

Impacts of beekeeping on wild bee diversity and pollination networks in the Aegean Archipelago

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First published: 24 June 2021 <https://doi.org/10.1111/ecog.05553>Citations: 13
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Abstract

Maintaining the diversity of wild bees is a priority for preserving ecosystem function and promoting stability and productivity of agroecosystems. However, wild bee communities face many threats and beekeeping could be one of them, because honey bees may have a strong potential to outcompete wild pollinators when placed at high densities. Yet, we still know little about how beekeeping intensity affects wild bee diversity and their pollinator interactions. Here, we explore how honey bee density relates to wild bee diversity and the structure of their pollination networks in 41 sites on 13 Cycladic Islands (Greece) with similar landscapes but differing in beekeeping intensity. Our large-scale study shows that increasing honey bee visitation rate had a negative effect on wild bee species richness and abundance, although the latter effect was relatively weak compared to the effect of other landscape variables. Competition for flowering resources (as indicated by a resource sharing index) increased with the abundance of honey bees, but the effect was more moderate for wild bees in family Apidae than for bees in other families, suggesting a stronger niche segregation in Apidae in response to honey bees. Honey bees also influenced the structure of wild bee pollination networks indirectly, through changes in wild bee richness. Low richness of wild bees in sites with high honey bee abundance resulted in wild bee networks with fewer links and lower linkage density. Our results warn against beekeeping intensification in these islands and similar hotspots of bee diversity, and shed light on how benefits to pollination services of introducing honey bees may be counterbalanced by detriments to wild bees and their ecosystem services.

Introduction

Honey bees, *Apis mellifera*, are used for honey production and also as crop pollinators of choice worldwide (Klein et al. 2007, Aizen and Harder 2009), because they are generalists, able to pollinate many different crops, and live in large colonies that are easily transported (Goulson 2003, Potts et al. 2010a). Although honey bees are native to Europe, Africa and western Asia (Michener 2007, Cridland et al. 2017, Requier et al. 2019), they have been managed for millennia in Europe (Crane 1999), where wild feral populations are now rare (Moritz et al. 2005, Requier et al. 2019). In recent decades, the number of managed honey bee colonies has increased exponentially in some Mediterranean European countries, such as Greece, Portugal and Spain, despite their decrease elsewhere (Potts et al. 2010a, Herrera 2020). Beekeeping intensification in the Mediterranean Basin has led to a gradual replacement of wild bees by honey bees at wild and cultivated flowers in this region (Herrera 2020). This replacement is alarming because honey bees can only complement, and never fully substitute, the pollination services provided by wild insects (Ollerton et al. 2012, Garibaldi et al. 2013, Page et al. 2021). From a conservation perspective it is essential to understand whether the pollination benefit of introducing honey bees is counteracted by negative effects on wild pollinators.

Beekeeping activities can produce unnaturally high local densities of honey bees, and therefore, there is a strong potential for competition between them and wild pollinators (Goulson 2003, 2004, Geslin et al. 2017). Managed honey bees reduce pollen and nectar availability (Dupont et al. 2004, Torné-Noguera

et al. 2016, Cane and Tepedino 2017), competitively displace wild pollinators from floral resources (Dupont et al. 2004, Shavit et al. 2009, Artz et al. 2011, Lindström et al. 2016, Ropars et al. 2019) and influence their foraging behaviour (Thomson 2004, Walther-Hellwig et al. 2006, Artz et al. 2011). Ultimately, the presence of honey bees can reduce the size, biomass and/or reproduction of wild bees (Thomson 2004, Goulson and Sparrow 2009, Elbgami et al. 2014, Torné-Noguera et al. 2016). Competition may be particularly strong when resources are limited (Martins 2004, Thomson 2006), under unfavourable climatic conditions (Thomson 2016) or in homogeneous landscapes (Herbertsson et al. 2016). All of this suggests that massive introductions of honey bees could potentially lead to depauperate wild pollinator communities, with important consequences for natural ecosystems and agricultural productivity (Winfree et al. 2007, Aizen and Harder 2009, Potts et al. 2010b, Breeze et al. 2011, Garibaldi et al. 2013). However, the effect of honey bee density on wild pollinator diversity has been little explored (Mallinger et al. 2017) and the results are so far inconclusive, with some studies reporting negative effects on pollinator richness (Valido et al. 2019, Ropars et al. 2020), while others failing to find any relationship with wild pollinator richness or species composition (Steffan-Dewenter and Tscharrntke 2000, Russo et al. 2015, Torné-Noguera et al. 2016, Reverté et al. 2019). To our knowledge, no previous study has evaluated the relationship between the density of honey bees and wild bee richness at large scales.

High numbers of introduced honey bees can also trigger effects on the structure and functioning of local foraging communities (Geslin et al. 2017), because honey bees generally visit a large proportion of flowering plants in a community (Petanidou 1991, Santos et al. 2012, Magrach et al. 2017) and occupy central positions in plant–pollinator networks (Petanidou et al. 2008, Giannini et al. 2015). Therefore, resource sharing between honey bees and those wild pollinators that resist local extirpation may increase with increasing honey bee abundance (Magrach et al. 2017). However, the degree of resource sharing will vary with intrinsic diet overlap (Wojcik et al. 2018) and with the ability of wild pollinators to shift their diet under strong competition (Magrach et al. 2017). The effects of honey bee competition on the structure of pollination networks can be direct, if competition leads to shifts in wild pollinator diets (Magrach et al. 2017); or, alternatively, the effect may be indirect, if high honey bee densities reduce pollinator diversity, which in turn influences network metrics (both effects may occur together; Valido et al. 2019). Understanding how these direct and indirect effects vary along beekeeping gradients may help us evaluate the potential of honey bees to modify the structure of plant–pollinator communities over large spatial scales.

Here, we explore whether intensive beekeeping in Greece impacts wild bees, which are honey bees' nearest relatives and the main pollinator group in the area (Petanidou and Ellis 1993, 1996, Potts et al. 2006, Nielsen et al. 2011). To do this, we examine Greek islands of similar climate and vegetation but varying intensity of beekeeping. In particular, we asked: 1) whether high honey bee visitation was negatively related to wild bee richness and abundance in ways that suggest interspecific competition; 2) whether high honey bee visitation increased competition for resources with wild bees and, if so, whether the strength of this effect differed between bee families; and 3) whether high honey bee visitation influenced the structure of wild bee pollination networks, either directly or indirectly, through changes in pollinator diversity.

Methods

Study sites

We conducted this study on 13 Cycladic islands, central Aegean Sea, Greece. Greece has one of the highest densities of honey bee hives in Europe (De la Rua et al. 2009, Potts et al. 2010a), but

beekeeping intensity differs substantially among sites, including the Cycladic Islands (Papas 2008). We selected specific islands to represent a wide range of variation in hive density, as recorded by the Greek Ministry of Rural Development and Food in the period 2005–2015 (Supporting information). We then selected study sites that covered the extent of each island, which were located in suitable habitats for wild bees (below) and separated by more than 1.5 km measured as straight-line distance. The number of sites was proportional to island area (1 site for each 50 km²; Supporting information). In total, we selected 41 study sites (Fig. 1), located in important habitats for wild bees, i.e. mainly low scrub (phrygana), in a few cases open olive groves and open oak forests that have also proven to be very rich in wild bees (cf. Potts et al. 2006; see specific habitat for each study site in Supporting information). Sites were separated on average (\pm SD) by 7.68 ± 2.24 km within an island, with a minimal distance of 1.7 km between closest sites (distances between each pair of study sites in Supporting information). Each of the selected sites covered an area ≥ 1 ha.

Bee richness and abundance

In each site we conducted three rounds of surveys during the main flowering season: round 1 from 20 March to 10 April; round 2 from 20 April to 10 May; and round 3 from 20 May to 10 June. Sampling rounds at a site were always separated by at least three weeks. Due to the many study sites, difficulties in reaching islands early in the season, and limits on experienced personnel, we sampled across three consecutive years, 2013 through 2015 (Supporting information).

Bees were collected both with pantraps and hand-netting (Nielsen et al. 2011). We arranged pantraps in triplets, each comprising one white, one blue and one yellow pantrap, all painted with UV-bright colours (Westphal et al. 2008). These colours account for different colour preferences among pollinating insects. In each site, we set up ten triplets as a standard, increasing this to as many as 17 in sites where we considered that pantrap loss could occur (e.g. due to nearby roads or passing animals). Pantraps were placed on the ground and were visible by flying insects even at a distance, and triplets were always separated by at least 15 m. Each pantrap was filled up with ca 350 ml of water plus 1 drop of odour-free dishwashing detergent, and was left in place for 48 h in each sampling round, to cover the entire flight period of diurnal and nocturnal pollinators. Hand-netting surveys in turn consisted of 120-min random walks during which any bee observed on any flower was collected or recorded if it could be easily identified, stopping the timer any time our attention was distracted from the flowers (e.g. while transferring captured insects into vials). To minimize bias two persons with prior experience did all hand-netting. All hand-netting was carried out during daytime, under good weather conditions, between 10:00 a.m. and 4:30 p.m., attempting to diversify collection times, so that both morning and afternoon collections were included in our collecting scheme at each study site. The total time devoted to collecting at any given plant species was closely related to its floral abundance. Wild bees collected by pantraps and hand-netting were respectively transferred to small plastic bags and vials, refrigerated during transportation, frozen until processing in the laboratory, identified to species and deposited in the 'Melissotheque of the Aegean' (Petanidou and Lamborn 2005, Petanidou et al. 2013).

To calculate wild bee richness and abundance we pooled all the data from the pantraps and hand-netting at a given study site across all sampling rounds. Because sampling effort in hand-netting surveys was identical across sites, whereas the number of effective pantraps differed, we standardized the number of insects collected based on the number of pantraps. We first chose a subsample from the original pantrap database for each site, randomly picking a number of pantraps equal to those in the site with the lowest number (Gotelli and Colwell 2001). The subsampled list of species was then merged with the list recorded in hand-netting surveys for that site, and overall species richness (i.e. number of species) and wild bee abundance (i.e. number of individuals) were calculated. We repeated this

procedure 1000 times for each study site and used the mean number of species and individuals respectively as standardized values of wild bee richness and wild bee abundance for the site. We also derived standardized values for richness and abundance at the level of different families of wild bees at each site.

As estimate of beekeeping intensity, we used honey bee visitation rate, calculated as the mean number of honey bee visits to flowers per 2-h survey at each study site, averaged over all hand-netting surveys at that site (Supporting information). Data on honey bees collected in the pantraps were not used, because honey bees seem to avoid the traps (authors' observations), so that numbers collected in traps did not reflect honey bee activity on flowers at the study sites. We also did not use honey bee hive density per island because: 1) hive density was correlated to the percentage of natural habitats in the island's landscape ('Resources at the local and landscape scales', below, for calculation of landscape variables) both when using average hive density in the period 2005–2015, or when using hive density in the year of study ($r = 0.43$, $t = 2.99$, $df = 39$, $p = 0.005$ and $r = 0.51$, $t = 3.71$, $df = 39$, $p = 0.001$, respectively; Supporting information), and therefore, there could be confounding factors between these two variables; and 2) there was no correlation between the average number of honey bee visits and hive densities reported in the period 2005–2015 or in the year of study ($r = 0.19$, $t = 1.21$, $df = 39$, $p = 0.234$ and $r = 0.19$, $t = 1.20$, $df = 39$, $p = 0.239$, respectively; Supporting information).

Pollination networks

We used the hand-netting data to construct 82 quantitative interaction networks: 41 for wild bees alone (one per study site pooling the observations of all the sampling rounds; Table 1), and 41 networks that included honey bees in addition to wild bees. In these networks the total number of visits per bee species to a plant species in a study site was the link weight.

Table 1. Results of the best models (GLMMs) showing the relationship between honey bee visitation rate (visits to flowers in 2 h), wild bee richness and wild bee abundance. For each variable that appear in the best models, the χ^2 , the degrees of freedom (df) and the p-value are shown. Significances are based on LRT and significant values are marked in bold. Honey bee visitation rate was fixed while selecting the best models, because we were specifically interested in testing the effects on the response variables

Model Variable	χ^2	df	p
(a) Wild bee richness			
Honey bee visitation rate	8.17	1	0.004
Flower abundance	4.43	1	0.035
Log (Island area)	3.47	1	0.063
R ² = 0.37			
(b) Wild bee abundance			
Honey bee visitation rate	4.20	1	0.040
Landscape heterogeneity	19.42	1	< 0.0001
% natural habitats in surroundings	5.11	1	0.024
Flower abundance	3.79	1	0.052
R ² = 0.26			

We analysed the networks at each site using the R-package bipartite ver. 2.15 (Dormann et al. 2020) in R ver. 4.0.2 (<www.r-project.org>), and calculated the following indices to characterize the structure of interactions: 1) number of links in the networks; 2) number of plant species in the networks; 3) linkage density (weighted), as the mean number of links per species weighted by the frequency of interactions (Bersier et al. 2002, Dormann et al. 2009; since networks differed in size, we chose this index to describe generalization, Tylianakis et al. 2010); 4) nestedness, as weighted NODF (Nestedness metric

based on Overlap and Decreasing Fill), a quantitative index in which high values indicate nestedness, i.e. high tendency for specialist species to interact with generalists (Almeida-Neto et al. 2008, Almeida-Neto and Ulrich 2011); and 5) modularity, as the modularity of weighted bipartite matrices (metacomputeModules function). Modularity describes the extent to which the networks are organized into subsets composed by strongly interlinked species which are weakly connected to other subsets (Olesen et al. 2007, Dormann and Strauss 2014). To evaluate whether any detected change in network metrics is due to changes in network size (the number of species in the networks), we also used z-scores ($z = [x - \mu]/\sigma$, where x is the observed value, and μ and σ the mean and standard deviation of 1000 random networks respectively, following the vaznull null model (Vázquez et al. 2007). This null model randomizes individual interactions in the network while maintaining the original values of connectance, total number of interactions and plant and pollinator richness. Therefore, link or plant number were not standardized. z-scores compare the observed network parameter to the distribution of simulated parameters, so that a negative value indicates a metric that falls below what is expected at random and a positive value reflects one that falls above.

Because our main objective was to study how honey bee activity influenced the structure of wild bee pollination interactions, only the 41 wild bee networks (one per study site; excluding honey bees) were used to evaluate this relationship. To illustrate how much a single managed species can affect the whole structure of networks depending on its abundance, we also compared the networks with and without honey bees (results shown in Supporting information). It might be noted though, that this is an unrealistic situation, since in absence of honey bees, wild bees might differ in number, abundance and behaviour, influencing network metrics.

Using the 41 networks including both honey bees and wild bees, we estimated the potential competition between honey bees and other bees via shared food plants. For this, we calculated the apparent competition index of Müller et al. (1999), using the PAC function on the transposed matrix with bee species in rows and plants in columns (Supporting information for details). This index measures species niche overlap between any two pair of species, accounting for the importance of the resources of each species in the pair; we then selected the values of potential resource competition between honey bees and wild bee species for further analyses.

Resources at the local and landscape scales

To assess resources for pollinators, we estimated the abundance and richness of flowers at each site on each day we sampled bees. We randomly placed 25 1×1 m squares within the hectare where bees were sampled; within these squares we counted the number of functional reproductive units (i.e. flowers or inflorescences depending on plant species). At least one sample of every flowering species was collected to allow identification and homogenise nomenclature across sites and islands following 'The Plant List' website (<www.theplantlist.org>). Specimens were deposited in the Herbarium of the Laboratory of Biogeography and Ecology at the University of the Aegean. To characterize the flower community at each site, we used an average for the three sampling rounds of the total number of flowers m^{-2} (hereafter flower abundance; Supporting information) and the total number of plant species with flowers (hereafter flower richness; Supporting information).

To assess the mosaic of landscape elements that could affect wild bee communities (Senapathi et al. 2017), we turned to ArcMap ver. 10.5 (ESRI 2016) and the CORINE Land-cover database from 2012 (European Environmental Agency 2018). We established 1-km and 2-km buffer zones around the centre of each study site and estimated the area covered by different landscape elements, including natural and semi-natural habitats (mainly sclerophyllous vegetation, grasslands and pastures), crops (mainly olive

groves, vineyards and other complex cultivation patterns) and urban elements (mainly urban structures and transport networks). With these data we calculated the total percentage of natural and semi-natural areas and landscape heterogeneity, as the diversity of all landscape elements calculated as Shannon's (1948) diversity index using the R-package *vegan* ver. 2.5-6 (Oksanen et al. 2019). Landscape variables are shown in Supporting information.

Statistical analyses

Statistical analyses were conducted in R 4.0.2 (<www.r-project.org>). To study the relationship between honey bee visitation rate and wild bee abundance and richness, we used separate generalized linear mixed models (GLMM, R-package *lme4* ver. 1.1.23; Bates et al. 2015) for each response variable, where the 41 study sites were the sampling units and island was included as random variable. As continuous predictor variables in the models, we included honey bee visitation rate, flower abundance and richness, and landscape variables. We also included the logarithm of island area (km²) as a predictor, since this variable has been shown to strongly influence the diversity of bees on these islands (Kaloveloni et al. 2018). Variation inflation factor (VIF) analyses identified collinearity (Zuur et al. 2009) between landscape variables in the 1- and 2-km buffer zones; models with variables calculated at 1 km performed best (AICc: 598.79 versus 633.39 for 1 versus 2 km), and their results are presented here. We used analogous models, including the interaction between honey bee visitation rate and wild bee family, to test for different responses by family. An additional GLMM was used to test whether potential competition of honey bees and wild bees along the gradient of honey bee density differed among bee families. In this model, honey bee visitation rate, bee family and their interaction were included as predictors, along with the density and richness of flowers, which could affect resource sharing (Thomson 2006). In this model, island and species were included as crossed random factors. Family *Melittidae* was excluded from any analysis testing for interactions with family, because this bee family appeared in very few study sites. Lastly, we also used GLMMs to compare network metrics when including and excluding honey bees, and to assess the relationship between the difference in metrics with and without honey bees and honey bee visitation rate (both using raw metrics and z-scores). Due to the nature of the data, we used gamma distribution and log link function for all the models except for the number of links that followed a poisson distribution. When needed, model selection was conducted using the dredge function (R-package *MuMIn* ver. 1.43.17; Barton 2020), setting the maximum number of variables to 4 to avoid over-parametrization, and fixing the variable honey bee visitation rate, as our main objective was to test the effect of this variable. We used a posteriori tests on the differences between the slopes for each wild bee family using *emtrends* function (R-package *emmeans* ver. 1.4.8; Lenth 2020). Significance of variables is based on likelihood ratio tests (LRT), using *Anova* function (R-package *car* ver. 3.0.8; Fox and Weisberg 2019). R² values were calculated using *r2beta* function (R-package *r2glmm* ver. 0.1.2; Jaeger 2017).

To sum up the direct and indirect effects (i.e. mediated by another variable) of honey bee visitation rate on wild bee network metrics, we conducted a piecewise structural equation model (SEM; R-package *piecewiseSEM* ver. 2.1.2, Lefcheck 2016), first with raw metric values and then with z-scores. Initially, component models for wild bee abundance and richness included honey bee visitation rate, log (island area), and floral and landscape variables as predictor variables, whereas component models for network metrics included all these variables and also the abundance and richness of wild bees. We also specified correlated errors between the different network metrics (Lefcheck 2016). The final SEM included only component models involved in significant or marginally significant causal relationships, to maximize model fit (lower AIC) and avoid over-parametrization. In all the component models, we included island as random variable, and used gamma distributions and log link functions after scaling the variables

(scale function) to increase model fit (Lefcheck 2016). Global goodness-of-fit is based on tests of directed separation (Shipley 2000, Lefcheck 2016).

Results

In total, we recorded 17 719 wild bees belonging to 229 species in 6 families (Halictidae: 8912 bees, 48 species; Megachilidae: 3476 bees, 69 species; Apidae: 2609 bees, 59 species; Andrenidae: 1913 bees, 35 species; Colletidae: 730 bees, 16 species; and Melittidae: 79 bees, 2 species). Wild bee richness varied from 16 to 75 across the 41 study sites (Supporting information). During hand-netting surveys we recorded 18 525 honey bee and 13 736 wild bee visits to flowers. Mean number of flower visits per 2 h in different sites ranged from 0.0 to 486.3 for honey bees, and from 9.3 to 269.0 for wild bees (Supporting information). Across all study sites we recorded honey bees foraging on 107 of the 159 plant species (73.6%); their visits amounted to a 57.4% of the 32 261 total recorded bee visits to flowers.

Wild bee richness and abundance

Wild bee richness was negatively related to honey bee visitation rate (Table 1a, Fig. 2a), and positively to flower abundance (Table 1a, Fig. 2b), and also tended to increase with island area, although this effect was only marginally significant (Table 1a, Fig. 2c). Wild bee families differed in their overall species richness ($\chi^2 = 126.21$, $df = 4$, $p < 0.0001$), however the effect of honey bee visitation rate on wild bee richness did not differ significantly among families (honey bee visitation rate \times family: $\chi^2 = 2.31$, $df = 4$, $p = 0.679$; full model in Supporting information).

Partial effects plots showing the relationships between wild bee richness and: (a) honey bee visitation rate (visits to flowers in 2-h surveys); (b) flower abundance; and (c) island area (km²), logarithmically transformed. The lines represent the estimates, the dots the partial residuals of the best model and the shaded area the confidence interval. Full model: $R^2 = 0.37$.

Wild bee abundance also decreased with honey bee visitation rate, but the effect was relatively weak (Table 1b, Fig. 3a). Wild bee abundance decreased significantly with landscape heterogeneity (Table 1b, Fig. 3b), and increased with the percentage of natural and semi-natural habitats in the landscape (Table 1b, Fig. 3c) and with flower abundance, although this last relationship was only marginally significant (Table 1b, Fig. 3d). Wild bee families differed in their overall abundance ($\chi^2 = 54.72$, $df = 4$, $p < 0.0001$), however the effect of honey bee visitation rate on wild bee abundance did not differ among families (honey bee visitation rate \times family: $\chi^2 = 3.65$, $df = 4$, $p = 0.456$; full model in Supporting information).

Partial effects plots showing the relationships between wild bee abundance and: (a) honey bee visitation rate (visits to flowers in 2-h surveys); (b) landscape heterogeneity (Shannon's (1948) diversity index of land-cover layers 1-km buffer zones); (c) % natural and semi-natural habitats in 1-km buffer zones; and (d) flower abundance. The lines represent the estimates, the dots the partial residuals of the best model and the shaded area the confidence interval. Full model: $R^2 = 0.26$.

Potential competition between honey bees and wild bees through shared resources

As expected, potential resource competition between honey bees and wild bees via shared plants increased with honey bee density, although at significantly different rates for different bee families (family: $\chi^2 = 11.19$, $df = 4$, $p = 0.025$; honey bee visitation rate \times family: $\chi^2 = 21.35$, $df = 4$, $p = 0.0003$). At low honey bee densities potential competition was highest for Apidae (Fig. 4). However, as honey bee visitation rate increased, the potential competition between honey bees and wild Apidae increased

moderately, whereas honey bee competition with wild bees in other families increased more strongly (Fig. 4).

Partial effects plot showing the relationship between honey bee visitation rate and potential competition between honey bees and wild bees through shared plant resources, calculated using the apparent competition index of Müller et al. (1999). Lines represent the estimates of the best model for each bee family, dots represent average partial residuals for each bee family at each study site, and vertical lines standard errors. Different letters indicate significant differences after a posteriori tests.

Structure of pollination networks

Networks including honey bees showed significantly higher number of links, plant species, raw linkage density and nestedness and lower raw modularity than the networks including only wild bees. Networks including honey bees also exhibited a larger departure from the expectation under random interactions than those containing only wild bees (Supporting information). The extent of difference between networks with and without honey bees at each study site was significantly related to honey bee visitation rate, except in the case of linkage density (both raw and z-score) (Supporting information). Supporting information, illustrates the pollination networks at each study site and the role of honey bees in them.

The relationships between honey bee visitation rate and wild bee raw network metrics were adequately represented by the parametrized piecewise SEM (global goodness-of-fit: $C = 10.56$, $df = 16$, $p = 0.836$; tests of directed separation in Supporting information). This causal model indicates that the effect of honey bee visitation rate on wild bee raw network metrics was indirect, through an effect on wild bee richness (Fig. 5). The relationship between honey bees and wild bee richness is negative – in keeping with the result from GLMM. In turn, wild bee richness was positively related to the number of links and linkage density. Thus, the negative effect of honey bee visitation rate on wild bee richness cascaded into a reduced number of links and linkage density in the wild bee pollination networks. Notice also that an additional effect of flower richness on the number of links in the networks was detected, and that the two network metrics – number of links and linkage density – were positively correlated. Raw modularity and nestedness were not significantly affected by any of the variables included in the SEM, and wild bee abundance did not directly affect any of the network metrics, nor was it correlated with wild bee richness; thus these variables were not included in the final model (Fig. 5).

Structural equation model (SEM) testing the direct and indirect effects of honey bee visitation rate (visits to flowers in 2-h surveys) on wild bee raw network metrics (number of links and linkage density). Boxes represent measured variables. Marginal (M) and Conditional (C) R²s for component models are given in the boxes of response variables (R-package piecewiseSEM; Lefcheck, 2016). Single-head arrows show significant ($p < 0.05$) unidirectional relationships among variables, whereas double-head arrows show significant correlated errors, being positives marked in black and negatives in red. The thickness of the arrows was scaled based on the magnitude of the standardized regression coefficient, given in the associated box. Marginally significant relationship ($p = 0.056$) is marked with a dashed line.

An analogous piecewise SEM using z-scores exhibited a similar overall pattern (Supporting information for full model and tests of directed separation), except that there was no significant effect of the number of wild bee species on z-score linkage density (estimate \pm SE -0.0309 ± 0.0693 , $df = 41$, critical value -0.4458 , $p = 0.656$), confirming that honey bee visitation rate affected network metrics via network size. Furthermore, the correlation between number of links and z-score (linkage density) is

negative, because the more links in the networks, the more strongly z-scores for linkage density fall below the expectation based on random interactions.

Discussion

In this large-scale study we discovered that increasing honey bee visitation rate had an unambiguous negative effect on wild bee richness and abundance, increased competition for flowering resources and influenced the structure of pollination networks indirectly through an effect on the number of wild bees in the communities.

Effects of beekeeping intensity on wild bee diversity and abundance

High honey bee visitation rate had a negative impact on wild bee richness, after controlling for the known positive influence of local flowering resources (Ebeling et al. 2008, Blaauw and Isaacs 2014) and island size (Kalooveloni et al. 2018) on the diversity of bees. Previous studies linking honey bee visitation rate to wild pollinator diversity, however, showed variable results. For instance, in a protected Mediterranean habitat, Ropars et al. (2020) showed that richness of large wild bee species was negatively affected by honey bee colony density, while communities of small wild bees were structured instead by local flowering communities. Conversely, in another protected Mediterranean natural park, Reverté et al. (2019) failed to find any relationship between honey bee visitation rate and the composition of wild pollinators in an area dominated by honey bees. A few other studies conducted in agroecosystems also failed to find any effect of honey bee density on wild bee richness (Steffan-Dewenter and Tscharntke 2000, Russo et al. 2015). Perhaps these previous studies did not include a sufficiently large gradient of variation in either beekeeping intensity or bee diversity. Here we assessed this relationship over a larger scale than previous studies, covering an area of ca 23 000 km² and including 41 sites dispersed across 13 islands, and also included a large gradient of variation in beekeeping intensity (Papas 2008), ranging from zero to high levels (Supporting information). Our study system might be also particularly suited for exploring how honey bees affect wild bees, since island systems limit species movements and ecological communities on islands are usually more fragile in the face of species introductions (Kaiser-Bunbury et al. 2010, Massol et al. 2017).

In agreement with several previous correlational and experimental studies (Dupont et al. 2004, Shavit et al. 2009, Artz et al. 2011, Goras et al. 2016, Lindström et al. 2016, Herrera 2020), we also detected a decrease in overall wild bee abundance with increasing honey bee visitation rate. However, the effect we detected was relatively weak compared to the effect on abundance of variables related to landscape disturbance. In our study, wild bee abundance increased with the percentage of natural habitats in the landscape and decreased with landscape heterogeneity. The first effect is unsurprising because bee populations are mainly regulated by the amount of high quality habitat, which provides flowering resources (Steffan-Dewenter and Tscharntke 2001, Potts et al. 2003) and nesting sites (Potts et al. 2005, Williams et al. 2010). Indeed, pollinator abundance and richness have been repeatedly reported to increase with proximity to, or proportion of, natural and semi-natural habitats, both for wild communities (Steffan-Dewenter et al. 2002, Öckinger and Smith 2007, Ekroos et al. 2013, Woodcock et al. 2013) and crops (Ricketts et al. 2008, Garibaldi et al. 2011, Alomar et al. 2018).

Turning to landscape heterogeneity, several studies show that it favours pollinator diversity (Ekroos et al. 2013, Aguirre-Gutiérrez et al. 2015, Senapathi et al. 2017), mainly due to a higher number of available habitats for pollinators and a higher landscape complementation and supplementation (respectively the proximity of landscape elements essential to a bee life cycle and of elements containing substitutable resources; Dunning et al. 1992, Holzschuh et al. 2008) in heterogeneous landscapes. Interestingly, landscape heterogeneity had no detectable effect on wild bee richness in our study, but instead a negative effect on abundance. Likely, the negative relationship between wild bee

abundance and landscape heterogeneity can be attributed to the large proportion of wild bees that are associated with certain habitats, such as phrygana and olive groves that if traditionally managed host a wealth of flowering plant and pollinator species (Potts et al. 2006, Nielsen et al. 2011). Indeed, Tscheulin et al. (2011) showed that wild bee abundance in a Greek island (Lesvos) was positively related to the extent of olive groves, while other habitats had mostly negative or no impact on the abundance of wild bees. For any given area, an increase in the number of different cover types leads to a decrease in the amount of each (Duelli 1997), which might not allow a large growth of populations of species that are benefitted from certain habitats. Finally, as was true for wild bee richness, we also found that flower abundance tended to be positively related to wild bee abundance, supporting the idea that a larger number of flowers in a community attract a larger number of pollinators (Hegland and Boeke 2006, Lázaro et al. 2020).

Overall, high beekeeping activity has negative consequences for both wild bee abundance and richness; however, the effects on richness may be stronger than those on abundance, as wild bee abundance was also largely influenced by aspects of the habitat. This suggests that a focus on overall pollinator activity – without considering species composition – may underestimate the damage to wild pollinators from managed honey bees. Thus, beekeeping intensification could result in biotic homogenization of communities in the long term, similar to intensive land-use (Gossner et al. 2016), and this might catastrophically affect the provision of pollination services which are known to depend on pollinator diversity as well as on pollinator abundance (Aizen and Harder 2009, Garibaldi et al. 2013).

Effects of beekeeping intensity on interaction networks

Competition between managed honey bees and wild pollinators has long been suggested to be a key factor in structuring plant–pollinator communities (Denno et al. 1995, Lázaro et al. 2010, Magrach et al. 2017). Honey bees can resemble invasive species in their impacts on pollination networks (Traveset et al. 2013, Albrecht et al. 2014, Stouffer et al. 2014): they can easily monopolize floral resources (Santos et al. 2012, Geslin et al. 2017, Magrach et al. 2017), maintain central roles in the networks (Giannini et al. 2015), contribute more to nestedness, and have an overall higher within-module degree and among-module connectivity than other species (Geslin et al. 2017). This was also the case in our communities, where honey bees visited ca 75% of available plants. Indeed, as in Santos et al. (2012), removing this single managed species from the networks significantly reduced the number of links, linkage density and nestedness and increased modularity (Supporting information). Moreover, we found the effect of honey bees on the structure of networks to increase significantly with their abundance, as expected for highly polylectic species having a very wide dietary niche (Requier et al. 2015) and thus with the potential to interact with diverse plant species. These results are also in line with Giannini et al. (2015), who found that nestedness in their pollination networks increased with increasing diversity of flowers used by honey bees.

Resource competition for flowering resources between honey bees and wild bees that persisted in the communities increased strongly with honey bee visitation rate for all bee families except Apidae, where it increased more moderately. Thomson (2016) showed that increasing density of honey bees was related to declines in bumble bees and to reduced diet overlap between these taxa. Indeed, our results suggest that there is a stronger niche segregation in Apidae than in the other families in response to high honey bee densities. What is the cause of this reduced diet overlap? This effect cannot be explained by differences among families in the loss of species or individuals, because we did not detect any stronger effect of honey bees on richness or abundance of wild Apidae than for other bee families (Supporting information). High floral diversity may also decrease plant use overlap between honey bees and other wild bees (Thomson 2006), but we found no evidence for this either. Perhaps changes in niche segregation within Apidae might result from changes in bee species composition along the

beekeeping gradient. Alternatively, wild Apidae could be better able to reshuffle their diet to reduce intense competition with honey bees. Unfortunately, the large variation in plant and wild bee composition along our study gradient, does not allow to properly evaluate interaction rewiring (CaraDonna et al. 2017).

Niche segregation between wild Apidae and their honey bee relatives can also be contemplated from a morphological and physiological perspective. For instance, wild Apidae often possess a long proboscis and other morphological adaptations that allow them to exploit narrow tubular and flag flowers with hidden nectar, such as in the Fabaceae, that are less accessible to honey bees (Michener 2007). In addition, many large Apidae have the ability to generate heat endothermically and so can exploit floral resources earlier in the day or in cold days (Stone et al. 1999), or emerge earlier in the year to be the first group exploiting the late winter flowering plants (Petanidou and Ellis 1996) and thus benefit from reduced competition. However, since functional diversity within bee families often overlaps with that found over the entire phylogeny, unraveling the mechanism(s) in our system might ultimately depend on finer functional and phylogenetic information than we possess at this point.

We expected changes in the structure of pollination interactions along the gradient of beekeeping intensity to be mediated by changes in the diversity of wild bees in the communities, but also by changes in diets of the wild bees that persisted in the communities (Hansen et al. 2002, Walther-Hellwig et al. 2006, Petanidou et al. 2008, Roubik and Villanueva-Gutiérrez 2009), because pollinators can vary their feeding choices in response to changes in floral abundances (Hegland and Totland 2005, Hersch and Roy 2007) and pollinator densities (Inouye 1978, Lázaro et al. 2011). However, our results indicate that honey bee density affected the structure of wild bee pollination interactions only indirectly, through changes in species richness. To our knowledge, there are only two previous experimental studies that have evaluated the effect of temporal increases in honey bees on network structure. One of them showed that honey bee spill-over led to a re-assembly of plant–pollinator interactions through shifts in wild species' diets (Magrach et al. 2017). The other reported that beekeeping reduced both the diversity of wild pollinators and the interaction links in the pollination networks, causing the loss of interactions by generalist species (Valido et al. 2019). In our study system, decreased generalization (number of links and linkage density) as a result of honey bee density was not due to changes in pollinator diets, but to local extirpation of species from the communities.

Conclusions

Our large-scale study demonstrated that honey bee abundance was negatively related to the richness and abundance of wild bees in the Cycladic Islands, and that these effects also cascaded into the pollination interactions at the community level, by increasing potential competition between managed and wild bees through shared resources, and by decreasing the number and generalization of wild bee interactions. Overall, our study indicates that the massive introduction of honey bees in the Cyclades can threaten the high diversity of wild bees these islands host, and warns against management actions aimed at increasing beekeeping in this insular system and other hotspots of wild bee diversity.