Anthophora dispar* and *Bombus terrestris*) became dominant in the insect-plant visitation network and the potential apparent competition significantly decreased. Accordingly, both species intensified their foraging activity and increased nectar suction time, a recognised proxy for quantity of probed nectar, and *B. terrestris* also shortened the time of pollen searching. Transect monitoring revealed an alarming ~80% decline in both species over four years, consistent with honeybee monopolisation of floral resources, thus reducing availability for wild pollinators and altering their foraging budget. These findings underscore the risks of introducing high densities of honeybees into protected areas and emphasise the need for rigorous preventive ecological assessments.

Keywords

Niche overlap, pollinator, conservation, *Apis mellifera*, *Anthophora dispar*, *Bombus terrestris*, beekeeping, foraging behaviour, population decline, resource availability

6.2 Introduction

Wild and managed bees play a pivotal role in pollination services worldwide[1,2]. *Apis mellifera* is also economically important, both for crop pollination and beehive products[3–5]. However, while wild bee populations are globally declining[6–8], honeybee colonies are thriving due to management[9,10]. This dynamic is particularly evident in the Mediterranean Basin, where honeybees are progressively replacing wild bees[11], raising concerns about their impact on biodiversity[12–14]. When kept in high densities, honeybees can consume large amounts of resources, reducing nectar and pollen availability for other pollinators[15–17]. The resulting exploitative competition[13,18–20] can alter wild bee flower-visiting rates and diets[21–24], influence development[25] and reduce solitary [26,27] and social wild bees'[28,29] fitness. The impacts of honeybees vary by context[30,31] and tend to be more pronounced in homogeneous and/or confined landscapes, like islands[15,32,33], urban green spaces[34] or altered agricultural environments[31,35].

Most studies on this topic are indicative rather than conclusive, as demonstrating exploitative competition requires complex experiments with manipulation and long-term population monitoring. Typically, this process includes i) demonstrating trophic resource overlap and reduced resource availability due to honeybees' activity; ii) observing reductions in wild bee flower visits iii) detecting altered pollen and nectar harvesting rates by wild bees. However, these steps only suggest potential competition while conclusive evidence of negative impacts requires to iv) link these effects to reduced survival, fertility or population size[19]. The first three steps are particularly challenging because honeybees are nearly ubiquitous, making it hard to design experiments comparing resource availability in their presence and absence. Consequently, studies often rely on correlative approaches rather than directly manipulating honeybee pressure[13,14]. Additionally, in many regions, managed honeybees have coexisted at high densities with wild pollinators for decades, suggesting any impacts on wild bees may have already occurred. The fourth step, measuring wild bee reproductive success, is challenging due to difficulties

in finding enough nests in nature and the need for invasive assessment techniques[20,26]. Previous studies found evidence of potential competition primarily by measuring flower visitation rates or foraging behaviour[13,20]. Nevertheless, no studies have conclusively demonstrated negative impacts on wild bees using all necessary steps[13,19,20].

Giannutri, a small Mediterranean island (2.6 km²) within the Tuscan Archipelago National Park (Italy), offers unique conditions to overcome these limitations. Since 2018, beekeepers have been introducing 18 hives of *A. mellifera* (Figure 1A) annually, from December to June, using the island as an isolated mating site, resulting in a density of about 7 hives/km², higher than the European average, 4.2 hives/km² [36]. Given the limited floral diversity in early spring, there is potential overlap in resource use with wild pollinators[37]. This island represented an ideal system to test resource competition because i) honeybees had been present for only three years by 2021, when experiment commenced, providing a unique opportunity to study early impacts on wild bees; ii) the island's limited size, smaller than the typical honeybee foraging range[38], ensured their influence across the entire island; iii) the absence of non-managed honeybees enabled a comprehensive island-wide removal experiment.

The island served as an open-air laboratory, with honeybees excluded temporarily by closing hive entrances before sunrise for 11 hours on selected days. Over three years (2022–2024, February-March), we examined the short-term effects of honeybee removal on resource availability (nectar and pollen) of the two most abundant plant species (*Teucrium fruticans* and *Salvia rosmarinus*), exploited by both *A. mellifera* and wild pollinators. We also investigated the foraging behaviour of the two most abundant wild bee species[37]: *Anthophora dispar* (solitary species) and *Bombus terrestris* (social species) (Figure 1B,C). Additionally, we monitored their abundance through transect walks over four years (2021–2024) to assess population trends in prolonged coexistence with honeybees.

Our experiment fulfilled all the necessary steps to demonstrate trophic exploitative competition between honeybees and wild bees[19]. We observed strong overlap in floral visits between honeybees and wild bees. Daily honeybee removals increased nectar and pollen availability, changed wild bee foraging behaviour and increased resource collection efficiency, indicating that even short-term removal causes detectable changes. Additionally, wild bee abundance significantly declined over four years, presumably due to trophic competition with *A. mellifera*. Our results underscore the need of thorough assessment of honeybee influence when managing protected areas to safeguard wild bee biodiversity.

6.3 Results

Honey bees show strong trophic resource overlap with wild bees

We analysed the degree of trophic resource overlap between wild bees and *Apis mellifera* (hereafter Am) using hierarchical flower-visitor networks based on transect walks. We created two networks by pooling days based on hive condition, open hives (HB+, Figure 1D) and closed hives (HB-, Figure 1E), and 29 daily networks to compare the effect of Am removals on network and species-level indices.

Am dominated flower visits in HB+, accounting for 59.95% of total visits, being the main visitor of *T. fruticans, S. rosmarinus* and *Euphorbia dendroides. Anthophora dispar* (hereafter Ad) primarily visited *T. fruticans* and *Bombus terrestris* (hereafter Bt) mainly *Borago officinalis* (Figure 1D). Network specialisation (H2', the degree of partitioning among nodes) increased significantly in HB- compared to HB+, while niche overlap and weighted NODF (a measure of nestedness) showed no significant differences (Figure 1F, Data S1B). Networks significantly differed from those computed for null models in 86% of cases for H2', 79% for niche overlap and 38% for weighted NODF (Data S1C). Species-level indices were more affected by Am removal, especially for

Ad, which in HB- showed a significant increase in species strength (species' relevance across all nodes) (W=32, p=0.021), interaction push-pull index (measuring pollinators-plants dependency) (W=23, p=0.004) and PSI (Pollination Service Index, pollinator importance for plants) (W=0, p<0.010) but not in the Bluthgen's d' (measuring species specialisation) (W=52, p=0.217). In HB-, Ad also showed a significant decrease in the Müller_modified index of Potential Apparent Competition (PAC, a measure of resource overlap between one species and all the others) (W=234, p<0.001) (Figure 1G, Data S1B). Bt showed a similar pattern but only for PSI (W=32, p=0.021) and Müller_modified index (W=163, p=0.015). PAC between Am and both Ad and Bt increased significantly over the season (Ad*:* Estimate=0.007, p=0.002; Bt: Estimate=0.006, p=0.005, Figure S1C, Data S1B).

PERMANOVAs on wild bees' daily visiting frequencies per plant did not show changes in flower resource use between HB+ and HB- (Ad: F=0.242, p=0.773; Bt: F=0.685, p=0.473, Data S1D).

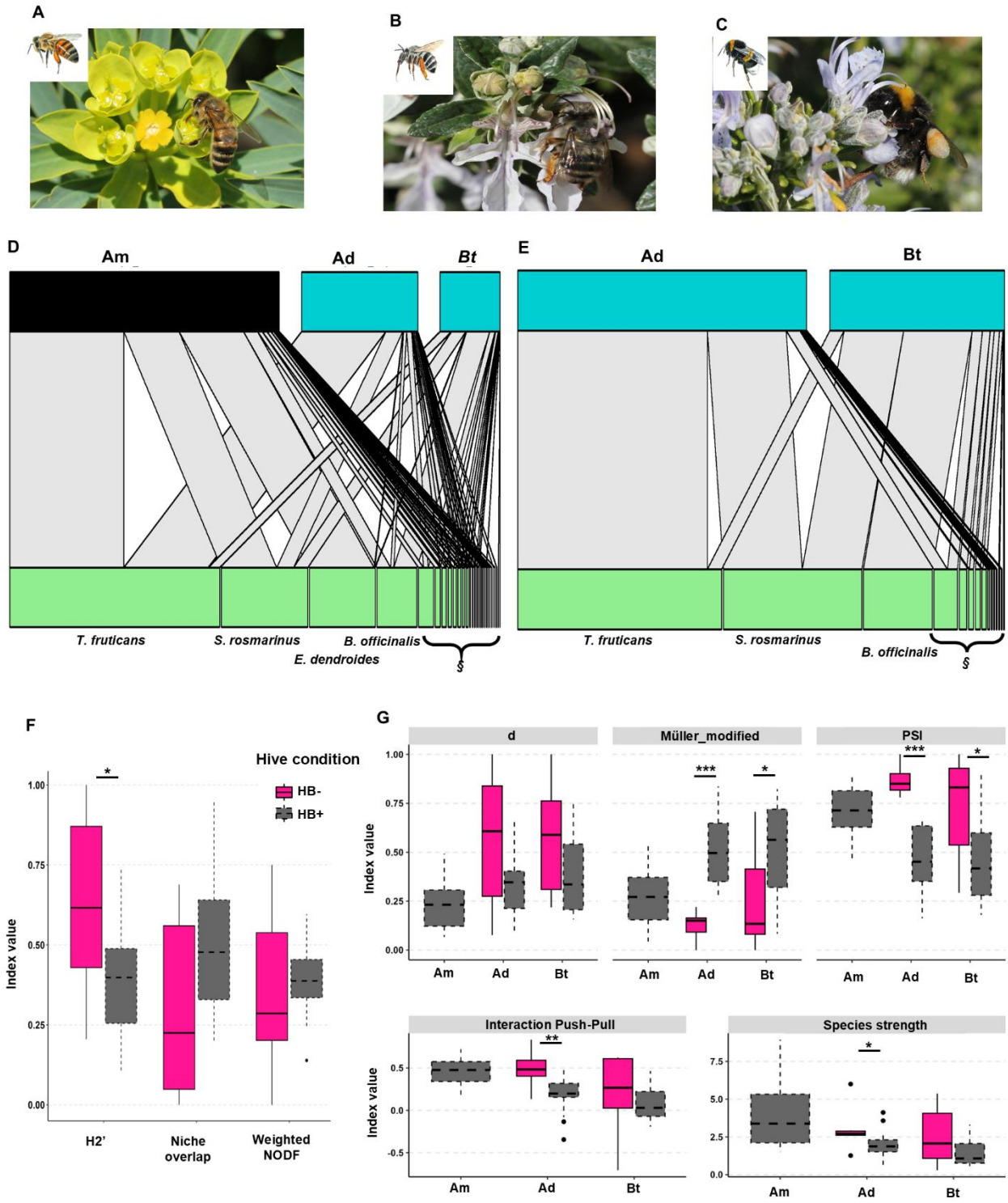

Figure 1. Resource overlap between wild bees and honeybees on Giannutri Island.

(A) *Apis mellifera* (Am) on *Euphorbia dendroides*. (B) *Anthophora dispar* (Ad) on *Teucrium fruticans*. (C) *Bombus terrestris* (Bt) on a *Salvia rosmarinus*. (D)-(E) Flower-visitation network for HB+ and HB- days, respectively. Link size between pollinators (upper level) and plants (lower level) reflects interaction strength. Box dimensions indicate the total number of visits (made/received). Plant species marked with § are listed in Data S1A. Additional results on full nodes networks are available in Figure S1. (F)-(G) Boxplots of network- and species-level indices for daily networks (Data S1B). Pink, solid-lines: closed hives (HB-); grey, dashed-lines: open

hives (HB+). Significant differences between hive conditions are indicated above comparisons: *p<0.05, **p<0.005, ***p<0.001.

Honeybees reduce the amount of available nectar and pollen

We analysed short-term effects of Am removal on resource availability of *T. fruticans* (nectar and pollen) and *S. rosmarinus* (nectar). Pollen availability was evaluated visually by counting the number of anthers bearing pollen[22,39] on 8425 *T. fruticans* flowers over 9 days (4 HB+ and 5 HB-) at 20 sites (Figure 2A). A few hours after Am removal, the number of pollen-bearing anthers increased (Generalised Additive Mixed Model (GAMM): Estimate=-0.326, p<0.001, expl.var=0.73%) (Data S1I).

A stronger increase of 35.7% in pollen availability was noted near the apiary (estimated number of pollen-bearing anthers in HB+=1.64±0.14 SE; and in HB-=2.23±0.10) compared to 16.8% at the maximum distance (HB+=2.30±0.13; HB-=2.70±0.10) (Estimate=-0.114, *p*=0.002, expl.var=0.65%, Figure 2C, Data S1I)

Nectar availability was evaluated over 25 days (13 HB+ and 12 HB-) at five experimental sites. To assess nectar availability in the absence of consumption, we bagged 4-6 branches per plant 24 hours before measurements. On each survey day, nectar volume was measured in three bagged and three unbagged flowers per plant, using microcapillary tubes, with a total of 1524 surveyed flowers (695 *T. fruticans* and 829 *S. rosmarinus*). Sucrose concentration was determined with a refractometer[40].

Pollinators consumed most of the available nectar and Am removal increased the nectar in both plants, with no effect on sucrose concentration (Data S1E-H). Bagged flowers retained more nectar than unbagged ones (GAMM, *T. fruticans,* Estimate=-0.440, p<0.001 and expl.var=3.80%; *S. rosmarinus*, Estimate=-0.314, p<0.001, expl.var=2.06%). Am removal significantly increased nectar volume in unbagged flowers (Tukey *post-hoc* test;

T. fruticans unbagged HB- vs unbagged HB+ p<0.001; *S. rosmarinus* p<0.005), but had no effect on bagged flowers (Tukey test; *T. fruticans* bagged HB- vs bagged HB+ p=0.762; *S. rosmarinus* p=0.974; Figure 2B,D), accounting for 15% of variance explained in *T. fruticans* and 8.8% in *S. rosmarinus* (calculated as the sum of expl.var in model terms where *hives.condition* was involved, Data S1E,F). The mean estimated volume of nectar increased by 52.5% for unbagged *T. fruticans* flowers and by 71.1% for *S. rosmarinus* between HB+ and HB-. The mean estimated nectar consumption dropped from 53.5% to 35.6% in *T. fruticans* and from 48.8% to 27.0% in *S. rosmarinus* (Table S1).

Distance from the apiary had no effect on nectar volume (*T. fruticans*, Estimate=0.222, p=0.221, expl.var=0.48%; *S. rosmarinus*, Estimate=0.441, p=0.511, expl.var=1.67%), but nectar volume increased over time (*T. fruticans*, Estimate=0.740, p<0.001, expl.var=6.64%; *S. rosmarinus*, Estimate=0.906, p<0.001, expl.var=4.47%) (Data S1E,F).

Sucrose concentration remained unchanged in HB- but was lower in unbagged flowers (*T. fruticans*, Estimate=-0.094, p=0.006, expl.var=1.63%; *S. rosmarinus*, Estimate=-0.112, p=0.007, expl.var=0.67%), likely due to evaporation of unconsumed nectar and the dynamics of nectar secretion[40] (Data S1G,H).

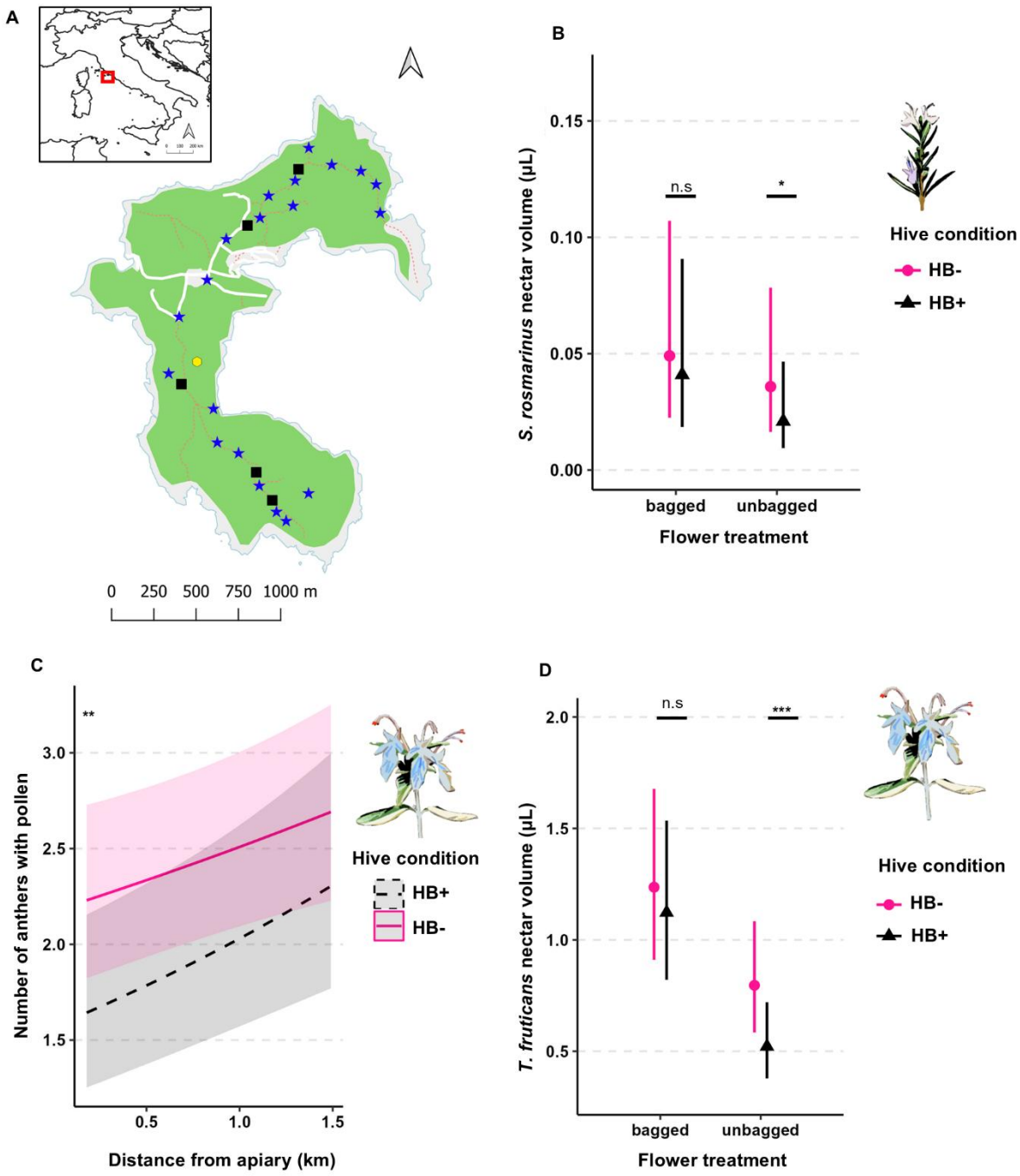

Figure 2. Effect of honeybees on trophic resource availability

(A) Map of Giannutri with sampling points: nectar surveys (black squares) and pollen surveys (blue stars); apiary location (yellow hexagon). (B)-(D) Interaction between hive condition (HB+, HB-) and flower treatment (bagged/unbagged) on estimated marginal means ± SEM of nectar volume (Data S1E,F and Table S1). Pink circles, HB-; black triangles, HB+. Post hoc tests: *p<0.05, **p<0.005, ***p<0.001. (C) Interaction between hive condition and distance from apiary on *T. fruticans* pollen availability (Data S1I). Solid pink curve: relationship with distance from the apiary in HB-; dashed black curve: relationship in HB+. Shaded areas: 95% confidence intervals. Interaction significance: *p<0.05, **p<0.005, ***p<0.001.

Wild bee activity is altered by honeybee presence

We tested whether short-term Am removals affected wild bee foraging by measuring their abundance, time spent, and movements in 2×1 m² flower plots. We conducted 2076 observations over 51 days (35 HB+ and 16 HB-). Am were more frequent near the hives (GAMM, edf=1.126, p<0.001, expl.var=5.97%) and in the central hours (Figure 3B, edf=2.777, p<0.001, expl.var=2.81%, Data S1L). Honeybees influenced both species' activity, often altering their hourly activity patterns (Data S1J-P). Bt and Ad reduced the time spent within flower patches in HB- (Bt, Estimate=-0.330, p<0.001, expl.var=4.59%; Ad, Estimate=-0.076, p<0.001, expl.var=0.29%, Figure 3E,F), with a significantly different hourly activity pattern between HB- and HB+ (Figure 3E,F and Data S1M-P). The increased time spent within patches by Ad strictly aligned with Am peak activity (Figure 3E).

Ad movements inside and outside the plots decreased in HB+ (Estimate=0.242, p<0.001, expl.var=0.54%), especially in response to the onset of Am activity (edf=0.827, p=0.009, expl.var=0.62% Figure 3D, Data S1O). Bt activity across patches was lower near the apiary (edf=0.958, p<0.001, expl.var=1.1%, Data S1P).

The short-term removal did not affect pollinator abundance in floral patches (Ad, Estimate=0.141, p=0.156, expl.var=0.22%; Bt, Estimate=-0.395, p=0.133, expl.var=0.34%) but in HB+, Ad concentrated their activity in the early hours (edf=0.839, p=0.013, Figure 3C) (Data S1J,K).

Flower visitation rates significantly decreased over the years (Ad, Estimate=-0.711, p<0.001, expl.var=6.67%; Bt, Estimate=-0.693, p<0.001, expl.var=4.97%, Data S1O,P), as did the time spent by Ad inside the plots (Estimate=-0.075, p=0.004, expl.var=0.48%, Data S1M) and the number of wild pollinators observed during scan sampling (Ad, Estimate=-1.275, p<0.001, expl.var=13.28%; Bt, Estimate=-1.248, p<0.001, expl.var=8.94%, Data S1J,K), the latter reflecting the population decline evidenced by transect data (see below).

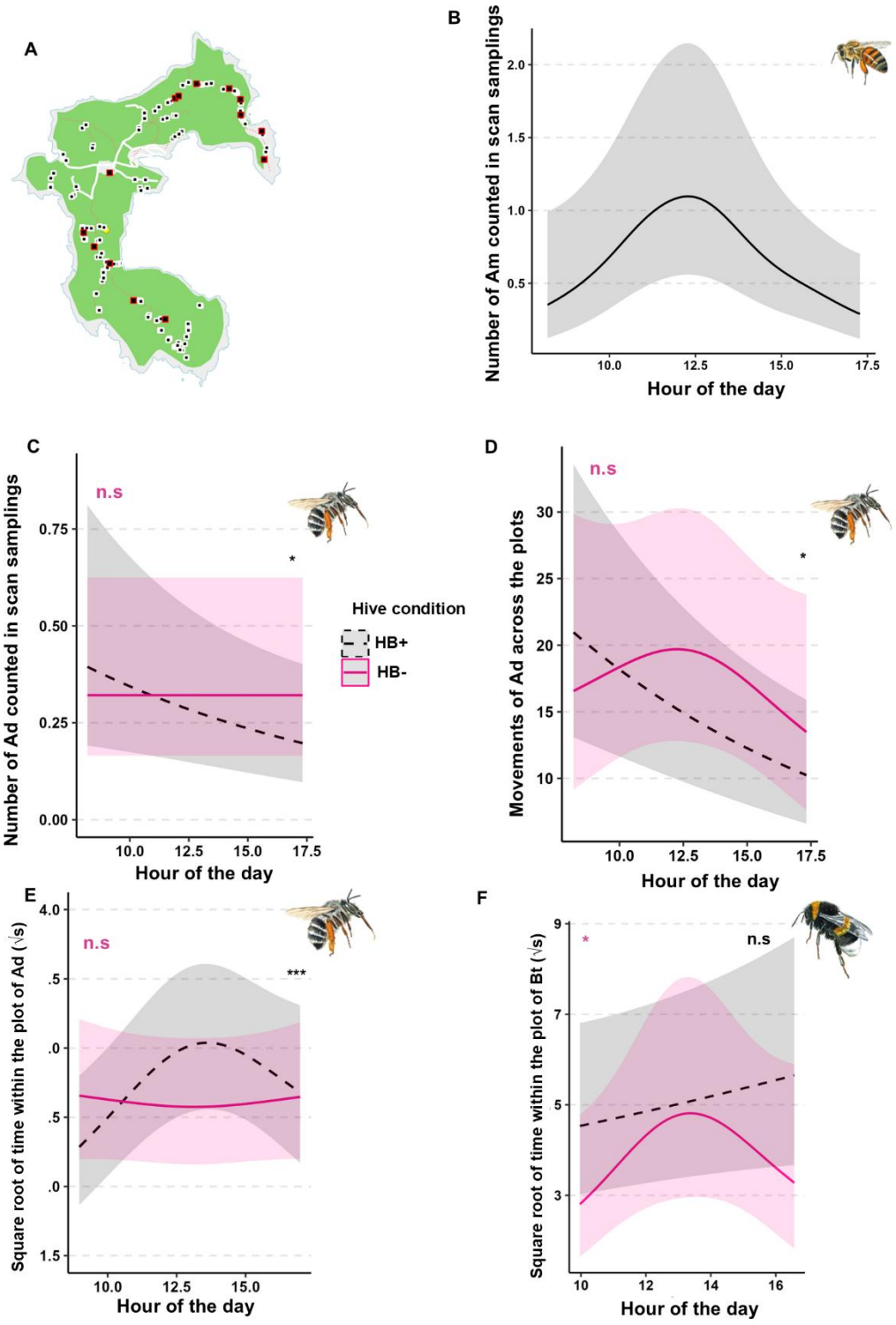

Figure 3. Effect of honeybees on wild bees' foraging activity

(A) Map of sampling points. White-bordered black squares: position of sampling points (focal and plot observations); red-bordered black squares: location of 15 opportunistically sampled focal points. Yellow hexagon: apiary location. (B) Daily activity of Am (number of individuals counted in scan samplings) (Data S1L). (C) Effect of hive condition (HB+, HB-) and time of day on Ad counts in scan sampling (Data S1J). (D) Effect of hive condition (HB+, HB) and time of day on Ad movements across plots (Data S1O). (E)-(F) Effect of hive condition and time of day on time spent within the plot by Ad (E) and Bt (F) (Data S1M,N). For (C)-(F): Pink, solid lines:

HB-, black, dashed lines: HB+. Shaded areas indicate 95% confidence intervals. Significance: top left (pink) for HB-, top right (black) for HB+, *p<0.05, **p<0.005, ***p<0.001.

Honeybee activity alters wild bee foraging behaviour

Focal observations, conducted alongside plot observations, revealed the effects of short-term Am removals on key aspects of wild bee foraging behaviour: nectar suction time and lag times between consecutive nectar probing events and pollen collection events. We performed 6168 sessions (4053 in HB+ and 2115 in HB-; each tracking a single bee's behaviour). In HB+, a significant relationship between distance from the apiary and suction time occurred in both species (GAMM, Ad, edf=1.573, p<0.001; Bt, edf=0.934, p<0.001) but not in HB- (Ad, edf=0, p=0.676; Bt, edf=0, p=0.914; Figure 4A,B). Overall, the interaction between distance and hives condition explained 4.55% of variance in Ad and 4.65% in Bt (Data S1Q,R).

In HB+, suction intervals were shorter near the apiary (Ad, edf=0.934, p<0.001; Bt, edf=1.476, p=0.012), with no effect in HB- (Ad, edf=0.597, p=0.114; Bt, edf=0.699, p=0.068; Figure 4C,D). This interaction accounted for 0.8% of explained variance in Ad and 2.45% in Bt. Suction intervals increased over the years (Ad, Estimate=0.167, p<0.001, expl.var.=3.42%; Bt, Estimate=0.257, p<0.001, expl.var.=4.83%, Data S1S,T).

The lag between pollen collection events was shorter in HB- only for Bt (Estimate=-0.207, p=0.015, expl.var.=3.63%; Figure 4E,F). This lag increased over the years for Ad (Estimate=0.074, p<0.001, expl.var.=2.36%, Data S1U,V).

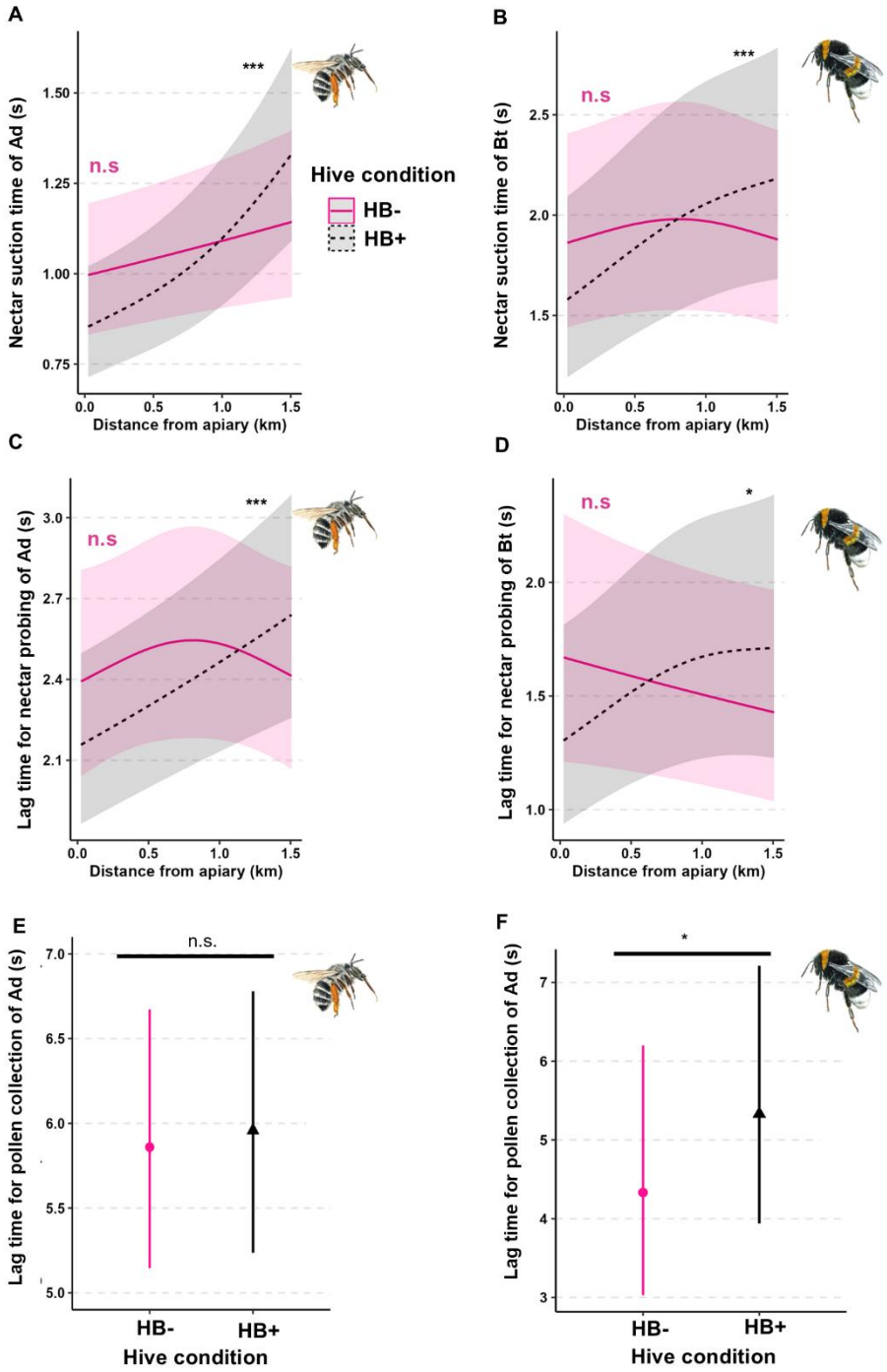

Figure 4. Effect of honeybees on wild bees' foraging behaviour

(A)-(B) Interaction between hive condition (HB+, HB-) and distance from the apiary on nectar suction time of Ad (A) and Bt (B) (Data S1Q,R). Relationship between suction time and distance from the apiary in HB- (solid pink line) and in HB+ (dashed black line). (C)-(D) Interaction between hive condition and distance from the apiary on Ad (C) and on Bt (D) lag time between consecutive nectar probes (Data S1S,T). Relationship between lag time and distance from the apiary in HB- (Solid pink line) and in HB+ (dashed black line). For (A)-(D): shaded areas indicate 95% confidence intervals. Significance: top left (pink) for HB-, top right (black) for HB+, *p<0.05, **p<0.005, ***p<0.001. (E)-(F) Hive condition effect on Ad (E) and on Bt (F) lag time

between pollen collection events (Data S1U,V). Data: estimated marginal means ± SEM. Pink circles: HB-; black triangles: HB+. Post hoc ps: *p<0.05, **p<0.005, ***p<0.001.

Anthophora dispar and *Bombus terrestris* populations are declining on the island

We monitored the abundance of the target species over four years using six walking transects established in 2021[37] (Figure 5A). The climate of this area did not show significant variation in daily maximum temperature and monthly precipitation from 2016 to 2024, during peak adult activity (February-April, Figure 5B,C, Data S1AB,AC). In summer months (with adults absent, Figure S4), maximum temperature increased over time, with no trend for precipitation (Figure 5D,E). From 2021 to 2024, median individual counts per transect in HB+ decreased by 77% for Ad and 87% for Bt (Figure 5F,H). A GAMM, accounting for environmental and weather variables, confirmed this decline (Ad, Estimate=-0.337, p<0.001, expl.var.=4.29%; Bt, Estimate=-0.336, p<0.001, expl.var.=1.44%, Figure 5G,I, Data S1W,X). Flower coverage also significantly decreased over the years (edf=-0.165, p<0.001, expl.var.=2.23%, Data S1Y).

Including HB- days in the analysis showed that Am removal did not affect wild bee abundance or cause displacement (Ad, Estimate=0.151, p=0.084, expl.var=0.19%; Bt, Estimate=-0.050, p=0.710, expl.var=0.01% for Bt, Data S1Z,AA).

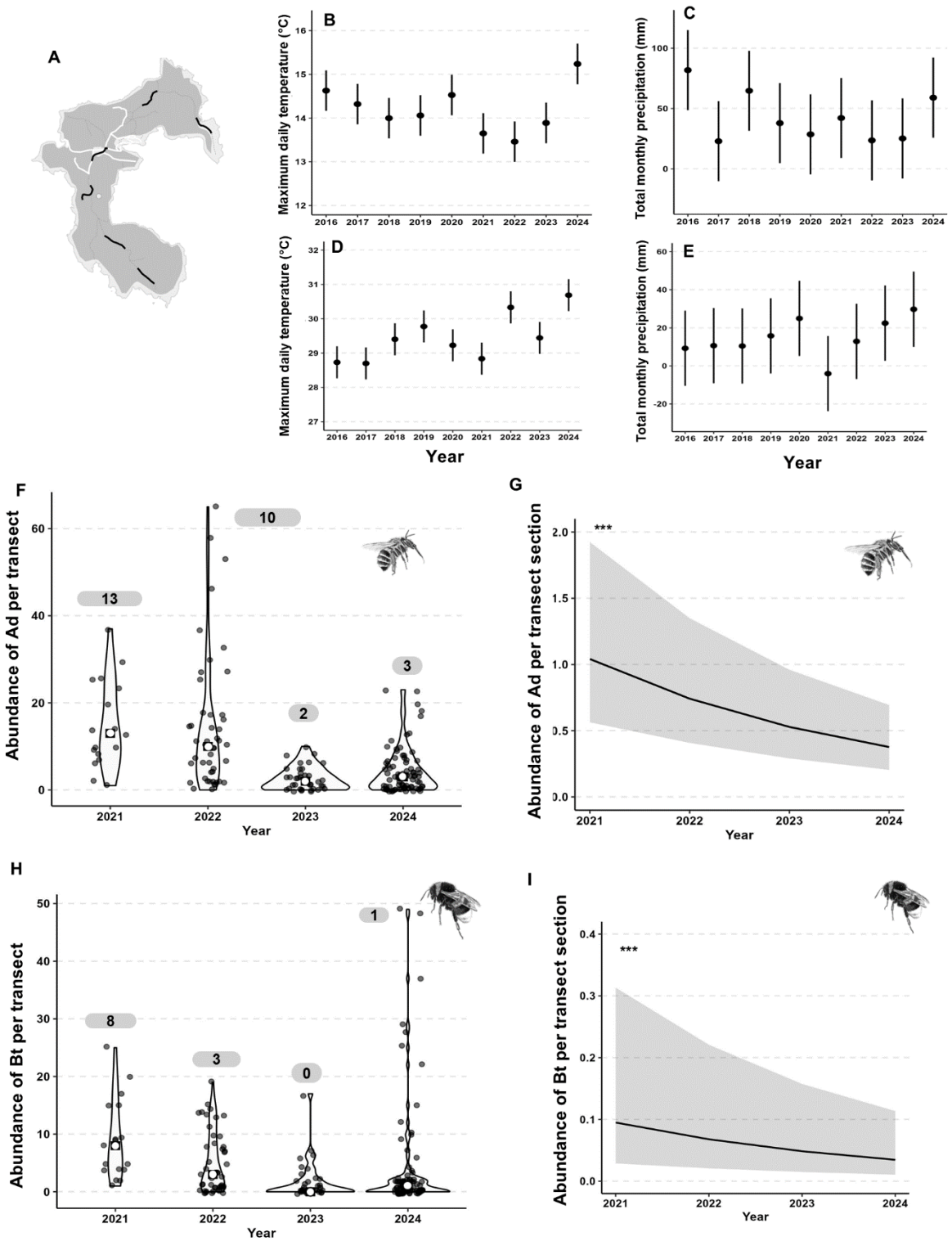

Figure 5. The decline of wild bees on the island

(A) Walking transects on the island (black lines). Yellow hexagon: apiary location. (B)-(E) Climatic trend since 2016 at Giglio Porto (the nearest weather station). Maximum daily temperature (B) and total monthly precipitation (C) during the experimental period (January-April). Maximum daily temperature (D) and total monthly precipitation (E) during summer (21st June - 21st September) (Data S1AB,AC). (F) Violin plot of Ad counts per year. Dots: daily transect counts; black squares: median. Median values are shown in grey boxes. (G) Yearly effect on Ad abundance (individuals per transect section) (Data S1W). (H) Violin plot of Bt counts per year.

Dots: daily transect counts; black squares: median. Median values are shown in grey boxes. (I) Yearly effect on Bt abundance (individuals per transect section) (Data S1X). For (G)-(I): statistical significance: *p<0.05, **p<0.005, ***p<0.001.

6.4 Discussion

Assessing impacts of honeybees on wild bees due to trophic resource competition is challenging and requires evidence from manipulative experiments and long-term monitoring with exceptional model systems[13,18,19,41,42]. We capitalised on the closed terrestrial ecosystem of Giannutri island, characterised by well-preserved maquis vegetation[43], where the local introduction of *Apis mellifera* (Am) in 2018 altered the pollinator community[37]. The wild bee fauna, dominated by *Anthophora dispar* (Ad) and *Bombus terrestris* (Bt), primarily foraged on floral species which were also heavily exploited by Am, determining strong resource overlap and high potential for competition. Experimental Am removals significantly altered resource availability and wild bees' foraging behaviour. Without Am, visitation networks became more specialised and potential apparent competition significantly decreased, whereas nectar and pollen availability increased. Accordingly, wild bees exhibited higher flower visitation rates, probed more nectar, and changed their resource collection timing and frequency. Competition with honeybees likely affected wild bees' fitness over six consecutive springs. During this period, both Ad and Bt populations declined by ~80%. These findings provide comprehensive evidence of the negative impact of high honeybee density on the wild pollinators of Giannutri.

Honeybee removal alleviated resource competition

Honeybees often monopolise a significant portion of resources, altering the structure of pollination networks[21,44]. On Giannutri, Am dominated pollinator-flower visits (~60% of events), functionally displacing wild bees. The apical role that Ad could have reasonably exhibited prior to Am introduction is highlighted by the significant increase, in HB-, in those indices measuring a species relevance in the flower-visit network (PSI, species strength and interaction push-

pull). The potential apparent competition (PAC) between Ad and Bt was much lower in HB−, likely resembling conditions before Am introduction. This reduction in PAC was due to resource partitioning observed between these species: Bt mostly exploited *Borago officinalis* while Ad focused on *T. fruticans*. PAC increased throughout the season, likely due to Am colony growth[21,22] and the introduction of mating hives, suggesting even stronger effects in later months, beyond the experimental timeframe. The displacement of Ad by Am, which became the island's main pollinator, may have negatively impacted pollination ecosystem services, as Am generally has lower per-visit pollination efficacy[12] and can reduce the fitness of plants it massively visits[45,46].

The strong potential competition observed in HB+ contrasts with expectations that polylectic pollinators such as *Bombus* and *Anthophora* species[47,48] can adjust diets in response to competition with other bees[49–51]. However, we did not observe any change in neither the frequency of floral resource use by wild bees between HB+ and HB- nor in the d' index of specialisation. The limited floral diversity of Mediterranean maquis likely forced managed and wild bees to rely on the same resources[31,52].

Honeybee removal increases trophic resources

Trophic resource depletion due to Am activity is well documented across different environments[22,53,54]. We documented significant reductions in trophic resources due to Am foraging pressure. In HB+, about half the nectar produced by both studied plants was consumed, compared to approximately 30% in HB-. Am removal increased nectar availability in unbagged flowers by approximately 50% and 70% for *T. fruticans* and *S. rosmarinus*, respectively. Pollen availability in *T. fruticans* increased to a lesser extent in HB- with distance from apiary and less variance was explained compared to nectar.

The variations between resources may stem from different production mechanisms: nectar replenishes quickly, within hours or overnight[55–57], so prolonged Am exclusion would hardly amplify its availability. Conversely, pollen

production is slower since it follows anther dehiscence[58]. Distance from apiary influenced pollen availability but did not influence nectar availability. This could be explained by the tendency of honeybees to forage for nectar at greater distances than for pollen resulting in a higher consumption of pollen near the apiary[59]. Although our setup did not allow prolonged Am exclusion, extended honeybee absence would likely increase pollen availability. Finally, our approach may underestimate pollen depletion, as it is reduced gradually over multiple visits[60]. Therefore, Am may significantly reduce pollen quantity without fully depleting its visible presence on anthers.

Honeybee removal boost wild bee foraging behaviour

Resource limitation triggers various behavioural changes [61,62] and the observed effects of honeybee presence on wild bee foraging behaviour align with previous findings (Table S2). Both Bt and Ad spent more time in floral patches in HB+, mostly when the activity of Am peaked. As a result, the daily foraging pattern of wild bees was shifted in HB+ towards the early hours of the day. The reduction in activity is consistent with findings in *Bombus* and other wild bee species, which exhibit fewer but longer foraging trips when resources are scarce[49,63,64]. Increasing energy costs and extended foraging times can negatively impact the fitness of solitary wild bees by reducing offspring abundance, brood cells production, hatching rate[65,66], and/or altering larvae sex ratio[67]. In addition, longer foraging trips can increase predation risks[68] or nest parasitism by cleptoparasites[69]. On Giannutri, Ad coexists with the bee *Melecta italica* and the Meloidae *Sitaris sp.*, two known cleptoparasites of *Anthophora*[70,71].

The combined effect of honeybee removal and the distance from the apiary explained a portion of variance consistent with that explained for nectar availability. Suction time, representing a proxy for the volume of probed nectar[72,73] (refer to our experiment on Bt, Data S1AD, Figure S2), increased in HB-. Shortened suction time in HB+ is a typical response to exploitative

competition dynamics[74,75] and to variation in nectar production[76]. Suction time also decreased near the apiary but only in HB+. This aligns with evidence of reduced nectar crop content of wild bees and honeybees collected near the apiary[16]. Insufficient nectar intake can have evident detrimental effects on wild bee survival, as nectar is the main source of energy for adults[77], impacting flight efficiency and survival[78,79]. Additionally, nutritional deficiency can increase susceptibility to other environmental stressors at both individual and colony level[77,80].

Foraging timing analysis revealed a varied pattern. Lag time for pollen collection significantly increased for Bt in HB+, documenting a longer time needed to find pollen, a typical response to exploitative competition[26,28]. The observed reduction of pollen collection per time unit likely translates into smaller food provisions for offspring, potentially reducing their number and/or adult body size[80,81].

Conversely, lag time for nectar probing was shorter near the apiary in HB+ for both target species and remained higher and unaffected by distance in HB-. Thus, high Am densities trigger both target species to visit more flowers to obtain nectar more frequently with shorter suction times, likely increasing their energetic expenses to obtain the same amount of resources. The diverging response in time lag between nectar and pollen collection can be explained by their different accessibility: pollen on anthers can be visually assessed by bees before collection and empty flowers discarded, whereas evaluating the presence of nectar in the calyx requires probing it.

Most behavioural effects showed a significant relationship with distance from the apiary in HB+, supporting the hypothesis that competition intensifies with high Am densities[16,29,82]. These effects disappeared when Am were removed, similarly to a study documenting an increase in *Bombus* species richness and abundance with distance from the apiary, an effect that disappeared after seasonal hive removal[54]. If Am pressure forces wild bees to forage for longer periods or over longer distances[49,63,64], Giannutri effectively became functionally smaller. The area-per-se hypothesis[83], suggests that smaller

islands host smaller populations, making them more vulnerable to stressors and extinction. Indeed, insular ecosystems with higher honeybee densities had lower wild pollinator richness[32,33], indicating local extinctions.

The decline of wild bee populations on the island: honeybee pressure as the most likely driver

Resource limitation caused by exploitative competition likely reduced wild bee fitness[42], driving population declines. At least four-year assessments are needed to confirm such trends, as pollinators typically show population fluctuations[84], which can be stochastic in nature[85]. While the lack of data prior to Am introduction on Giannutri makes it impossible to evaluate the fraction of population loss since then, we revealed a decline of ~80% in median wild bee abundance in four years. Being consistent for both species, this decline can hardly be due to stochastic variations or species-specific impacts. The significant effects of Am on trophic resource availability and foraging behaviour of wild bees, indicate that its introduction is a strong candidate to explain this drop in wild bee abundance. Several studies have documented the negative effect of Am on wild bee populations[14,86] and on their reproductive success or development through cage experiments[26], trap nests for solitary wild bees[27], or artificial bumblebee nests[28,29,87].

Other factors may have concurred with honeybee competition and contributed to wild bee populations declines. The Mediterranean, a climate change hotspot, is experiencing rising temperatures and aridity[88], which can reduce floral rewards[76,89] and impact pollinator foraging and survival[76,90]. Significant temporal changes in various aspects of wild bee activity indicate a potential influence of environmental conditions. Flower abundance, which declined during our study timeframe, may be among possible drivers of the observed changes. However, no significant temperature trend was observed during the adult activity period over the past nine years. Moreover, aridity did

not notably increase in the last four years since rising summer temperatures from 2021 to 2024 were accompanied by more intense precipitations.

Notably, the effect of the 'year' variable on foraging behaviour mirrored shifts triggered by Am presence, such as a decrease in visitation rates among wild species and longer lag times for pollen collection in Ad. Two exceptions were observed: increased nectar volume over time and longer lag times for nectar probing in both species. We can speculate that the former can be ascribed to increased precipitations, the latter to reduced consumption of nectar due to the decline of wild pollinator abundance. Disentangling the relative contribution of honeybee density and ecological determinants in a four-year period is challenging, with the only solution being the removal of honeybees from the island for a sufficiently long time. This would also allow testing of the effects of prolonged Am exclusion on wild bee population abundance.

Another unmeasured potential impact on wild bee behaviour and survival[91,92] is pathogen spillover from Am, for which evidence has been growing in recent years[13,18,93]. Viruses vectored by *Varroa destructor* (DWV, KBV, and IAPV) and quasi-ubiquitous pathogens (BQCV, SBV and *Nosema ceranae*) have been reported in bumblebees, mason bees and leafcutter bees[93], with *N. ceranae* demonstrating an increased virulence in Bt[94]. On Giannutri, limited resources and high density of Am likely increase the potential for pathogen spillover to wild bees, which have not been exposed to these stressors in recent decades[95]. Further studies should increase our understanding of this phenomenon.

Pesticides are effectively absent from the island due to the lack of agriculture. A localized spraying of FLYTRIN 6.14 Colkim is restricted to about 7% of the island around the main village for mosquito control during summer season when adults of the target species are absent (except for a few Bt, Fig. S4). Additionally, ground-nesting preimaginal stages (as Ad and Bt) are less susceptible to pesticides[96]. We are thus confident that these limited treatments cannot account for the observed decline across the entire island.

Conclusions

Using a manipulative approach in a confined ecosystem, we provide strong evidence for the negative impacts of exploitative competition between honeybees and wild bees. Honeybee competition, alongside potential changes in resource availability due to climate change, led to an alarming 80% decline in wild bee abundance over four years. In an increasingly demanding environment, high densities of honeybees can amplify damages caused by other stressors, especially in fragile ecosystems such as small islands[33,97]. These findings, obtained in a Mediterranean maquis model system, are likely scalable to larger islands and mainland areas, particularly in conservation-critical zones such as National Parks. Among the many threats to wild bees from human activities and climate change, competition with honeybees is one that can be managed or removed. We thus highly recommend that beekeeping should not be allowed unless evidence proving no harm to local populations is provided, especially in small, protected areas. Globally, our study paves the way for further research to evaluate competition based on eco-ethological monitoring and address key methodological challenges highlighted in recent reviews[13,18,20].

6.5 Resource availability

Lead contact

Further information and requests for resources should be directed to and will be fulfilled by the lead contact, Leonardo Dapporto (leonardo.dapporto@unifi.it)

Materials availability

This study did not generate new unique reagents

Data and code availability

- The data supporting the findings of this study have been deposited at Zenodo and are publicly available as of the date of publication. DOIs are listed in the key resources table

- All original code has been deposited at Zenodo and is publicly available as of the date of publication. DOIs are listed in the key resources table
- Any additional information required to reanalyse the data reported in this paper is available from the lead contact upon request.

6.6 Acknowledgments

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Author contributions

Conceptualization; LP, AC, LD; Methodology; LP, AC, LD, CB; Formal Analysis; LP, LD, AC; Investigation; LP, AC, CB, MB, SS, EM, VS, SP, GS, GV, MP, EvT, FB, MSV, LD; Data Curation; LP; Writing–Original Draft; LP, LD, AC, CB; Writing–Review & Editing; LP, AC, CB, MB, SS, EM, VS, SP, GV, MP, EvT, FB, MSV, FG, LD; Visualization; LP; Supervision; LP, AC, LD, FG; Project Administration; LD, AC, FG; Funding acquisition; LD, AC, FG, LP

Declaration of interests

The authors declare no competing interests

Supplemental information titles and legends

Data S1. Statistical analyses related to Figures 1-5 and STAR METHODS.
Supplemental PDF. Figure S1-S4 and Tables S1-S6, related to Results and STAR Methods.
Table S2. Expected direction of the effect of competition on the parameters measured in this study based on available literature, related to Discussion and STAR Methods.
Table S5. Variables used in the main text and their sample size, range or levels specified for each analysis, related to STAR Methods.

Key Resources Table

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Deposited data		
All data used for the analyses	This paper, available on Zenodo	10.5281/zenodo.14855496
All R scripts (Codes) used for the analyses	This paper, available on Zenodo	10.5281/zenodo.14855496
Experimental models: Organisms/strains		
Anthophora dispar Lepetelier, 1841	Wild population on Giannutri Island, Italy	N/A
Bombus terrestris Linnaeus, 1758	Wild population on Giannutri Island, Italy	N/A
Apis mellifera ligustica Linnaeus, 1758 hives	La Pollinosa	N/A
Software and algorithms		
R software v 4.1.3	R Core Team[104]	https://www.r-project.org/
R package: mgcv v 1.8-41	Wood and Wood[106]	10.32614/CRAN.package.mgcv
R package: corrplot v 0.92	Wei and Simko[105]	10.32614/CRAN.package.corrplot
R package: emmeans v 1.10.1	Lenth[108]	10.32614/CRAN.package.emmeans
R package: DHARMa v 0.4.6	Hartig[107]	10.32614/CRAN.package.DHARMa
R package: gam.hp v 0.0-2	Lai et al.[109]	10.32614/CRAN.package.gam.hp
R package: sjPlot v 2.8.15	Lüdecke[110]	10.32614/CRAN.package.sjPlot
R package: fossil v 0.4.0	Vavrek[111]	10.32614/CRAN.package.fossil
R package: bipartite v 2.18	Dormann et al.[112]	10.32614/CRAN.package.bipartite
R package: vegan v 2.6-4	Oksanen et al.[117]	10.32614/CRAN.package.vegan
R package: irr v 0.84.1	Gamer[98]	10.32614/CRAN.package.irr

BORIS (Behavioral Observation Research Interactive Software)	Friard and Gamba[118]	https://www.boris.unito.it/download/
Other		
Hand refractometer calibrated for small volumes	Bellingham + Stanley - Xylem Inc, UK	45-81

6.7 STAR Methods

EXPERIMENTAL MODEL

This study was conducted on Giannutri Island (decimal Latitude: 42.251 and decimal Longitude: 11.100), the southernmost and second smallest island of the Tuscan Archipelago (2.6 km^2). Situated 11.5 km from Mount Argentario, a fossil island connected to the Italian mainland, and 16 km from the nearest island, Giglio island, Giannutri island is entirely within the Tuscan Archipelago National Park since 1996. Its southern part is protected as a strict reserve and the island is also part of the Natura 2000 network (site code IT51A0024) as SIC (Site of Community Interest) and ZPS (Zone of Special Protection) for birds. The island's vegetation is dominated by maquis of *T. fruticans*, *S. rosmarinus*, *E. dendroides* and *Juniperus turbinata*, with patches of woodland of *Quercus ilex* and *Phillyrea latifolia*. The evolution of the island's vegetation over 50 years, in the absence of human impact such as logging, agriculture, and fires, was very slow. It primarily consisted of the expansion of *Juniperus* thickets within the maquis, without leading to a significant transition from maquis to oak woodland[43]. Giannutri had no recorded beekeeping activities at least between the establishment of the National Park in 1996 and 2018. There are no known records of beekeeping in earlier years either, although the island has been inhabited since ancient times, as evidenced by the presence of a Roman villa. Since December 2018, in agreement with the National Park, a single apiary has been placed every year approximately in the centre of the island by professional beekeepers (Figure 2A). The island is used as an isolated mating site for selection purposes. Hives are brought to the island in mid-December and removed in mid-June. From 2021, when we started our preliminary analysis, the

apiary usually consisted of 18 hives (18 hives in 2021 and 2022, 17 hives in 2023, 18 in 2024, resulting in a density of ~ 7 hives/km^2). In mid-April, after the experimental period, *Apis mellifera* (Am) density increases as approximately 200 mating hives with one queen and ~650 workers each are added (Gianni Alessandri and Paola Bidin, personal communication) which corresponds to the worker force of about 4-7 colonies. The island isolation prevents the arrival of Am from elsewhere, and there are no unmanaged bee colonies[37] as we did not observe Am individuals after the seasonal removal of the hives (transect data from mid-June to December). Consequently, all Am observed on the island belong to the managed colonies located at the apiary. The small size of Giannutri allows Am to forage throughout the entire island (the maximum linear distance from the apiary on Giannutri Island is 1.7 km, widely within the foraging range of honeybees[38]).

Climate is known to drive variations in pollinator abundance across different regions, although the direction of these effects is not always negative[98] . Our goal was to describe recent trends in two key climatic variables—temperature and rainfall—associated with one of the most significant climatic alterations in the Mediterranean region: increasing aridity[88].

We obtained recent data on daily maximum temperature and weekly rainfall from the nearest weather station on Giglio Island and modeled data for three distinct periods of the year: 1)The main activity period of adult individuals of the target species (January 1 – April 30, Figure 5B, D), 2) The complementary period covering diapause, aestivation, and immature stages (May 1 – December 31, Figure S3), and 3) The hottest period, between the summer solstice (June 21) and the autumn equinox (September 21) (Figure 5C, E).

We performed GAMs using the following formula:

gam(temperature ~ s(Julian day) + year)

Experimental design framework

Giannutri had no recorded beekeeping activities at least from 1996, but likely since much earlier, until 2018, only three years before our study, allowing potential negative effects on wild populations to still be observable as declining abundance trends. To investigate this, we adopted a manipulative approach by temporarily preventing honeybees from accessing resources. In collaboration with beekeepers, we closed the hive entrances on specific days. Closures occurred early in the morning (around 6 AM, sunrise) to ensure all workers were still inside the hives. Hives were re-opened in the afternoon of the same day (around 5:00 PM) to minimise stress imposed upon the honeybees and reducing the possibility of a generalised increase in the activity of honeybees as a compensatory behaviour following the closure. An *ad hoc* analysis showed that the Am abundance and activity did not increase the day after the hive closures (see Validation methods and analyses section). The experimental closure of the hives created two experimental conditions: i) hives open, with Am free to forage on the island (HB+) and ii) hives closed, with Am prevented from foraging (HB-). We performed all the observations under both experimental conditions to assess the effect of a short absence of Am on trophic resources as well as the activity and foraging behaviour of wild bees. To preserve the health of honeybee colonies, hives couldn't be kept closed for two consecutive days. In the first year (2022), HB- days were followed by at least two HB+ experimental days to allow the validation described in the Validation methods and analyses section. After verifying that honeybee activity did not increase the day after the closure (Data S1AE-AG), for 2023 and 2024 we ensured that HB- days were still preceded and followed by at least one day with hives open. These experimental cycles were subjected to weather conditions and in case of forecasted rain and/or strong wind the hives were left open, thus altering the temporal sequences of the closures. This resulted in the overall number of HB+ days being approximately double than of HB- days. A detailed list of experimental days is given in Table S3.

We conducted four different types of surveys (see below for details): i) transect walks (to monitor abundance trend and study resource overlap), ii) resource

availability (to detect changes in nectar and pollen availability after the short-term removal), iii) plot observations (to detect changes in wild bee activity) and iv) focal observations (to detect changes in target wild bees key aspects of foraging behaviour). The expected effects of Am on the measured parameters were literature-based and are summarised in Table S2. Surveys were conducted only when wind intensity was equal to or less than 5 on the Beaufort wind force scale, as is standard practice in pollinator studies[99]. A preliminary analysis using walking transects[37] showed that target wild bee populations on the island are active through April, with peak abundances occurring in February–March. As a result, our experiment was conducted from February to early April. We could not extend our experiments into April due to rising temperatures, which would pose a risk to colony health during hive closures and to the mating activity managed by beekeepers using the 200 small mating hives. Therefore, after early April, only walking transects for demographic trend investigations were conducted. Overall, we carried out 54 experimental days, 16 HB- days and 38 HB+ days (5 HB- days and 8 HB+ days in 2022, 5 HB- days and 13 HB+ days in 2023, 6 HB- days and 17 HB+ days in 2024). Monitoring for wild bee demographic trends began in 2021. For this analysis, we included an additional 7 HB+ days from February–April 2021 (Table S3). Sampling efforts for each method are detailed in their respective sections.

Animal and plant models used

A one-year monitoring study[37], revealed that managed honeybees are the most abundant bees recorded on the island. *Anthophora dispar* (Ad) and *Bombus terrestris* (Bt), together accounted for 91% of the total counts of wild bees[37]. Due to their abundance, easy field identification, and contrasting social behaviours (Ad is solitary, while Bt is social), they were selected as target species. Preliminary observations on Giannutri island identified *S. rosmarinus* and *T. fruticans* as the most visited flowering plants by both wild and managed bees[37]. Given their widespread distribution on the island, these plants were chosen to assess the impact of honeybees on trophic resource availability.

Three types of aforementioned surveys involved animals. In transect walks and plot observations, all encountered individuals were recorded without being handled. Since no marking was performed, the exact number of individuals remained unknown, and the number of observations is indicated in the result section. The same applied to focal observations, where the behavior of the first individual encountered was described in each session.

For the resource availability surveys involving measurement of nectar *T. fruticans*, *S. rosmarinus* flowers were randomly selected at three (2022-2023) and five (2024) sites in each survey day for a total of 1524 flowers (see below for details); for pollen *T. fruticans* flowers at 20 sites were randomly selected in each survey day for a total of 8648 flowers (see below for details).

To validate the assumption that a longer suction time corresponds to a greater nectar intake, we randomly collected 32 Bt workers on the island and released them after the experiment.

METHOD DETAILS

Blindness of the observations

To minimise operator bias regarding the direction of the expected results, we took steps to limit the knowledge of the hive condition. For logistical reasons (maintaining the experimental days sequence, considering weather conditions and the need of two people to close the hives for safety reasons), four authors were aware of the established hive condition for the following days. Hive closures were not communicated to other operators. While it was not possible to maintain full blindness, since the absence of Am could be easily detected through brief observations on the island, several precautions were taken to limit bias: i) the hive condition was not communicated in advance of the experimental days, ii) preliminary results about the direction of the relationship with the variable of interest were not shared with the operators, iii) operators were unaware of the hypotheses about the potential effect a short-term removal of Am could have on wild bees on Giannutri island.

Recording of environmental variables

Before starting any observation session, the operators recorded their name, the date, the time of day (standard time), sampling point, wind intensity (according to the Beaufort scale), estimated cloud coverage. Air temperature was taken on the island using a data logger recording temperature every 5 minutes placed close to the centre of the island at about 1.5 m from the ground. However, temperature at the observation time was excluded from models because it is highly related with Julian day, hour and cloud coverage. Accordingly, temperature concurvity was higher than 0.74 (average 0.86) for all GAMs (see next sections). We thus decided to maintain in the GAMs the variables about Julian day, hour and cloud coverage and to remove temperature (details in Validation methods and analyses section and results in Table S6).

Trophic resource availability

Pollen

We aimed to evaluate whether the short-term removal of honeybees could affect pollen availability[22,39]. We conducted this survey in March 2024 on *T. fruticans* flowers, when the species was in full bloom. This species was selected because it has bigger flowers and anthers than *S. rosmarinus* and could be investigated more precisely with a visual method. Twenty sites spread across the island were chosen across the island (blue stars in Figure 2A, coordinates in Table S4). One bush per site was selected opportunistically, georeferenced and tagged in the field using red and white tape. On each survey day, an operator checked for the presence or absence of pollen on each anther of maximum 50 random flowers per bush by visually examining them[22,39]. We planned these assessments over five pairs of days. Pollen availability was assessed in the two experimental conditions, with the first condition for each pair alternating between HB+ and HB-. For each pair of days, the same bushes were investigated. In successive pairs, other bushes were selected within 15 meters radius of the original

georeferenced point. While within each pair of days, the direction of bush assessments remained consistent (e.g. from north to south), it varied between different pairs of days to avoid linking a specific site to a specific hour of the day. Due to weather conditions, we were unable to perform pollen assessments on the last planned day. As a result, 9 days were assessed, with 5 HB- days and 4 HB+ days. Overall, it was possible to visually inspect 8648 flowers (3949 in HB+ days and 4699 in HB- days). We assessed the reliability of the visual method to assess the presence of pollen on *T. fruticans* anthers by computing inter-rater agreement[100]. Seven authors were presented with 28 photos of *T. fruticans* flowers from Giannutri. Each operator independently evaluated the number of anthers with pollen out of the four anthers, and then the absolute inter-rater agreement was computed using the "icc" function of the "irr" R package[101], using two-way model and single unit of analysis, following[102]. We found that good degree of inter-rater absolute agreement[102](ICC (A,1)=0.849, with 95%-Confidence Interval for ICC Population Values: 0.764 & lt; ICC & lt; 0.916, F(27,139)=44.1 , p=3.13e-55).

Nectar

We assessed nectar availability on *T. fruticans* and *S. rosmarinus* in 2022, 2023 and 2024, over 7 (4 HB+ and 3 HB-), 11 (6 HB+ and 5 HB-) and 7 (3 HB+ and 4 HB-) days, respectively. We selected three sites with at least two distinct plants of each target species. In 2024 we added two more sites at different distances from the apiary (black squares in Figure 1A, coordinates in Table S4). In each site, we sampled two bushes for each species except for one of the two sites added in 2024, where only *S. rosmarinus* bushes were available. These bushes were marked and surveyed in different days and years. In addition to the pollen availability analysis, we bagged 4-6 branches (depending on the number of flowers on them) with a fine mesh bag 24 hours before measurements[40] to evaluate the baseline nectar standing crop and study the potential nectar volume available in the absence of consumption. The flowers were chosen randomly from those that appeared undamaged and were at the same life stage (with vivid

colours and well-developed, turgid anthers). Nectar was extracted from flowers using 1µL microcapillary tubes (Drummond Microcaps®, length=32mm, capacity=1µL, accuracy=1%). The proportion of nectar inside the capillary was measured with a ruler to obtain the volume of the nectar[40,89]. In 2023 and 2024 we measured sucrose concentration with a hand refractometer calibrated for small volumes (Eclipse refractometer, Bellingham+Stanley, 0-50% Brix). We could not measure sucrose concentration for extremely small volumes (mostly in the 0.03-0.06µL range) due to intense evaporation[40]. To improve the accuracy of the Brix measurement, we diluted the nectar sample with 1µL of distilled water and then converted the result based on the initial nectar volume before dilution. Overall, we sampled 1524 flowers; 829 *S. rosmarinus* flowers (212 bagged in HB+, 201 bagged in HB-, 215 unbagged in HB+ and 201 unbagged in HB-) and 695 *T. fruticans* flowers (178 bagged in HB+, 165 bagged in HB-, 181 unbagged in HB+ and 171 unbagged in HB-). The different number of experimental sites between nectar and pollen is due to logistic constraints, in particular, the different time required for pollen (visual inspection) and nectar measurements (capillary + refractometer). Therefore, a limited number of flowers can be assessed by nectar examination during the relatively short HB- period. In this respect we minimised the movement of the operator among sampling points. Moreover, we could not use the same bag approach to inspect pollen, as pollen could accidentally be removed when bagging branches, especially of flowers touching the bag for long periods (24 hours).

Activity and behaviour

General framework

To verify the effect of the short-term removal of Am and evaluate their influence on the activity and foraging behaviour of wild bees, we conducted two types of observations: plot observations and focal observations. We first identified suitable areas on the island, excluding inaccessible regions (e.g., dense Mediterranean maquis, private properties, and areas without flowering plants

during the experimental period, such as the rocky coast). To evenly distribute sampling points, we divided the island into six macro-areas of similar size. We established that each macro-area should contain between 8 and 10 different sites. We then created 1000 random points on the island and selected only those within suitable areas. For each macro-area, we randomly selected 10 points (among the random points within suitable areas). We obtained 60 sites (random points) across the entire island. These sites were then inspected in the field and their position was adjusted so that every site consisted of the nearest flowering spot. If no flower patches were found within a 40-meter radius, the point was excluded. This resulted in a final selection of 57 sites. For each site, we randomly chose another suitable sampling point (consisting of a flower patch) within a 40-meter radius from it. We thus established a total of 57 pairs of points (Figure 3A) that were used for both plot observations and focal observations. Each day, one sampling point of each pair was surveyed using the plot observation technique, while the other was surveyed using the focal observation technique by different operators. After three days (having sampled each point in both experimental conditions), the sampling methods were switched between points. The order of the sampling points and the operators conducting the observations were also rotated to avoid bias related to time of day or specific operators. For focal observations, 15 sampling points (not coupled with plot observations points) were added for opportunistic sampling, this led to a different number of replicates for each sampling method. Sampling points were marked in the field with white and red warning tape to ensure that different operators could consistently locate them.

The sampling points were selected in 2022 and maintained over all three experimental years with some logistical modifications (e.g. cut of bushes by local people) that forced us to substitute some two sampling points and eliminate nine pairs of sampling points after the first year of sampling. Table S4 summarise all sampling points with their coordinates, their status (regular or opportunistic) and linear distance from the apiary.

Plot observation sampling method

We conducted plot observations[103] to assess the abundance and activity of bees across the island. Plots were rectangular (1 m x 2 m) and consisted of flower patches of shrubs or herbaceous plants; they were permanently marked during the sampling period with red and white warning tape and delimited during the observation with plastic tubes to precisely visualise the edges. We observed wild bees using two sampling methods commonly used to study pollinators' behaviour and flower visitation rates [99]: "all occurrences sampling" and "scan sampling"[104,105]. All-occurrence sampling involved recording movements inside and outside the observation plot for 2 minutes. Due to the low abundance of the target species on the island, we could track the entrance and exit of most individuals (without focusing on their behaviour inside the plot). Bees inside the plot were followed to assign each movement (inside and outside) to a specific individual. The observation of each bee was defined as an "observation session" and the same individual could be observed multiple times across different sessions. Every movement inside or outside the observation plot that was not possible to assign to a specific individual was promptly indicated on the field (i.e. if two or more individuals physically interacted with each other). The scan sampling consisted of an instantaneous count (<10s duration) of all the bees inside the plot at that moment and the flowers they were visiting (distinguished by species). During each plot observation, we performed four scans alternated by three all-occurrences. The total duration of a plot observation was a maximum of 6.5 minutes. The observations were recorded in the field with an audio recorder together with the additional data described above. All bees were identified to species level when possible. We specified the sex for Ad as it is easily distinguishable. The operator carrying out the plot observation made sure not to disturb the pollinators by maintaining a sufficient distance and avoiding casting a shadow onto the plot. We aimed to obtain three metrics from the observation plot that will be more precisely described below: the abundance of individuals counted during scan sampling, time spent inside the plot, and the

number of movements inside and outside the plot. Overall, we conducted 2076 plot observation replicates: 1397 in HB+ days (414 in 2022, 451 in 2023 and 532 in 2024) and 682 in HB- days (240 in 2022, 204 in 2023 and 238 in 2024).

Focal observation sampling method

Focal animal observation involves following a single individual and describing its behaviour[104]. We used this method to study key aspects of the foraging behaviour of bees. The duration of each focal observation was approximately the same as for the plot observations (6.5 minutes). Unlike plot observations, focal observations were not strictly tied to the position of the plot. While the observation starts at the marked sampling plot (with white and red warning tape), the operator was allowed to move up to 20 meters from the point to locate foraging pollinators. For each sampling point, we tried to describe the foraging behaviour of both target species (Ad and Bt), when possible. For Ad*,* we specified the sex. During each focal observation, multiple individuals were observed (each one singularly).

The observation of an individual started when it was seen approaching the flowers and ended when it flew out of the observer's sight or after two consecutive minutes of behavioural description. The observation of every single animal was called an "observation session" and the same individual could have been observed multiple times in different observation sessions. The foraging behaviour of wild bees was described using an audio recorder. We identified the main foraging behaviours that could be observed, and we used field-code words to promptly describe them. This allowed us to precisely indicate the beginning, end and occurrence of each behaviour for each observation session. We recorded wild bee behaviours on every plant they visited. The plant on which a certain behaviour occurred was always specified. Overall, we identified four distinct behaviours: probing nectar, collecting pollen, non-foraging and flying away (representing the end of an observation session). The "probing nectar" behaviour refers to the action of a wild bee probing nectar inside the calyx with

its tongue after landing on a flower, this is particularly visible on flowers of *T. fruticans* and *S. rosmarinus*. As the tongue is not visible inside the flowers, we also recorded the action of exploring the calyx to check for the presence of nectar as "probing nectar". "Collecting pollen" indicates the activity of collecting pollen from flowers by a wild bee. Due to the shape of *T. fruticans* and *S. rosmarinus* flowers, the anthers are exposed, and this behaviour is usually easily distinguishable from nectar probing in the field (as bees use their legs to collect pollen). Ad usually performs this behaviour in hovering flight, especially on *T. fruticans* flowers. "Non-foraging" refers to all other behaviours not included in the previous categories (e.g. flying or walking from one flower to another, resting on vegetation). "Flying away", although not strictly a behaviour, indicates that the individual has flown out of the operator's sight marking the end of the observation session. We aimed to obtain three metrics from the focal observation: the time of nectar suction from flowers, the nectar search time and the pollen search time, with search time defined as the lag between an event of collecting the trophic resource (nectar or pollen) and the consecutive behaviour of the same type. Suction time represents the duration of foraging for nectar on a flower.

We verified our assumption that suction time is positively correlated with the amount of probed nectar, an assumption supported by literature[72,73]. We tested it on wild pollinators of Giannutri island using suction time on flowers as a proxy of the amount of nectar probed by wild bees. The validation procedure of this behavioural metric is precisely described in the Validation methods and analyses section.

Overall, we conducted 2084 focal observation samplings: 1382 in HB+ days (352 in 2022, 503 in 2023 and 527 in 2024) and 702 in HB- days (244 in 2022, 215 in 2023 and 243 in 2024).

Walking transects

To monitor pollinator populations on the island, we established six walking transects in 2021 (Figure 5A). Walking transects are a common and effective method to investigate pollinator trends across the years[103,106]. We followed a standardised transect protocol for Italy according to the first ISPRA (Institute for Environmental Protection and Research in Italy) guidelines, which were already tested on Giannutri Island during our preliminary study[37]. The walking transects are fixed 250m long walks, 4m wide and 2m high. Each transect is divided into 10 subunits, 25m each, and must be surveyed for 50 minutes (5 minutes per subunit). During the walk, each Anthophila interacting with a flower is recorded and identified to species level (when possible) and their sex is specified, if possible. As requested by the ISPRA protocol, for each transect subunit for each replicate, we recorded the percentage of area covered by flowered vegetation at three levels (grassland, shrubland and arboreous) and converted them into classes of coverage (0=0-10%; 1=11-30%; 2=31-50%; 3=51-100%). At the beginning and at end of each replica of the transects, we recorded the hour (standard time), the wind strength (according to the Beaufort scale) and cloud coverage, the name of the operator, transect ID and date. Cloud coverage is converted into coverage classes according to the following aggregation: 0=0%, 1=<10%, 2=10-29%, 3=30-29%, 4=50-69%, 5=>70%. We replicated walking transects for three different purposes: i) to evaluate trophic resource overlap between wild and managed bees and study whether the removal of Am changes the diet of wild bees, ii) study the variation in target wild bee abundance across seasons and years and iii) study the short-term and long-term effects of Am presence on wild bee distribution and abundance on flowers. To study the abundance trend, we replicated all established walking transects from February to October between 2021 and 2024. To study changes in the distribution and diet of wild bees in relation to Am presence, we conducted transect surveys more frequently during the experimental period (February–March, starting in 2022) under both experimental conditions (HB+ and HB-). Overall, we conducted 407 transect replicates from March 2021 to September 2024, including 199 during the experimental period. Of these, 142 replicates were carried out on HB+ days

(42 in 2022, 30 in 2023, and 70 in 2024), and 57 on HB- days (24 in 2022, 7 in 2023, and 26 in 2024).

QUANTIFICATION AND STATISTICAL ANALYSES

General

All analyses were performed in the R environment (version 4.1.3)[107]. We checked for correlation among numerical variables using the R package "corrplot"[108]. Generalized Additive Mixed Models (GAMMs) were generated with the "gam" function of the "mgcv" R package[109]. We used the method="REML" as a smoothness selection method, selecting the model with the built-in "double selection approach" (select=T in the model formula). Differences in model parameters and variable type are specified in the relative paragraph (Table S5). The model assumptions were tested using the DHARMa R package[110]. Based on these tests, we adapted the model structure. Only the final models used in the analysis are reported in the original code. For interactions with categorical variables, we conducted Tukey *post hoc* tests using the "emmean" R package[111]. We calculated the fraction of deviance explained using the gam.hp function of the "gam.hp" R package[112]. This function calculates the deviance explained by each variable of the total deviance explained by the model (I.perc), we then calculated the deviance attributed to each effect (expl.var in the text) using the following formula: *(I.perc/100)*deviance explained by the model*. The statistical details for the GAMs (Estimate or Estimated degree of freedom (edf), Standard Error, z-value, p-value and explained variance (I.perc and expl.var) are available in Data S1 including the DHARMa test for the assumptions. We plotted the predicted values (marginal effects) for specific model terms of the GAMMs with the "plot_model" function of the "sjPlot" R package[113]. Linear distances were calculated using the "earth.dist" function of the "fossil" R package[114].

Trophic niche overlap and visiting frequencies

To study resource overlap between Am and wild bees on the Giannutri Island, we used a hierarchical approach to construct a flower-visit network using the "bipartite" R package[115]. Due to their numerical dominance, we only used the data of Am, Ad and Bt in the network analyses[37]. To provide a biologically relevant picture of the flower-visit network we built daily networks, thus avoiding the creation of "forbidden links" by analysing network based on pooling together several days[116]. In this case, we constructed a flower-visit network for each day from 2022 to 2024, encompassing the period February-April, using both HB+ and HB- days. For each day, with 3 walking transects performed at least, we pooled all bee-plant interactions observed across the six transects. If some days had a limited number of transect walks, we pooled together data from days with the same experimental conditions, either two consecutive days or days separated by at most three days. For daily networks, we used days with at least 10 recorded visits by wild bees to flowers for the target wild species. We computed the network-level indices H2', Niche overlap and weighted NODF, using the "networklevel" function of "bipartite", and the pollinator species-level indices species.strength, interaction push-pull index, PSI and the Bluthgen's d' using the "specieslevel" function of "bipartite". H2' is an index of specialisation which depends on the level of specialisation of the nodes[117], it ranges from 0 (highly generalised network) to 1 (highly specialised network), it and it is known to be robust in variations of matrix size, shape and sampling effort[117,118]. Niche overlap is based on the Horn's index, and it evaluates similarities in interaction patterns between species of the same trophic level, pollinators in our case, and it ranges from 0 (no overlap in resource use) to 1 (perfect overlap). The weighted NODF (Nestedness based on Overlap and Decreasing Fill) is a quantitative (weighted) measure of nestedness which indicates the tendency of specialist species to interact with a subset of species that also generalist species interact with. It ranges from 0 (no nested pattern) to 1 (highly nested pattern). The species.strength quantifies species relevance across all nodes in the other trophic level. The interaction push-pull index is a measure of interaction asymmetry that ranges from -1 to +1, with positive values indicating that a plant is more dependent on the pollinator's presence ("pusher" species), while

negative values indicate that a pollinator is more dependent upon the plants ("pulled" species). The PSI (Pollination Service Index) measures the importance of a pollinator to all plant species, quantifying the pollination service provided by pollinators, it ranges from 0 (the pollinator is irrelevant to all plant species) to 1 (all visits are made to a single plant species that is completely dependent on that pollinator). The Bluthgen's d' index measures the specialisation of a species, it ranges from 0 (no specialisation) to 1 (perfect specialist) and depends on the relative abundance of other species of the same trophic level. The PAC (Potential Apparent Competition) was calculated using the function PAC of "bipartite". It is based on the Müller index[119,120] and represents a measure of the degree of niche overlap between two species and is being used to investigate the potential influence of Am on wild bees via the trophic resource they share[21–23]. We computed this index only in HB+ days and we tested whether its value increased during the season (effect of day of the year), following the growth of Am colonies, through linear regression, using the "lm" function. For daily networks, we also modified the Müller index to calculate PAC differently, accounting for the collective impact of all other pollinators on a given species rather than pairwise interactions. Müller index formulas, used to calculate the PAC, and our Müller_modified index are presented below:

$$\text{Müller index}_{i,j} = \sum_{k=1}^{n} \frac{a_{i,k}}{\sum_{k=1}^{n} a_{i,k}} \times \frac{a_{j,k}}{\sum_{m=1}^{p} a_{m,k}}$$

$$\text{Müller\_modified index}_{\,i} = \sum_{k=1}^{n} \frac{a_{i,k}}{\sum_{k=1}^{n} a_{i,k}} \times \frac{\sum_{\substack{m=1 \\ m \neq i}}^{p} a_{m,k}}{\sum_{m=1}^{p} a_{m,k}}$$

where $a_{i,k}$ represents the number of visits by pollinator *i* to plant species *k,* divided by the summation of visits by pollinator *i* to all plant species (*k* to *n*). This first term indicates the frequency in which pollinator *i* utilizes plant *k*. In our

Müller_modified index the numerator of the second term, instead of using the number of visits to plant *k* by pollinator *j*, $a_{j,k}$, provides the summation of visits to plant *k* by all other pollinators in the network, except for pollinator *i*. In both indices, $a_{m,k}$ represents the number of visits to plant species *k* by pollinator *m* of community *p* and the summation indicates all visits observed for plant species *k* by all of the pollinators (from *m* to *p*). Both indices range from 0 (resources visited by the examined species are only visited by it) to 1 (resources used by the examined species are largely and predominantly visited by (the) other pollinator(s)). We calculated this index for each day.

We tested whether the computed indices differed between HB+ and HB- with a Mann-Whitney test using the wilcox.test (paired=F) function. In total, we used n=29 networks (22 HB+ and 7 HB-) in this analysis, the statistical details of the analyses (W, and p. values) are available in Data S1A-D. Quantiles of daily index values have been plotted using boxplot (0%,25%,50%,75%.100% quantiles). For the daily network-level indices we also tested whether the observed value was significantly different from values obtained for random network. To test this, for each daily network we created 1000 random networks using the nullmodel function with the *vaznull* null model to maintain, in the randomly generated networks, the plant and pollinator richness, total number of interactions and the observed value of connectance[33]. For each random network we calculated the three network-level indices. We then tested the percentage of indices values (obtained from the random networks) that was greater or lower than our observed values (two-tail p-value). We considered the indices for the daily networks as significantly different from a random network when the p-value was lower than 0.05 (statistical details in Data S1B). To provide a readable data visualisation of the networks in the two experimental conditions, we built one network pooling together all data from the HB+ days, and another pooling together all data from the HB- days. Comprehensive flower-visitor networks (including all the pollinator and plant species) are available in Figure S1A (HB+ days) and S1B (HB- days).

To assess the effect of short-term removal of honeybees on dietary choices, we calculated flower visitation frequency to each plant species by the two target wild bee species on each day. The frequency was calculated by dividing the number of visits by each target species to each plant species on a given day by the total number of flower visits by that species on the same day. We tested whether visitation frequencies of each wild bee species to each plant species differed between HB+ and HB- using a PERMANOVA (Permutational Multivariate Analysis of Variance). This was done using the “adonis2” function from the "vegan" R package[121] using n=29 days of transect walks (see above) and 9999 permutations. The analysis examined the effect of hive condition on the frequency matrix across 45 plant species (not all species were visited on the same day). Statistical details (sum of squares, R^2, F and p values) are reported in Data S1D.

Trophic resource availability

Pollen

Among the 8648 *T. fruticans* flowers inspected, we only included those with all four anthers in our analysis, excluding flowers that had lost at least one anther. This resulted in a total of 8425 suitable flowers. Instead of using the wind intensity recorded at the time of observation we used the mean daily wind intensity which can have a greater impact on determining pollen availability (i.e. limiting or promoting the daily activity of pollinators). We considered each flower as a sample. We used a*nther.pollen* (the number of anthers carrying pollen on each flower) as the response variable in our GAMM. As predictive variables, we used *hour* (standard time converted into decimal values to use as a numeric variable), *day* (Julian day of the year, numeric), *wind* (the mean daily wind intensity), *dist* (linear distance from the apiary, numeric), *hives.condition* (categorical variable with 2 levels, indicates if hives were open or closed) and the interaction between *dist* and *hives.condition*. We considered the *site* (site ID, categorical) and *plantID* (ID of each sampled plant, categorical) as random

factors. We controlled for spatial autocorrelation and used the negative binomial family. Accordingly, we used this formula (n=8425 flowers):

gam(a*nther.pollen*~s(*hour*,k=5,bs="cr")+s(*day*,k=5,bs="cr")+s(*wind*,k=5,bs="cr")+*dist***hives.condition*+s(*site*,bs="re")+s(*plantID*,bs="re")+s(*plantID*,bs="re"),correlation=corSpatial(form=~long+lat,type="gaussian"),family="nb",method="REML",select=T)

We calculated the increase in pollen availability between HB+ to HB- at the closer and more distant sites using predicted values (marginal effects) for distance in GAMMs. For each distance value (minimum and maximum) we calculated this increase by subtracting from the predicted value in the HB- the value in HB+ and then dividing it by the value in HB-. Statistical details (estimates, p values and explained variance) are reported in Data S1I.

Nectar

We modelled the nectar *volume* (µL) as the response variable. We used *hour* (as defined above)*, day* (as defined above)*, hives.condition* (categorical variable with 2 levels)*, wind* (numeric variable, wind intensity on Beaufort scale)*, cloud* (percentage of cloud coverage, numeric)*, dist* (as defined above)*, treatment* (categorical variable with 2 levels, indicates if the flowers were unbagged or bagged) and *yearor* (numeric variable) as predictive variables. We added an interaction term between *day* and *hives.condition* and an interaction term between *hives.condition* and *treatment*. We used *plantID* (ID of each sampled plant, categorical) and *site* (site ID, categorical) as nested random factors (*plantID* nested into *site*) and the *operator* (name of the operator that measured the volume, categorical) as a random factor. We used the "tweedie" family. Accordingly, we adopted the following formula for both species:

gam(*volume*~s(*hour*)+s(*day*)+s(*day*,by=*hives.condition*)+s(*wind*,k=5)+s(*cloud*,k=5)+*dist*+*hives.condition***treatment*+*yearor*+s(*site*,bs="re")+s(*plantID*,bs="re")+s(*site*,*plantID*,bs="re")+s(*operator*,bs="re"),family="tw",method="REML",select=T)

For *T. fruticans,* the sample size was n=695 flowers, while for *S. rosmarinus,* it was n=829. We also modelled the sucrose *concentration* of nectar (in °Brix values) as the response variable using the same GAMM formula to verify if honeybees' removal could affect sucrose concentration. For this analysis, we excluded all empty flowers (volume equal to 0µL) and all flowers for which it was impossible to measure the concentration (extremely small volumes or unreadable concentration measures). This led to a sample size of n=438 flowers for *T. fruticans* and n=533 for *S. rosmarinus.*

We calculated the consumption between bagged and unbagger flowers in the HB+ and HB- days and the increase in nectar availability between unbagged flowers in HB- and unbagged flowers in HB+ using the mean values estimated by GAMM. In particular, the consumption of nectar was calculated by subtracting the estimated nectar volume of unbagged flowers from the estimated volume of bagged flowers and then dividing it by the estimated volume of the unbagged flowers. The increase in nectar availability for unbagged flowers between the HB+ and HB- days was calculated by subtracting from the estimated value in the HB- the value in HB+ and then dividing it by the value in HB-. Statistical details (estimates, p values, explained variance and Post-hoc Tukey tests) are reported in Data S1E-H.

Activity and behaviour

General data preparation

Defining the sky's cloud coverage can be subjective. To mitigate this problem, we recalculated each *cloud* value for each observation weighting the records by the operators considering the hour of the day and the distance from each

operator. For a given replicate, we considered all the cloud coverage recorded 15 minutes before and after on the island. We then calculated the linear distance from the focal replicate to all available measurements and weighted their cloud values by distance. Distances were converted into an integer values, *class.distance*, by expressing the distance class of 100 metres (e.g. 1=0-100m and 10=900-1000m) of a point from the measurements of interest. To assign the recalculated cloud (rec.cloud) value to each replicate in each day, we used the following equation:

$$rec.cloud_{\mathrm{d,s}} = \frac{\sum_{1}^{length(s')} \left(\frac{cloud}{class.distance}\right)}{\sum_{1}^{length(s')} \frac{1}{class.distance}}$$

Where s' are all replicates surveyed in a 30-minute interval. We then merged the recalculated values of *cloud* into the proper tables. We called this variable *cloud* and used it in the analysis.

Plot observation sampling method

We analysed the audio recording with BORIS software[122]. This software was used to listen to audio recordings of plot observations at speed 0.5. By analysing the audio recordings, it was thus possible to obtain: the number of individuals of the target wild bees observed during each scan sampling, the number of movements inside and outside the plot done by each observed individual and the time each individual spent inside the plot. Accordingly, we considered the following variables: i) *quantity* (sum of animals observed during the four scan samplings of each observation plot replicate), ii) *permanence* (square root of time spent by individuals inside the observation plot) and iii) *activity* (total movements inside and outside each observation plot replicate). All these variables were analysed for both Ad and Bt, while for Am we analysed the *quantity* to study their abundance in relation to the distance from the apiary. In addition, all three variables were analysed for Am only for 2022 to validate our experimental design against possible compensatory effects after HB- days (Data S1AE-AG). For the

permanence analysis, we excluded all those individuals for which the moment of entrance or exit from the plot was not seen or could not be attributed to a specific individual (i.e. after their interaction), we also excluded all values lower than 0.5 seconds, as they represent movement across the plot and not time spent searching for (or exploiting) resources inside the observation plot. We modelled the *quantity* testing for the effect of the following predictive variables: *hour* (as defined above), *wind* (as defined above), *cloud* (as defined above), *day* (as defined above), *yearor* (as defined above), *hives.condition* (as defined above), *dist* (as defined above), *flowercov* (surface of the observation plot covered by flowers, numeric); we also included two interaction terms of *hour*hives.conditions and dist*hives.conditions* to test if the activity of Am led to changes in spatial and temporal activities of wild bees; *operator* was included as a random factor. We also controlled for spatial autocorrelation of the observation plot and used the negative binomial family.

gam(*quantity*~s(*hour*)+s(*hour by=hives.condition,k=3*)
+s(*wind*)+s(*cloud*)+s(*day*)+*year*
+*hives.condition*+s(*dist,k=3*)+s(*dist*,by=*hives.condition,k=3*)+s(*flowercov*)+s(
operator,bs="re"),correlation=corSpatial(form=~Longitude+Latitude,type="ga
ussian"),family="nb",method="REML",select=T).

We adopted the same formula to analyse *permanence* and *activity*. The only difference is in the *permanence* analysis, where we used the tweedie family instead of the negative binomial. For the *quantity* and *activity* models, we used each plot observation replica as a sample (n=2076 plots for both species). For the *permanence* analysis, we used each bee that remained for more than 0.5 seconds inside the plot as analysis-unit, this led to different sample sizes for Ad *and* Bt analyses (n=3309 and n=271 observation sessions (bees, see above), respectively).

Focal observation sampling method

We used the software BORIS to obtain the behavioural sequences as described above. The behavioural sequences can be classified as either state events

(events with a start and stop time) or point events (events that happen at a certain time).

We calculated the suction duration of each nectar-collecting behaviour (state event) by subtracting the time of the recording at the start from the time of the recording at the end of the behaviour, we called this variable *suctime*. We removed all values shorter than 0.005 seconds and all values longer than 100 seconds (11 foraging events removed) from this analysis, as they could represent mistakes in describing the behaviour or non-foraging behaviours. In each observation session, we described the foraging behaviour of one individual. Since there was no way of knowing whether the same individual was focal-observed multiple times over the days, each observation session ID (*spec* column in the provided dataset) is not repeated on different experimental days. Consequently, session ID is completely nested within all other variables, making it impossible to use as a random factor. Indeed, in an exploratory analysis of the data with session ID as random factor, this variable alone explained almost all variance. For this reason, we aggregated the *suctime* by using its mean value of each session ID on each plant species it visited. For Ad*,* we removed all the individuals for which the sex was not specified and we used *sex* as a predictive variable, we could not do the same for Bt and did not consider the sex in the analysis, which is a widely used approach when queens, workers and males cannot be easily distinguished[31].

For each observation session, we also analysed the search time for a specific resource (nectar or pollen) by each *spec*. The pollen searching time, *searchpol*, was calculated by subtracting from the time of a pollen collecting event (point event) on a certain plant species the time of the previous event of collecting pollen from the same species. For this analysis, we considered only those intervals between two events of collecting pollen from the same species where no other behaviours that could have altered the searching time (such as collecting pollen from other species or collecting nectar) occurred. We used the same approach to calculate the searching time of nectar, *searchnec*, between two events of probing nectar from the same species. Since collecting nectar is a

state event, we calculated the duration of these intervals considering the time passed from the end of suction events to the start of the following suction events. We calculated the time between two events of collecting nectar only if no other behaviours occurred meanwhile (such as collecting nectar from other species or collecting pollen). We used the mean value of both *searchnec and searchpol* of the corresponding session ID in these analyses. For each target species, we considered the number of events occurring on different plant species for each analysed parameter (*suctime*, *searchnec* and *searchpol*). We minimised the number of plant species used in the analysis while comprehending at least 95% of the total events for each variable (*S. rosmarinus*, *T. fruticans* and *Borago officinalis* for Bt and for *searchpol* and *searchnec* for Ad). For *suctime* of Ad*,* we considered *S. rosmarinus*, *T. fruticans* and *Bellevalia romana*. For each analysis, we removed data collected by operators that observed less than 0.5% of the total observations. We modelled the *suctime* using *hour*, *wind*, *cloud, day*, *yearor*, *hives.condition*, *dist* (all defined above) and the interaction between *dist* and *hives.condition* as predictive variables and *operator* as a random factor. We accounted for spatial autocorrelation:

gam(*suctime*~*yearor*+*hives.condition*+s(*dist*,k=3)+s(*dist*,by=*hives.condition*,k=3)+s(*hour*,k=3)+s(*wind*,k=3)+s(*cloud*,k=3)+s(*day*,k=3)+*sex*+*Behavior*+s(*operator*,bs="re"),correlation=corSpatial(form=~*Longitude*+*Latitude*,type="gaussian"),weights=weight, family="tw",method="REML",select=T)

We used this GAM formula for all analyses (substituting *suctime* with *searchnec* or *searchpol*), we didn’t use the predictive variable *sex* for the *searchpol* of Ad (as only females collect pollen). The sample size was represented by observation sessions (bees, see above): for Ad nectar suction time n=2653, for Bt nectar suction time n=733, for Ad *searchnec* n=1858, for Bt *searchnec* of n=540, for Ad *searchpol* of n=1378, for Bt *searchpol* N=107.

Walking transects

We replicated the transects from January to October and verified that the adult phenology of Ad and Bt on Giannutri island is mostly encompassed in the period from February to April, as shown by the GAM estimates (Figure S4). Accordingly, we used the walking transects replicated in these months (from 2021 to 2024) to study the abundance trend of both target species. We first analysed the counting data without considering other variables. We summed the number of observed individuals of each target species in each transect replicate, considering only replicates using only HB+ days (the only conditions in 2021) and compared their median values across years (n=279 transects). We visualised distribution by violin plots also representing median values.

To model the abundance trend of wild bees more precisely, we also considered the effect of environmental variables that could have impacted wild bee abundance. In this way, the effect of the year (e.g. the abundance trend) was studied, after accounting for the effect of other variables. We used *abundance* (number of individuals of each species observed inside a transect subunit) as the response variable and *yearor, day, hour, wind, cloud, day, dist* (all as defined above)*,cloud* (cloud coverage in coverage classes, numeric), *flowerab* (flower coverage as classes of abundance, obtained by summing the classes of each level for each subunit, to a maximum of 3), as predictive variables. We considered the transect *subunit* identity (ID of each subunit, categorical) and the *operator* as random factors. We also checked for spatial autocorrelation among transect subunits by considering their middle point (each subunit is 25 meters long). We used a negative binomial family. Accordingly, we used the following formula for both Ad and Bt analyses:

gam(*abundance*~s(*year*,k=3)+s(*day*,k=3)+s(*flowerab*,k=3)+s(*wind*,k=3)+s(*cloud*,k=3)+s(*hour*,k=3)+s(*dist*,k=3)+s(*subunit*,bs="re")+s(*operator*,bs="re"),correlation=corSpatial(form=~*longi*+*lati*,type="gaussian", family="nb", method="REML" ,select=T)

To test whether the flower coverage of walking transects subunits changed over the years, we conducted another GAMM using the variable *flowerab* as the response variable and *yearor* and *day* as predictive variables and the *subunit* identity and the *operator* name as random factors. We used the "tw" family and we accounted for spatial autocorrelation. We used the following formula:

gam(*flowerab*~s(*day*,k=3)+*yearor*+s(*subunit*,bs="re")+s(*operator*,bs="re"), correlation=corSpatial(form=~longi+lati,type="gaussian"),family="tw",method ="REML",select=T)

Additionally, to test if the presence of Am could produce rapid changes, detectable by the walking transect method, in wild bee distribution and activity, we conducted other GAMMs. In this case, we considered only the experimental years (2022-2024) and both experimental days (HB+ and HB-). To the previous formula, we added the parametric term *hives.condition* (as defined above) and an interaction term between *dist* and *hives.condition*, leading to the following formula:

gam(*abundance*~s(*year*,k=3)+s(*day*,k=3)+s(*flower*,k=3)+s(*wind*,k=3)+s(*clou d*,k=3)+s(*hour*,k=3)+s(*dist*,k=3)+*hives.condition*+s(*dist*,by=*hives.condition*,k =3)+s(*subunit*,bs="re")+s(*operator*,bs="re"),correlation=corSpatial(form=~lo ngi+lati,type="gaussian", family="nb", method="REML" ,select=T)

For both the analyses of demographic trend and the effect of honeybees, our sample was the number of bees counted in each 25m long transect subunit. Therefore, the sample sizes of the two analyses were n=1910 transect units for the demographic trend (for both Ad and Bt) and n=1990 for the effect of Am presence analysis (for both species).

Validation methods and analyses

Using probing time on flowers as a proxy of the amount of nectar collected

We validated the use of suction time on flowers as a proxy of the amount of nectar probed by wild bees. This assumption was tested on Giannutri Island in 2023 using 32 workers of bumblebees (*B. terrestris,* Bt. We attempted to perform the same experiment on the other target species, *A. dispar*, but this was not possible as they failed to feed in captivity whether in a foraging arena or inside Falcon tubes.

Bt were captured on Giannutri Island with an entomological net. Immediately after collection, each bumble bee was placed inside a 20 ml Falcon tube with the tip cut off and fed ad libitum. Then, each Falcon tube was kept in the darkness for 2 hours to standardise the hunger level of bumblebees before the experiment. Bumblebees were then trained to obtain artificial nectar (50% sugar solution in water) from a microcapillary inserted through the cut tip. We chose this concentration value as it was a value frequently observed in flowers on Giannutri. The training was done by presenting 2 µL of artificial nectar two times with a 30-second interval between them. We tested the suction time using four different volumes: 0,1, 2 and 4 µL, which are volumes that can be found on the target flowering species, according to our nectar availability analysis. Each bumblebee was tested for all four volumes and the order in which the volumes were presented changed every time. The first 24 bumblebees received all 24 combinations of the four volumes, while a second group of eight bumblebees replicated eight selected combinations based on their representativeness among the previous 24. A 30-second interval separated each volume presentation. We used the same concentration of 50% for all volumes. We measured the volume probed by bumblebees for each presentation by assessing the remaining volume in the capillary, even though the bumblebees always consumed the entire volume available. Therefore, the volumes presented correspond to the volumes probed. All experiments were video recorded with a video camera positioned above the experimental setup. The bumblebees were released after the experiments.

The video recordings were analysed with BORIS. Three different behaviours were identified and coded into the following ethogram: suction (the subject moves its antennae and abdomen, the body is still, the tongue is extended and moving within the capillary probing nectar), exploration (the subject moves its entire body, the tongue is extended and moves both inside and outside the capillary) divided into exploring inside the capillary and outside the capillary and ignore (the subject either moves or remains still, the tongue is in a resting position). We modelled the suction time (*probingtime* in the analysis) and the *interactingtime* (*probingtime* + time spent exploring inside the capillary) as the response variables using GLMMs. We used the "glmmTMB" function of the glmmTMB[123] package. We modelled as predictive variables the volume of nectar and the order in which it was presented. We used the bumblebee ID as a random factor:

*glmmTMB(*probingtime ~ volume + order + *(1* | Bumblebee_ID*))*

*glmmTMB(*interactingtime ~ volume + order + *(1* | Bumblebee_ID*))*

The sample is represented by each presentation therefore the sample size was N= 128 for both analyses.

The GLMM analyses revealed that the *probingtime* was significantly and positively affected by the volume probed (Data S1AD and Figure S2A). The order in which the volumes were presented did not affect the time needed to probe them (Data S1AD). The *interactingtime* was significantly affected by both the volume probed and the order in which it was presented (Data S1AD). Notably, it was positively correlated with the volume probed and negatively correlated with the order (Data S1AD and Figure S2B,C).

Our hypothesis was verified considering both the suction time alone and the suction time summed with the exploration inside the capillary. As expected, the time exploring the capillary decreased as the familiarity with the presentation

method (the order of presentation) increased. The order did not affect the suction time alone. These results validate the adoption of suction time in field observations as a proxy for nectar probed by wild bees.

Testing whether honeybees show an increase in foraging activity the day after hive closure

The key aspect of our experimental framework was the closure of hives on specific days to investigate the effect induced by *A. mellifera* activity. The hives were closed before sunrise (before 6:00 AM) and re-opened in the afternoon of the same day (around 5:00 PM), leaving a couple of hours of daylight to forage, to minimise the stress and avoid harming the colonies. This also allowed us to lower the possibility of a generalised increase in the activity of honeybees as a compensatory behaviour on the day after the closure. To ascertain this and to validate the experimental approach's efficacy, we tested whether the experimental closure could trigger the honey bees to increase their activity on days after closure. For this reason, during the first year (2022) we repeated cycles of HB+, HB-, HB+, HB+ days to obtain HB+ preceded by an HB-, and HB+ preceded by an HB+. This allowed us to test whether, during the former, honey bees' activity was higher than during the latter. We measured the activity of honey bees with the observation plot used to measure the foraging activity of wild bees on flowering patches. During the observation plots survey, we recorded all *A. mellifera* occurring in the scan samplings, the time they spent inside the observation plot (when possible) and movements inside and outside the plot.

We selected only those experimental days in which hives were open (HB+) and created a variable named type to indicate the type of HB+ day; we assigned the value 1 to a day if it was preceded by an HB- day (hives closed) and value 2 if it was preceded by another HB+ day (hives open), we used this variable as a factor in the analysis.

We analysed the data with GAMMs using the same approach described the plot observation analysis: we modelled the *quantity* (sum of animals observed during

4 scan samplings of each observation plot replicate), *permanence* (square root of time spent inside the observation plot by observed individual) and *activity* (movements inside and outside each observation plot replicate) as response variables and *type* (the type of HB+ day, 1 if preceded by an HB- day and 2 if preceded by an HB+ day, factor variable), *hour* (standard time converted to decimal values to use as a numeric variable), *wind* (wind intensity on Beaufort scale, numeric variable), *cloud* (percentage of cloud coverage recalculated as described in the STAR methods, numeric), *day* (Julian day of the year, numeric), *dist* (linear distance from the apiary, numeric), *flowercov* (surface of the observation plot covered by flowers, numeric) as predictive variables and *operator* (the operator that performed the observation) as a random factor. We accounted for spatial autocorrelation. We accounted for spatial autocorrelation. Accordingly, we used the following formula using the "mgcv" R package.

gam(responsevariable~s(hour,k=3)+s(wind,k=3)+s(cloud,k=3)+s(day,k=3)+type+s(dist,k=3)+s(flowercov)+s(operator,bs="re"),correlation=corSpatial(form=~Longitude+Latitude,type="gaussian"),family = "nb",method="REML",select = T)

The *responsevariable* term shown in the formula is substituted by the variables: *quantity*, *permanence* or *activity* according to the analysis. The only difference is in the *permanence* analysis where we used the tweedie family instead of the negative binomial shown in the formula. For the quantity and activity models, we used each plot observation replica as a sample (N= 414). For the permanence analysis, we used each honeybee for which it was possible to calculate a permanence time and that remained for more than 0.5 seconds inside the plot as a sample (N=433).

The GAMMs revealed that the variable type had no significant effect on any of the three parameters studied (Data S1AE-AG). These results indicate that the foraging activity of honey bees is independent of their condition (freely foraging or closed in the hives) on the preceding day. The foraging activity of honey bees the day after the closure of the hives is statistically the same as the activity on the day after an HB+ day (hives open). This allowed us, for the second and third

year of the field experiment, to modify the hives' closure sequences (see STAR methods).

Exclusion of temperature from the GAM models

Since 2022, we have measured air temperature using a portable weather station (Eurochron Funk Wetterstation ECWC1080) placed approximately at the centre of the island (decimal latitude: 42.253275, decimal longitude: 11.100401). The station recorded temperature every five minutes throughout the experimental period.

For each observation or measurement (including nectar and pollen analyses, focal samplings, and plot observations), we assigned the temperature value recorded at the closest timestamp. We retained temperature data only if it fell within a one-hour window of the observation.

However, due to periods when the weather station failed to record temperature, we were unable to assign temperature values to 12% of our observations. To reconstruct the missing values, we used the mice function from the "mice" R package[124], incorporating publicly available temperature data from the weather station at Giglio Porto (https://www.sir.toscana.it/consistenza-rete) as a reference (temperature and precipitation trends in the last 10 years are in Figure 5B-E and in Figure S3). We selected this station because it is the closest weather station (~18 km) to Giannutri with public available data and with similar climatic condition: is located on an island (Giglio Island) at the sea level (our portable weather station was located at 39 meters a.s.l.). Statistical details are reported in Data S1AB, AC).

During our preliminary study on the island, we verified that temperature was influenced by hour of the day, day of the year and cloud coverage[37]. We tested for this in our analyses assessing the concurvity values, which indicate when a variable can be approximated by one or more other variables. Specifically, we tested whether including temperature in our statistical models introduced high concurvity.

For each analysis, we created a GAM model with the gam function of the "mgcv" R package. We included in these models all the variables selected for the final analysis plus temperature, but excluded random effects and interaction terms, as they inflated concurvity of the involved terms. We calculated the concurvity using the concurvity function of the "mgcv" R package. Then, from the same model we removed the temperature term to calculate the concurvity without it.

In Table S6 we report the concurvity value of temperature in each analysis. Temperature consistently showed high values of concurvity (mean ± standard deviation: 0.858 ±0.078) indicating it was highly correlated with a combination of other variables.

Based on these finding, we opted to exclude temperature from the final models to keep the information about the effect hour of the day, day of the year and cloud coverage.

6. 8 References

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Chapter 7. Final conclusions

Insular biodiversity conservation represents a challenging but crucial task in order to protect irreplaceable fragments of global biodiversity. Insular faunal compositions are usually unique (Cronk, 1997; Dapporto et al., 2017; Hausdorf & Hennig, 2005) and thus extremely valuable in a biodiversity conservation optic. The same factors that have contributed to their uniqueness and irreplaceability make island particularly vulnerable to anthropogenic pressure (Graham et al., 2017; Purvis et al., 2000; Russell & Kueffer, 2019). The combination of extremely valuable biodiversity (i.e. endemic taxa or isolated population or lineages) and high susceptibility makes the protection of insular taxa particularly important (Fernández-Palacios et al., 2021). Yet, there are still several gaps in our understanding of biodiversity patterns or in our knowledge of the actual faunal composition of many islands.

Indeed, before our surveys, Giannutri was one of the less studied islands of the Tuscan Archipelago. In this thesis I improved the knowledge about Giannutri by i) investigating its main pollinator species richness (Chapters 2-3), ii) exploring a sub-endemic taxa of particular interest (Chapter 4), iii) testing and validating a powerful, but still seldom used, method to estimate the size of pollinator populations (Chapter 5) and iv) studying the presence of a high density of honey bees as a potential driver of the pollinator decline on the island with an innovative and manipulative approach (Chapter 6).

The first step on the island

Until 2021, there was no scientific knowledge about the Anthophila species occurring on the island. The first step was to conduct a preliminary analysis to provide a first list of species and to test the new standardised monitoring protocol for bees proposed by the Italian Institute for Environmental Protection and Research (ISPRA) (Bonelli et al., 2020; D'Antoni et al., 2020). This method, accurately described in all its components in Chapter 2, consists of walking transect (250 m long and 4 m wide) in which the minimum required taxonomic

resolution is to separate *A. mellifera* (AM) from *Bombus* (B) species to other wild bee species (AA, other bees in the Italian acronym). Accordingly, we started monitoring the Anthophila fauna of the island with this method to immediately accumulate data on species presence and their relative abundance. The homogeneous environment of the island and the few bee species recorded allowed us to increment the taxonomic resolution of the method by specifying also the species identity of the wild bees. The ISPRA method provided reliable data (confirming known relationships with pollinator abundance on flowers and environmental and meteorological conditions). Therefore, the preliminary study describe in Chapter 2 provided important keystones for this thesis: i) delineated the main features of the island and established six monitoring transects that was used to study pollinator species richness on the island (Chapter 3) and to document the abundance trend of *Bombus terrestris* and *Anthophora dispar* over four years and ii) identified the species that could be used as target species for estimating population size (Chapter 5) and for evaluating the impact of honey bees (Chapter 6). The simplified monitoring scheme acted as a propellent for most of the studies reported in this thesis.

Unveiling insular biodiversity

Understanding the patterns of insular biodiversity has always fascinated scientists worldwide. In particular, the relationship between species richness and island area and isolation has been crucial for biogeographical studies since 1967 with the keystone paper of R. H. MacArthur and E. O. Wilson (MacArthur & Wilson, 1963). It postulated that immigration and extinction rate on islands were mainly driven by the size and the isolation of the island with larger and less isolated islands bearing more species than small and isolated ones due to different pattern of immigration and extinction rates (MacArthur & Wilson, 1963; Warren et al., 2015). Although this theory has been largely supported and redefined in many details by analysing data of several islands (see Warren et al., (2015) and the literature therein), the small islands provided consistent exceptions. Indeed, small islands, below a certain size threshold, show a pattern

of species richness that seems to be not affected by species-area relationships consistently among diverse taxa (Lomolino & Weiser, 2001; Morrison, 2014). Indeed, species richness on small islands appeared to be shaped by ecological features while larger islands' richness appears to be regulated by colonisation-extinction patterns (Chisholm et al., 2016). Clearly, the distribution of different taxa (with different size and dispersal ability) is differently influenced by island area. An island that may considered large for invertebrates could at the same time be small for big mammals (Chisholm et al., 2016).

Giannutri island, as discussed in Chapter 3, perfectly fits in these biodiversity patterns. Indeed, we showed that the bee richness of Tuscan Archipelago follows the species-area relationship and the richness we found on Giannutri island fits with the expected values (Forbicioni et al., 2019). The species richness of Giannutri was coherent with the pattern showed in the Tuscan Archipelago also for butterflies, with a number of species recorded similar to that on Gorgona island which has a similar size (Dapporto et al., 2017). As already mentioned, our checklist of hoverflies is the first for the Tuscan Archipelago and thus Giannutri island can not be compared to the other island. The presence of migratory species of hoverflies, *Episyrphus balteatus*, *Eristalis tenax*, and *Scaeva pyrastri,* as well as of butterflies, *Vanessa cardui* and *Vanessa atalanta*, and the occurrence as singletons of the butterfly species *Aglais io, Gonepteryx rhamni* and *Argynnis paphia* prompts reflection on which species should be actually considered as part of the core faunal composition of an island. Migratory species can arrive every year on the island, following precise migratory routes, and thus really contribute to the ecosystem functioning of the island, with the pollination service they provide or by representing supplemental food for birds (Bauer & Hoye, 2014; López-Hoffman et al., 2017). For these reasons they should be included in the typical faunal composition. The erratic arrival of an individual (as *A. io, G. rhamni* and *Ar. Paphia* observed only with a single individual per species) however, could produce an inflation of the number of species recorded, thus producing misleading faunal compositions. It is worth mentioning that the presence of singletons may also be a result of undersampling in more

inaccessible environments (Coddington et al., 2009) or of rapid species turnover (Fitzgerald & Carlson, 2006). In the case of Giannutri island, we accurately surveyed, for the presence of butterfly species, most of the island surface from February to October for several years and these three species have been collected in a single individual only once, suggesting stochastic events of dispersal. Nonetheless, the presence (and recording) of these species on Giannutri could provide useful information on the dispersal abilities of these species and/or the stochastic events that may lead to new colonisation and should be a target of future studies.

Conservation emergencies: the case study of the sub-endemic Bombus xanthopus

This thesis remarkably increased the knowledge about the pollinators of the island highlighting many key species, especially among hoverflies and bees. The crucial role of Giannutri island in the biodiversity protection of the Tuscan Archipelago is expressed by the presence of a new species of Syrphidae for Italy, *Eumerus narcissi*, for which Giannutri actually represents the only known station in Italy, and by three new species for the Archipelago, *Callicea rufa* (never recorded before in Tuscany)(Paparatti, 1997), *Melanostoma mellinum* and *Scaeva dignota* (Bezzi, 1926; Razzauti, 1917). Among bees, we recorded for the first time in the Tuscan Archipelago the brood parasite *N. sheppardana*. The presence of typical Sardo-Corsican-Tuscan endemisms such as *C. corinna* and *B. xanthopus* remarked the importance of pollinator protection on Giannutri island and confirmed the unique faunal composition of the Tuscan Archipelago, which is influenced by Corsica and the Tuscan mainland faunas (Dapporto et al., 2017; Dapporto & Cini, 2007; Fattorini, 2009). Increasing knowledge on species diversity is considered a pivotal step to protect insular biodiversity as it creates a baseline for future monitoring and more in-depth studies (Fernández-Palacios et al., 2021). In this optic, the results reported in this thesis (in particular Chapters 3-4) should be seen as a starting point rather than an end point, and they aim to promote i) the sampling of still underrepresented taxa (such as

hoverflies) also on other islands of the Archipelago and ii) molecular studies based on both mitochondrial and nuclear markers. This will allow to unveil other facets of the biodiversity of the pollinator of the Tuscan Archipelago fostering its protection (Fernández-Palacios et al., 2021).

From this perspective, our study (Chapter 4) focusing on one of the most characteristic species of the Tuscan Archipelago, the sub-endemic *Bombus xanthopus*, represents a further step towards pollinator protection. This species was previously known to be present in Corsica, Capraia and Elba islands, where it was observed hybridising with the more common *B. terrestris* and thus producing individuals with intermediate colour patterns (Boni et al., 2023; Rasmont et al., 2008; Rasmont & Quaranta, 1997). Our surveys on the Tuscan Archipelago unveiled a wider distribution of this species and of the hybrids across all other islands except for Giglio. Through DNA-barcoding analyses we demonstrated that there is great accordance between the morphology (colour pattern), that can be typical of *B. terrestris*, typical of *B. xanthopus* or exhibiting intermediate colour patterns, and the DNA-barcoding identity with the exceptions of a few specimens. Indeed, *B. xanthopus* individuals of the Tuscan Archipelago showed haplotypes typical of the Corsica and Elba islands specimens (Williams, 2021) with a clear, although due to few mutations, distinction from the typical *B. terrestris* haplotypes exhibited across the western Palearctic region (Williams, 2021). Introgression between the two taxa appeared to be not frequent and the main reasons of this should be investigated by further analyses involving nuclear markers or testing the existence of reproductive barriers that may reduce the fitness of the hybrids. Overall, *B. xanthopus* emerged as an important, and potentially charismatic, taxon on Giannutri. We recorded its presence (of all the castes) only in 2022 (with a hybrid specimen in 2023), which suggests a potential arrival, promoted by unknown reasons, and a consequent failure in the establishment of a resident population or apparent loss of the *B. xanthopus* characters due to hybridisation with *B. terrestris* and demographic swamping (Todesco et al., 2016). The reasons behind this can only be hypothesised -too small founding population, more susceptibility to

environmental or parasite stressors (de Jonghe, 1986)- but continuous monitoring could reveal recurrent arrivals of other individuals over the years. Therefore, the existing populations of *B. xanthopus* in the Tuscan Archipelago deserves special conservation efforts, achievable by regulating the importation of commercial bumble bees and honey bees, which may compete with or transmit pathogens to local bumble bee populations (Alger et al., 2019; Iwasaki & Hogendoorn, 2022; Lázaro et al., 2021; Mallinger et al., 2017), as demonstrated for honey bees in Chapter 6. The failure in establishing a stable population on Giannutri, at least until nowadays, raises concerns on the hypothetical loss of its populations on other islands, that may lead to a complete disappearance of this unique insular taxon in the next decades.

Quantifying abundance of pollinators

The description of pollinators of an island is the first crucial step for effectively monitoring their abundance over the years (Fernández-Palacios et al., 2021). The application of powerful techniques to monitor their populations can significantly enhance the possibility for their protection as it provides more reliable data. Trade-offs between sampling efforts, spatial, temporal and taxonomic resolutions are needed in accordance with the aims of the monitoring (Breeze et al., 2021; O'Connor et al., 2019). Walking transect monitoring is the most common method to estimate population abundance trends over the years for both butterflies and wild bees (Potts et al., 2021; van Swaay et al., 2008) and has been proven effective in detecting species declines (Van Dyck et al., 2009). However, classic transect method (Pollard, 1977) does not provide estimates of population size. To produce precise population assessments other methods must be kept into account. Thanks to the unique condition of Giannutri island, we were able to test the efficacy of the distance sampling method in estimating pollinator populations size (Chapter 5). This method is based on the walking transect method with the difference that also the distance at which the bee has been observed is recorded. Under the assumption that the increase of the distance from the operator decreases the detectability of insects, it is

possible to estimate the density of the individuals observed (within the fixed area of the transects) and then predict the size of the population on the entire site. We effectively applied this method to estimate the population size of *Anthophora dispar* and *Bombus terrestris*. We validated the method using the *Apis mellifera* population size (estimated with an independent method (Dainat et al., 2020)) as a reference against the estimates of the distance sampling. This method resulted particularly powerful in estimating population size as well as in documenting the effect of different environments on species abundance and detectability. Its effectiveness aligned with the few other studies that successfully applied this method on insects (Darshetkar et al., 2023; Hamm, 2013; Isaac et al., 2011; Patterson et al., 2023; Steffen, 2006), which however usually lack the validation with a reference population. A wider use of distance sampling in future studies is desirable, given the positive trade-offs between costs, sampling efforts, and the quality of information it provides. Distance sampling emerged as another arrow in the quiver of conservation biologists, especially on small islands.

The threat of trophic exploitative competition on wild pollinators

Monitoring pollinator abundance, although extremely valuable (Breeze et al., 2021), can miss important information about the potential drivers of an observed decline and therefore targeted experiments are essential to fully address the loss of biodiversity (Weisser et al., 2023). Indeed, we studied for four years, on the island of Giannutri, the potential effects of exploitative competition between managed honey bees and wild bees, *An. dispar* and *B. terrestris* (Chapter 6). We adopted an innovative and manipulative approach (closure of the hives) to experimentally measure the effect of honeybees on trophic resource use by wild bees, trophic resource availability of flowers, and foraging behaviour of wild bees. In addition, we monitored the population abundance of wild bees as a proxy for fitness (Thomson, 2016). In this study, we encompassed all the evidence required to demonstrate the presence of negative impacts of honey bees on wild bees (Paini, 2004). This result is remarkable as very few studies

experimentally demonstrated an impact on wild bee fitness due to competition (Elbgami et al., 2014; Goulson & Sparrow, 2009; Hudewenz & Klein, 2015; Meeus et al., 2021; D. Thomson, 2004) and it thus provides valuable insights into the competitive dynamics in Mediterranean environments. Indeed, we revealed the negative impact of high hive density of honey bees on trophic resource availability and on several behavioural parameters of wild bees. The population abundance of both target species declined of about 80% in four years indicating that a generalised threat (such as exploitative competition) has been acting on them. In this scenario, the presence of 18 hives stood out as the main candidate for the worrying decline of wild bees. Climate change also may play a synergistically role in threatening wild bee populations of the island. In fact, droughts periods can exacerbate the negative effects of competition worsening the trophic resource scarcity and further impacting local wild bee populations (Thomson, 2016). Climate change is a global threat and clearly cannot be removed from a single location. On the other hand, the presence of honey bees is a removable threat, since the hives are moved to the island each year, and a few years of complete absence of this pressure should allow to i) ascertain if the observed decline was primarily driven by exploitative competition and ii) further describe the behaviour and trophic resource availability of the island when only the native and local pollinator are present.

In conclusion, in this thesis I delineated a comprehensive and logical path to effectively promote pollinator protection in small islands (Fernández-Palacios et al., 2021; Weisser et al., 2023). This approach is extremely scalable in its main key steps ranging from incrementing the knowledge of specific taxa to experimentally addressing the potential drivers of the decline. This thesis unveiled the importance of Giannutri island for the biodiversity of the Tuscan Archipelago and will hopefully inspire others researchers to investigate neglected islands and/or taxa. This thesis also documented the detrimental effects of exploitative competition in confined and homogeneous landscapes, alerting about the need to accurately evaluate the addition of a disproportionately high amount of hives in protected areas.

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Appendix

Here are reported the Supplementary material of the paper in Chapter 6. As Data S1, Table S2 and Table S5 (Chapter 6) are Excel Tables too big to coherently fit into a PDF, I provide here a Google Drive link to those supplementary files: https://drive.google.com/drive/folders/1kXxaCC2TIVc0rFVtfvopD3IWA79wE1Ts?usp=sharing

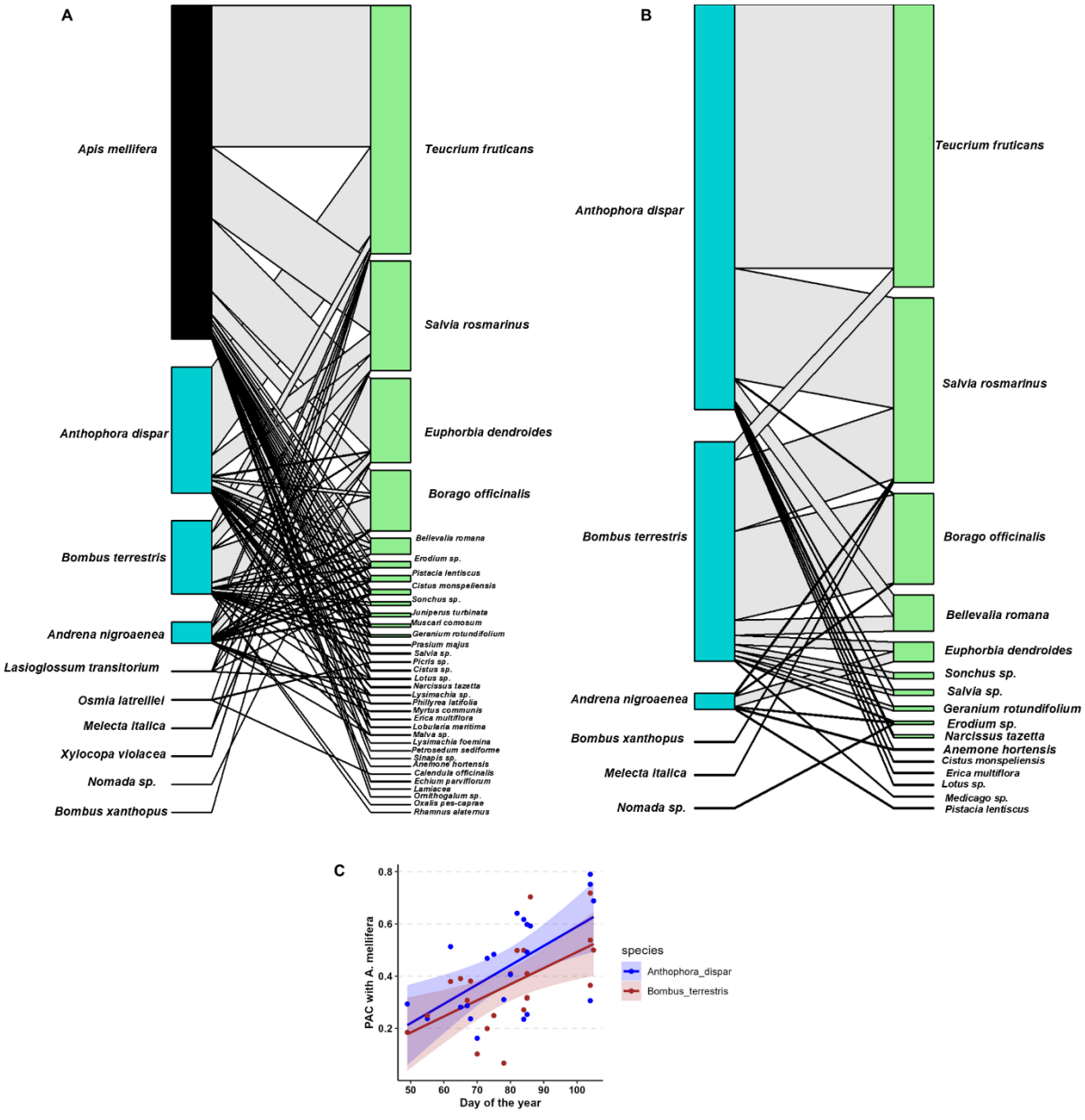

Figure S1. Supplementary result of network analysis, related to Results (Figure 1).

(A) Flower-visitor network of HB+ days (22 days) showing al the pollinators and plants species, link size between pollinators (upper level) and plants (lower level) reflects interaction strength. Box dimensions indicate the total number of visits (made/received). (B) Flower-visitor network of HB- days (7 days) showing al the pollinators and plants species. (C) Daily values of the PAC index for both target species against *A. mellifera*. In blue *An. dispar* data and in brown *B. terrestris* data. The linear regression showing the relationship between PAC and day of the year is represented by solid lines. The shade represents the 95% confidence interval. Both relationships are statistically significant (p-value=0.002 for *An. Dispar* and p-value=0.005 for *B. terrestris*).

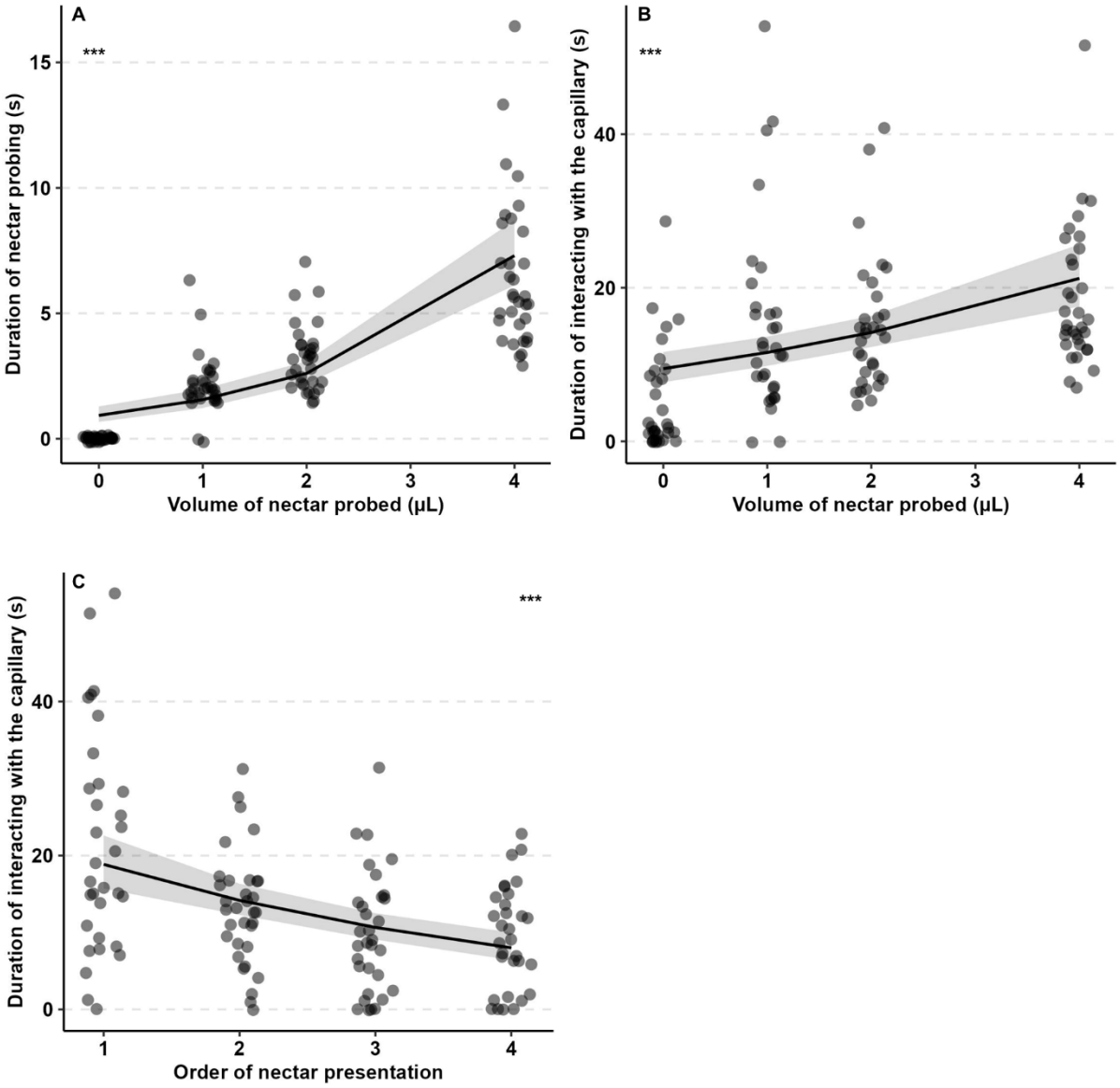

Figure S2. Results of the validation experiment, related to STAR Methods.

(A) Effect of the volume probed on the probing time of bumble bees. (B) Effect of the volume probed on the *interacting* time with the capillary of bumble bees. (C) Effect of the order of presentation on the *interacting* time with the capillary of bumble bees. For (A)-(C) Black dots are the raw data, the black curve indicates the estimated relationship between the variables and the shaded area indicates the 95% confidence interval. Asterisks indicate a statistically significant effect: *** p-value<0.001.

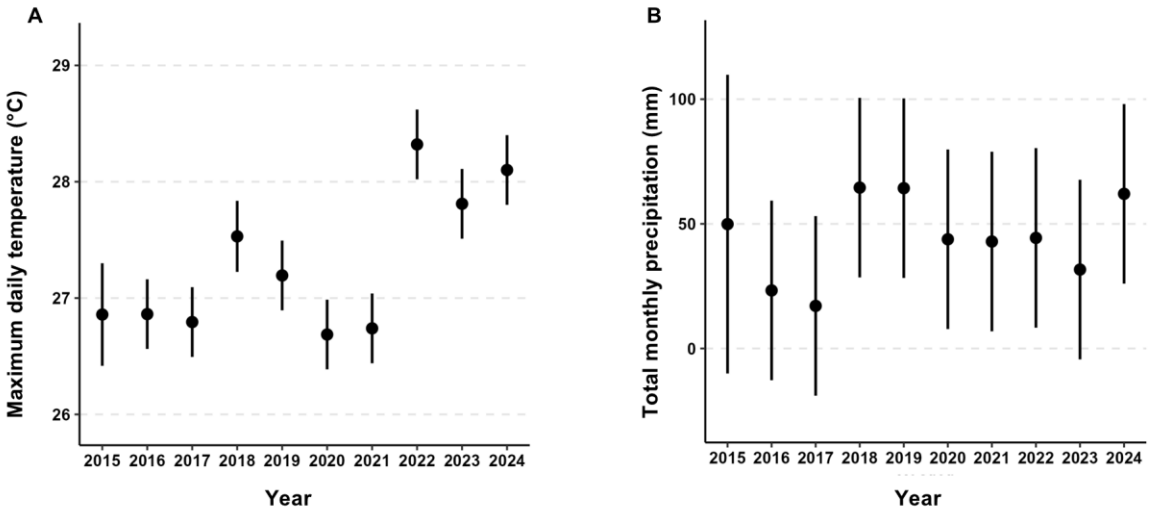

Figure S3. The estimated maximum daily temperature and monthly precipitations on Giglio Porto, related to STAR Methods.

(A) Maximum daily temperature on Giglio Porto during the period May- December from 2015 to 2024, an increase in temperature. (B) Total monthly precipitation on Giglio Porto during the period May- December from 2015 to 2024. These months correspond to the absence of adults (most of them) of both Ad and Bt as visible in Figure S4.

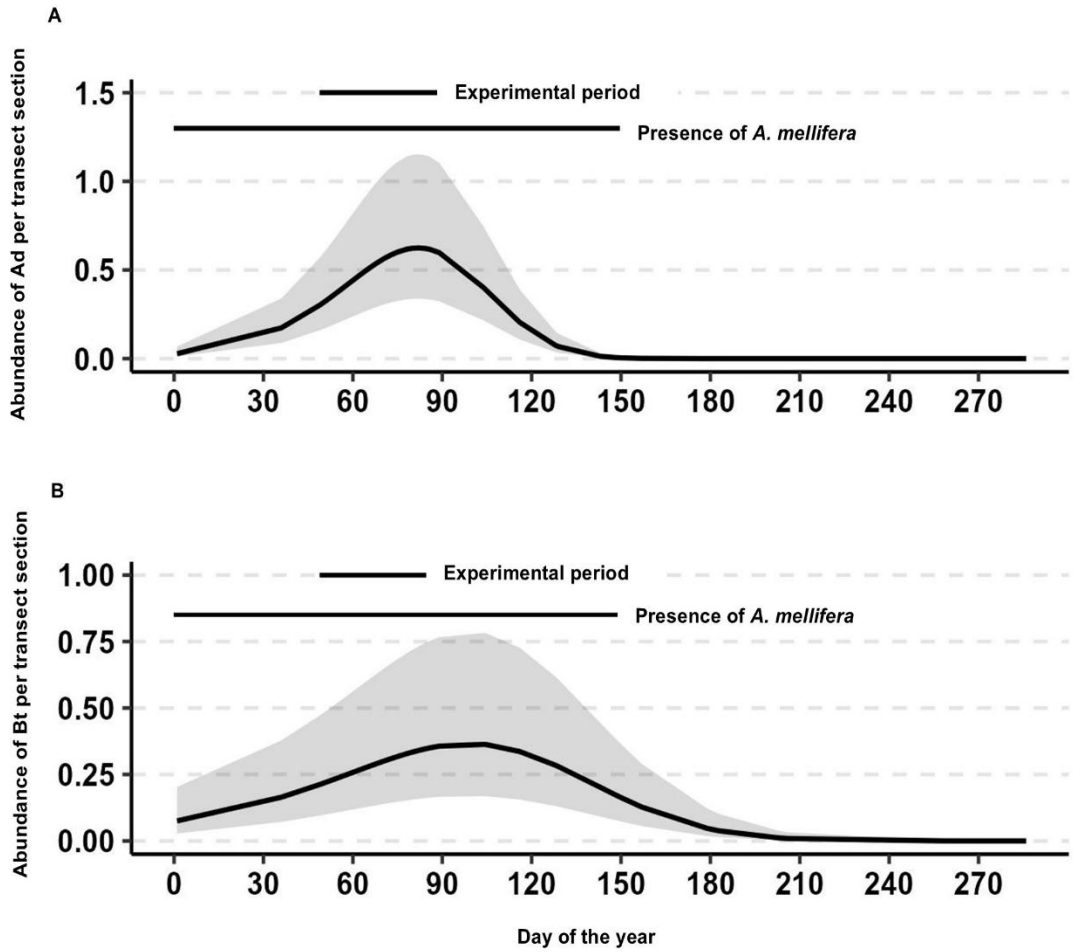

Figure S4. The mean number of bees counted each survey day in each subunit of walking transects, related to STAR Methods.

(A) Number of *A. dispar* (estimated by GAMM) during the over the seasons per transect section. (B) Number of *B. terrestris* (estimated by GAMM) during the over the seasons per transect section. For (A) and (B) the black bar solid line represents the estimated marginal mean for each day of the year and the shadow area represent the 95% confidence interval. The black lines above the curves indicate the periods of experiments (resource availability, plot and focal observations) and the presence of *A. mellifera* during the year.

Species	Flower treatment	Hives condition	Nectar volume (µL) (mean ±SE)
Teucrium fruticans	bagged	closed (HB-)	1.236 ± 0.156
Teucrium fruticans	bagged	open (HB+)	1.122 ± 0.159
Teucrium fruticans	unbagged	closed (HB-)	0.796 ± 0.157
Teucrium fruticans	unbagged	open (HB+)	0.522 ± 0.164
Salvia rosmarinus	bagged	closed (HB-)	0.049 ± 0.398
Salvia rosmarinus	bagged	open (HB+)	0.041 ± 0.406
Salvia rosmarinus	unbagged	closed (HB-)	0.036 ± 0.399
Salvia rosmarinus	unbagged	open (HB+)	0.021 ± 0.408

Table S1. Estimated mean nectar volumes, related to results (Figure 2).

Table showing the estimated (from GAMM) mean nectar volume values and their Standard Error of the two investigated species for bagged and unbagged flowers in HB+ and HB-.

Date (dd-mm-yyyy)	Year	Month	Hive condition	Nectar availability	Pollen availability	Plot sampling	Focal sampling	Walking transects
25-03-2021	2021	March	open (HB+)					X
26-03-2021	2021	March	open (HB+)					X
14-04-2021	2021	April	open (HB+)					X
23-02-2022	2022	February	closed (HB-)	X		X	X	X
24-02-2022	2022	February	open (HB+)	X		X	X	X
06-03-2022	2022	March	open (HB+)			X	X	X
07-03-2022	2022	March	closed (HB-)			X	X	X
08-03-2022	2022	March	open (HB+)			X	X	X
09-03-2022	2022	March	open (HB+)			X	X	X
10-03-2022	2022	March	closed (HB-)			X	X	X
11-03-2022	2022	March	open (HB+)			X	X	X
24-03-2022	2022	March	closed (HB-)	X		X	X	X
25-03-2022	2022	March	open (HB+)	X		X	X	X

26-03-2022	2022	March	open (HB+)	X		X	X	X
28-03-2022	2022	March	closed (HB-)	X		X	X	
29-03-2022	2022	March	open (HB+)	X		X	X	
14-04-2022	2022	April	open (HB+)					X
03-03-2023	2023	March	open (HB+)			X	X	X
06-03-2023	2023	March	open (HB+)			X	X	X
09-03-2023	2023	March	open (HB+)	X		X	X	x
11-03-2023	2023	March	closed (HB-)	X		X	X	X
12-03-2023	2023	March	open (HB+)	X		X	X	
16-03-2023	2023	March	open (HB+)	X		X	X	
17-03-2023	2023	March	closed (HB-)	X		X	X	
18-03-2023	2023	March	open (HB+)			X	X	
20-03-2023	2023	March	open (HB+)			X	X	X
23-03-2023	2023	March	open (HB+)	X		X	X	
24-03-2023	2023	March	closed (HB-)	X		X	X	
25-03-2023	2023	March	open (HB+)	X		X	X	
26-03-2023	2023	March	closed (HB-)	X		X	X	
27-03-2023	2023	March	open (HB+)			X	X	X
30-03-2023	2023	March	open (HB+)	X		X	X	X
31-03-2023	2023	March	closed (HB-)	X		X	X	X
01-04-2023	2023	April	open (HB+)			X	X	
04-04-2023	2023	April	open (HB+)			X	X	
26-04-2023	2023	April	open (HB+)					X
18-02-2024	2024	February	open (HB+)					X
19-02-2024	2024	February	open (HB+)			X	X	
20-02-2024	2024	February	open (HB+)			X	X	X
21-02-2024	2024	February	open (HB+)			X	X	X
22-02-2024	2024	February	closed (HB-)			X	X	X
25-02-2024	2024	February	open (HB+)			X	X	X
28-02-2024	2024	February	open (HB+)			X	X	X
04-03-2024	2024	March	open (HB+)			X	X	
05-03-2024	2024	March	closed (HB-)	X	X	X	X	
06-03-2024	2024	March	open (HB+)	X	X	X	X	X
08-03-2024	2024	March	open (HB+)			X	X	
10-03-2024	2024	March	open (HB+)					X
11-03-2024	2024	March	open (HB+)					X
13-03-2024	2024	March	open (HB+)		X	X	X	X
14-03-2024	2024	March	closed (HB-)	X	X	X	X	X
15-03-2024	2024	March	open (HB+)	X		X	X	X
18-03-2024	2024	March	open (HB+)			X	X	X
19-03-2024	2024	March	closed (HB-)	X	X	X	X	X
20-03-2024	2024	March	open (HB+)	X	X	X	X	X
21-03-2024	2024	March	closed (HB-)	X	X	X	X	X
22-03-2024	2024	March	open (HB+)		X	X	X	X
24-03-2024	2024	March	closed (HB-)		X	X	X	X
25-03-204	2024	March	open (HB+)			X	X	X
13-04-2024	2024	April	open (HB+)					X
14-04-2024	2024	April	open (HB+)					X

Table S3. List of all the experimental days (from February to April, including the year 2021 for walking transects data) used in the data analysis of this paper, related to STAR Methods. For each day, the survey techniques carried out are reported (with an x in the appropriate cell).

Sampling point ID	Survey	Latitude	Longitude	Distance from apiary (km)	Status	Changes
A_1_1	Focal and plot	42.2405	11.1069	1.079	regular	no
A_1_2	Focal and plot	42.2405	11.1070	1.084	regular	no
A_10_1	Focal and plot	42.2403	11.1076	1.134	regular	no
A_10_2	Focal and plot	42.2403	11.1074	1.121	regular	no
A_2_1	Focal and plot	42.2408	11.1072	1.066	regular	no
A_2_2	Focal and plot	42.2406	11.1071	1.082	regular	no
A_3_1	Focal and plot	42.2421	11.1082	0.993	regular	no
A_3_2	Focal and plot	42.2418	11.1082	1.021	regular	no
A_4_1	Focal and plot	42.2412	11.1080	1.064	regular	no
A_4_2	Focal and plot	42.2418	11.1082	1.021	regular	no
A_5_1	Focal and plot	42.2397	11.1080	1.2	regular	no
A_5_2	Focal and plot	42.2407	11.1078	1.102	regular	substituted in 2024
A_5_3	Focal and plot	42.2417	11.1082	1.033	regular	substitution from 2024
A_6_1	Focal and plot	42.2434	11.1094	0.964	regular	no
A_6_2	Focal and plot	42.2434	11.1093	0.955	regular	no
A_7_1	Focal and plot	42.2411	11.1064	1.002	regular	no
A_7_2	Focal and plot	42.2410	11.1063	1.006	regular	no
A_8_1	Focal and plot	42.2417	11.1056	0.914	regular	no
A_8_2	Focal and plot	42.2417	11.1055	0.908	regular	no
A_9_1	Focal and plot	42.2424	11.1052	0.827	regular	no
A_9_2	Focal and plot	42.2425	11.1056	0.832	regular	no
A_9_opportunistic	Focal	42.2425	11.1060	0.853	opportunistic	opportunistic
A_9_opportunistic1	Focal	42.2425	11.1060	0.853	opportunistic	opportunistic
B_1_1	Focal and plot	42.2454	11.1001	0.408	regular	no
B_1_2	Focal and plot	42.2456	11.1002	0.383	regular	no
B_10_1	Focal and plot	42.2433	11.0997	0.633	regular	no
B_10_2	Focal and plot	42.2433	11.0997	0.642	regular	no
B_2_1	Focal and plot	42.2460	11.1002	0.339	regular	no
B_2_2	Focal and plot	42.2461	11.1002	0.328	regular	no
B_3_1	Focal and plot	42.2466	11.1018	0.289	regular	no
B_3_2	Focal and plot	42.2465	11.1016	0.288	regular	no
B_4_1	Focal and plot	42.2465	11.1014	0.284	regular	no
B_4_2	Focal and plot	42.2466	11.1012	0.278	regular	no
B_5_1bis	Focal and plot	42.2445	11.0994	0.515	regular	no
B_5_2bis	Focal and plot	42.2445	11.0995	0.512	regular	no
B_6_1	Focal and plot	42.2464	11.1006	0.289	regular	removed after 2022
B_6_2	Focal and plot	42.2463	11.1006	0.302	regular	removed after 2022
B_7_1	Focal and plot	42.2439	11.1036	0.622	regular	no
B_7_2	Focal and plot	42.2438	11.1037	0.633	regular	no
B_7_opportunistic	Focal	42.2439	11.1030	0.603	opportunistic	opportunistic
B_8_1	Focal and plot	42.2428	11.1044	0.76	regular	no
B_8_2	Focal and plot	42.2427	11.1044	0.771	regular	no
B_9_1	Focal and plot	42.2468	11.1005	0.249	regular	no
B_9_2	Focal and plot	42.2467	11.1005	0.256	regular	no
B_9_opportunistic	Focal	42.2466	11.1008	0.268	opportunistic	opportunistic
C_1_1	Focal and plot	42.2471	11.0996	0.226	regular	no
C_1_2	Focal and plot	42.2471	11.0999	0.22	regular	no
C_10_1	Focal and plot	42.2520	11.0979	0.397	regular	removed after 2022
C_10_2	Focal and plot	42.2522	11.0978	0.417	regular	removed after 2022
C_2_1	Focal and plot	42.2473	11.1001	0.189	regular	no
C_2_2	Focal and plot	42.2473	11.1000	0.189	regular	no
C_3_1	Focal and plot	42.2492	11.0983	0.185	regular	no
C_3_2	Focal and plot	42.2492	11.0983	0.186	regular	no
C_4_1	Focal and plot	42.2487	11.0983	0.184	regular	no
C_4_2	Focal and plot	42.2487	11.0983	0.184	regular	no
C_4_opportunistic	Focal	42.2489	11.0984	0.177	opportunistic	opportunistic
C_5_1	Focal and plot	42.2484	11.0985	0.179	regular	removed after 2022
C_5_2	Focal and plot	42.2484	11.0984	0.188	regular	removed after 2022
C_6_1	Focal and plot	42.2492	11.1003	0.024	regular	no
C_6_2	Focal and plot	42.2493	11.0999	0.055	regular	no
C_7_1	Focal and plot	42.2482	11.0997	0.109	regular	no
C_7_2	Focal and plot	42.2483	11.0999	0.093	regular	no
C_7_opportunistic	Focal	42.2478	11.0993	0.162	opportunistic	opportunistic
C_8_1	Focal and plot	42.2491	11.0991	0.115	regular	removed after 2022
C_8_2	Focal and plot	42.2492	11.0987	0.151	regular	removed after 2022
C_9_1	Focal and plot	42.2516	11.0975	0.376	regular	no
C_9_2	Focal and plot	42.2514	11.0973	0.379	regular	no
D_1_1	Focal and plot	42.2540	11.0969	0.636	regular	removed after 2022
D_1_2	Focal and plot	42.2545	11.0966	0.689	regular	removed after 2022

D_10_1	Focal and plot	42.2526	11.1034	0.462	regular	no
D_10_2	Focal and plot	42.2525	11.1044	0.507	regular	no
D_2_1	Focal and plot	42.2563	11.0985	0.832	regular	no
D_2_2	Focal and plot	42.2566	11.0988	0.851	regular	no
D_3_1	Focal and plot	42.2551	11.0995	0.68	regular	removed after 2022
D_3_2	Focal and plot	42.2549	11.0994	0.659	regular	removed after 2022
D_4_1	Focal and plot	42.2532	11.1013	0.475	regular	no
D_4_2	Focal and plot	42.2532	11.1011	0.47	regular	no
D_4_opportunistic	Focal	42.2531	11.1010	0.457	opportunistic	opportunistic
D_5_1	Focal and plot	42.2573	11.1045	0.98	regular	removed after 2022
D_5_2	Focal and plot	42.2569	11.1045	0.938	regular	removed after 2022
D_6_1	Focal and plot	42.2562	11.1039	0.854	regular	no
D_6_2	Focal and plot	42.2561	11.1039	0.838	regular	no
D_7_1	Focal and plot	42.2523	11.0953	0.56	regular	removed after 2022
D_7_2	Focal and plot	42.2522	11.0953	0.553	regular	removed after 2022
D_8_1	Focal and plot	42.2528	11.0953	0.599	regular	no
D_8_2	Focal and plot	42.2532	11.0956	0.612	regular	no
D_9_1bis	Focal and plot	42.2518	11.1040	0.42	regular	substituted in 2023
D_9_2bis	Focal and plot	42.2519	11.1046	0.467	regular	no
D_9_3bis	Focal and plot	42.2520	11.1039	0.431	regular	substitution from 2023
E_1_1	Focal and plot	42.2554	11.1072	0.899	regular	no
E_1_2	Focal and plot	42.2556	11.1074	0.923	regular	no
E_10_1	Focal and plot	42.2592	11.1083	1.303	regular	no
E_10_2	Focal and plot	42.2594	11.1088	1.344	regular	no
E_10_opportunistic	Focal	42.2585	11.1078	1.212	opportunistic	opportunistic
E_2_1	Focal and plot	42.2571	11.1073	1.058	regular	no
E_2_2	Focal and plot	42.2571	11.1075	1.067	regular	no
E_3_1bis	Focal and plot	42.2593	11.1105	1.41	regular	no
E_3_2bis	Focal and plot	42.2593	11.1110	1.435	regular	no
E_4_1	Focal and plot	42.2574	11.1055	1.024	regular	no
E_4_2	Focal and plot	42.2577	11.1059	1.065	regular	no
E_5_1	Focal and plot	42.2592	11.1100	1.379	regular	no
E_5_2	Focal and plot	42.2593	11.1098	1.374	regular	no
E_5_opportunistic	Focal	42.2593	11.1096	1.368	opportunistic	opportunistic
E_6_1	Focal and plot	42.2589	11.1122	1.457	regular	no
E_6_2	Focal and plot	42.2587	11.1132	1.504	regular	no
E_6_opportunistic	Focal	42.2589	11.1127	1.491	opportunistic	opportunistic
E_7_1	Focal and plot	42.2559	11.1081	0.989	regular	no
E_7_2	Focal and plot	42.2557	11.1078	0.958	regular	no
E_8_1	Focal and plot	42.2568	11.1063	0.991	regular	no
E_8_2	Focal and plot	42.2569	11.1067	1.013	regular	no
E_9_1	Focal and plot	42.2581	11.1070	1.146	regular	no
E_9_2	Focal and plot	42.2580	11.1068	1.126	regular	no
E_9_opportunistic	Focal	42.2583	11.1075	1.183	opportunistic	opportunistic
F_3_1	Focal and plot	42.2566	11.1140	1.393	regular	no
F_3_2	Focal and plot	42.2563	11.1142	1.39	regular	no
F_4_1	Focal and plot	42.2567	11.1139	1.397	regular	no
F_4_2	Focal and plot	42.2568	11.1138	1.399	regular	no
F_4_opportunistic	Focal	42.2570	11.1138	1.407	opportunistic	opportunistic
F_6_1	Focal and plot	42.2543	11.1161	1.412	regular	removed after 2022
F_6_1bis	Focal and plot	42.2587	11.1133	1.507	regular	no
F_6_2	Focal and plot	42.2546	11.1162	1.433	regular	removed after 2022
F_6_2bis	Focal and plot	42.2586	11.1134	1.506	regular	no
F_6_opportunistic	Focal	42.2538	11.1159	1.374	opportunistic	opportunistic
F_7_1	Focal and plot	42.2556	11.1157	1.446	regular	no
F_7_1bis	Focal and plot	42.2573	11.1137	1.426	regular	no
F_7_2	Focal and plot	42.2555	11.1158	1.455	regular	no
F_7_2bis	Focal and plot	42.2572	11.1138	1.425	regular	no
F_7_opportunistic	Focal	42.2558	11.1157	1.464	opportunistic	opportunistic
F_9_1	Focal and plot	42.2573	11.1138	1.432	regular	no
F_9_2	Focal and plot	42.2578	11.1138	1.472	regular	no
F_9_opportunistic	Focal	42.2581	11.1138	1.489	opportunistic	opportunistic
N_1	Nectar availability	42.2562	11.1044	0.861	regular	no
N_2	Nectar availability	42.24784	11.09936	0.16	regular	no
N_3	Nectar availability	42.2478	11.0994	0.741	regular	no
N_4	Nectar availability	42.24154	11.10567	0.932	regular	added in 2024
N_5	Nectar availability	42.2431	11.1046	1.288	regular	added in 2024
1	Pollen availability	42.24185833	11.10827167	1.02	regular	no
2	Pollen availability	42.2415	11.1057	1.079	regular	no
3	Pollen availability	42.24092	11.10594333	1.004	regular	no
4	Pollen availability	42.2591	11.1081	0.821	regular	no

5	Pollen availability	42.24408667	11.10333	0.594	regular	no
6	Pollen availability	42.2419	11.1083	0.492	regular	no
7	Pollen availability	42.2404	11.1066	0.295	regular	no
8	Pollen availability	42.2409	11.1059	0.179	regular	no
9	Pollen availability	42.2423	11.1048	0.286	regular	no
10	Pollen availability	42.2441	11.1033	0.488	regular	no
11	Pollen availability	42.2447	11.1018	0.746	regular	no
12	Pollen availability	42.2465	11.1016	0.93	regular	no
13	Pollen availability	42.2484	11.0985	1.083	regular	no
14	Pollen availability	42.2514	11.0993	1.07	regular	no
15	Pollen availability	42.2533	11.1014	1.219	regular	no
16	Pollen availability	42.2555	11.1028	1.427	regular	no
17	Pollen availability	42.2566	11.1053	1.411	regular	no
18	Pollen availability	42.2572	11.1077	1.486	regular	no
19	Pollen availability	42.2577	11.1059	1.49	regular	no
20	Pollen availability	42.2585	11.1079	1.395	regular	no

Table S4. List of all the sampling points used in the nectar availability, pollen availability, plot and focal observations, related to STAR Methods. Their decimal coordinates and distance from the apiary are reported. The type of survey they were involved in is specified in the “Survey” column. It is specified whether they were regular points (regularly sampled) or opportunistic (opportunistically sampled). Potential changes in their replication (such as points removed or substituted) are reported in the “Changes” column.

Analysis	**Concurvity of temperature**
Nectar volume of *T. fruticans*	0.923
Nectar volume of *S. rosmarinus*	0.922
Sucrose concentration of *T. fruticans*	0.960
Sucrose concentration of *S. rosmarinus*	0.961
Pollen availability	0.920
Scan sampling	0.796
Permanence time - *An. dispar*	0.858
Permanence time - *B. terrestris*	0.936
Movements across the plot	0.796
Probing time - *An. dispar*	0.768
Probing time - *B. terrestris*	0.792
Lag time for nectar - *An. dispar*	0.781
Lag time for nectar - *B. terrestris*	0.748
Lag time for pollen - *An. dispar*	0.792
Lag time for pollen - *B. terrestris*	0.926

Table S6. Concurvity of temperature and of the related variables in models with and without temperature, related to STAR Methods. The mean concurvity values in model with temperature is consistently higher than in the same model without temperature. Temperature has very high values of concurvity.

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