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Assessing bee species richness in two Mediterranean communities: importance of habitat type and sampling techniques

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Abstract The decline of bees has raised concerns regarding their conservation and the maintenance of ecosystem services they provide to bee-pollinated wild flowers and crops. Although the Mediterranean region is a hotspot for bee species richness, their status remains poorly studied. There is an urgent need for cost-effective, reliable, and unbiased sampling methods that give good bee species richness estimates. This study aims: (a) to

assess bee species richness in two common Mediterranean habitat types: semi-natural scrub (phrygana) and managed olive groves; (b) to compare species richness in those systems to that of other biogeographic regions, and (c) to assess whether six different sampling methods (pan traps, variable and standardized transect walks, observation plots and trap nests), previously tested in other European biogeographic regions, are suitable in Mediterranean communities. Eight study sites, four per habitat type, were selected on the island of Lesbos, Greece. The species richness observed was high compared to other habitat types worldwide for which

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comparable data exist. Pan traps collected the highest proportion of the total bee species richness across all methods at the scale of a study site. Variable and standardized transect walks detected the highest total richness over all eight study sites. Trap nests and observation plots detected only a limited fraction of the bee species richness. To assess the total bee species richness in bee diversity hotspots, such as the studied habitats, we suggest a combination of transect walks conducted by trained bee collectors and pan trap sampling.

Keywords Hymenoptera · Apoidea · Sampling methods · Pan traps · Transect walks · Abundance-based Coverage Estimator (ACE) · Subunit-based rarefaction curves · Olive groves · Phrygana · Mediterranean scrub · Biodiversity hotspot

Introduction

Animal pollination is recognized as an important ecosystem service as it ensures the production of animal-pollinated crops as well as the reproduction of animal-pollinated wild plants (Fontaine et al. 2006; Kremen et al. 2007; Klein et al. 2007). In recent years increasing concerns have been raised regarding the potential decline of pollinators, both in terms of their species richness and abundance (Steffan-Dewenter et al. 2005; Biesmeijer et al. 2006; Committee on the Status of Pollinators in North America 2007; Potts et al. 2010), although the extent and impact of the decline have been questioned (Ghazoul 2005). The lack of consensus on these matters reflects the lack of systematic pollinator monitoring programs. An important prerequisite for any such scheme is a tested and reliable method to survey pollinator stocks objectively and efficiently (by efficient we mean here, sampling a high number of individuals and species with the use of a low number of man-hours). As a result, long-term initiatives with the goal of detecting the nature and extent of pollinator declines and their ultimate causes have been initiated around the world (e.g., International Initiative for the Conservation and Sustainable Use of Pollinators, COP 5 Decision V/5, section II, <http://www.cbd.int/decision/cop/?id=7147>). However, there is still an urgent need for standardized monitoring schemes to assess whether the proposed

erosion of many pollinator communities is real. A significant contribution to the development of standardized tools is the recent test of sampling methods by Westphal et al. (2008), which focused on cultivated and semi-natural habitats in northern and central Europe. However, the main diversity hotspots for many pollinating insects, in particular bees, occur in more southern semi-arid regions (Michener 2007) and the appropriateness of monitoring methods needs also to be tested in such environments.

Bees (Hymenoptera: Apiformes) are the most important pollinators worldwide (Kearns et al. 1998). Although the Mediterranean is one of the world's highest bee diversity centers (Michener 2007), its bee species richness is relatively poorly documented (but see Petanidou and Ellis 1993, 1996; O'Toole and Raw 2004; Petanidou and Lamborn 2005; Petanidou and Potts 2006; Potts et al. 2006). The dry climate and frequent fires maintain the availability of bare soil (Potts et al. 2003a), a habitat characteristic on which many bee species rely for nesting (Potts et al. 2005). In addition, high floral diversity enhances the bee diversity in the area (Petanidou and Ellis 1996; Potts et al. 2003b). High bee diversity may be further facilitated by traditional Mediterranean land management, which employs light grazing by animals, regular rotation of cultivated crops and, most important in areas of arable crops, light plowing that prevents vegetation from closing. For instance, Potts et al. (2006) found that actively managed olive groves had higher bee species richness than olive groves that were left unmanaged or abandoned.

To obtain reliable estimates of species richness, sampling methods must be unbiased and comparable across sampling sites and events. The sampling method(s) must also provide data relevant to the questions posed. For instance, several methods might be included for comprehensive inventories aiming at detecting as many species as possible, while a single method may suffice to obtain a relative and comparable measure of species richness through time and across sites (Westphal et al. 2008; Wilson et al. 2008).

Sampling methods vary in how well they perform, i.e., how well they can estimate the true species richness with a given sampling effort. However, a particular method may be biased towards sampling species with particular traits (Roulston et al. 2007; Westphal et al. 2008; Wilson et al. 2008). The sampling methods most often employed in pollination studies are active censuses involving field workers recording insects visiting flowers in observation plots or along transect walks (e.g., Petanidou and Ellis 1996; Nielsen and Ims 2000; Potts et al. 2006; Totland et al. 2006; Westphal et al. 2008), or strategically collecting from areas (e.g., potential nesting habitats) where pollinators are most easily encountered. However, these methods are associated with several types of biases. First, the slow-moving, large and colorful insects are often detected and caught more easily than the fast, small, and inconspicuous ones. Second, different insect collectors with contrasting skills and

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experience in catching and handling insects may cause bias if involved in the sampling of different sites. Third, different insect collectors may show different collecting preferences towards species that are new or familiar to them or species groups in which they are particularly interested. Fourth, the knowledge of species-specific preferences for micro-habitats, i.e., where to search for particular species, may vary among surveyors.

Pan traps have been shown to be the most efficient and unbiased method for assessing bee species richness in several habitat types, including a montane valley with cereal grain and grasses, an irrigated fruit production area and sage brush, a high desert sage scrub (Stephen and Rao 2005 [using Asian Ladybug- and Japanese Beetle traps functioning with the same principle as pan traps]), as well as annual crop fields and semi-natural grasslands (Westphal et al. 2008). However, passive sampling methods, such as pan traps and trap nests, although avoiding some of the issues of collector bias, are associated with other biases, as they capture species with unequal probability. Acting as visual attractors, pan traps capture bees with contrasting nesting preferences and life histories, but have the tendency to capture smaller bees disproportionately relative to larger ones (Cane et al. 2000; Roulston et al. 2007). Moreover, some bee taxa are attracted preferentially to traps of certain colors (Leong and Thorp 1999; Toler et al. 2005). There are also indications that pan traps may catch bees in inverse proportion to the availability of competing flowers for their attention (Mayer 2005; Wilson et al. 2008). Trap nests capture only cavity nesting bees, a group usually representing only a small fraction of the total bee species richness in an area (Dafni et al. 2005; Westphal et al. 2008). Neither pan traps nor trap nests generate any floral host data, making them useless in studies of e.g., pollination networks.

Here, we assess the bee species richness in two common habitat types of the Mediterranean, phrygana (low shrub) and olive groves, using the same sampling methods and protocols as Westphal et al. (2008). These habitat types are considered not only representative but also valued cultural landscapes of the Mediterranean (Allen et al. 2006). Over recent decades, traditional olive grove cultivations have increasingly been abandoned, transforming them into grazing pastures, i.e., degraded phrygana (Petanidou et al. 2008). This transformation may have impacts on pollinator diversity, but little is known about whether olive groves and phrygana are comparable in terms of their bee species richness (but see Potts et al. 2006). Compared to other managed habitat types, there are several ecological features of olive groves that may facilitate bee species richness. They contain a variety of microhabitats, including rocky surfaces, stone fences, and terraces. In addition, regular management of the olive groves, in particular light plowing, increases the occurrence of bare soil. All these landscape structures are important for bees as nesting sites (Potts et al. 2004, 2005). In addition, the regular soil dis-

turbance prevents perennial plants and shrubs from dominating, thus allowing high densities of annual plants to flourish, many of which provide substantial floral resources for bees (Potts et al. 2003b, 2004). Consequently, we expected to find comparable, or even higher, bee species richness in the managed olive groves than in the semi-natural phrygana habitats. Indeed, other studies have shown that intermediate disturbance levels are positive for bee species richness (e.g., Vulliamy et al. 2006). This expectation contrasts with the pattern in most other European agricultural landscapes: Westphal et al. (2008) found that intensively managed, annual agricultural fields contained lower bee species richness than semi-natural grasslands across Europe. The less intense disturbance regime of the managed olive groves as compared to other agricultural habitats may have positive effects on the bee species richness.

The Mediterranean region is a hotspot for bee species diversity (Michener 2007). However, comparisons with species richness estimates from other regions based on similar sampling techniques are scarce (but see Westphal et al. 2008 compared to this study).

The aims of this study were, first, to compare bee species richness in two focal habitats in a region harboring a rich bee fauna. Second, to compare the bee species richness in our focal systems to other biogeographic regions where comparable data exist. Third, to identify the best sampling method, or combination of methods, for successful large-scale and long-term bee monitoring schemes in species-rich communities.

Methods

Study area and study sites

The study was conducted on the Greek island of Lesbos in the north eastern Aegean Sea (N 38°57'32"–N 39°23'15"; E 25°49'46"–E 26°36'51"). Details on the vegetation, habitat types and the island in general can be found in Detsis et al. (2000), Marathianou et al. (2000) and Potts et al. (2006). A long history of human land use has significantly transformed the island's landscape (Kizos et al. 2010). In particular, the land coverage by olive trees has increased from 2% in the antiquity (3rd century BC) to 27% in the 1880s and to 41% today (Marathianou et al. 2000).

Phrygana is a habitat composed of low shrubs and is equivalent to the west Mediterranean garrigue. It is the main semi-natural habitat in the eastern Mediterranean. Olive groves represent the main perennial cultivation throughout the Basin. Both these habitat types have dominated the landscape in this part of the world since the antiquity and have evolved under the presence of humans managing them for animal grazing, bee-keeping, and olive production.

To answer questions related to bee species richness and the effectiveness of different bee sampling methods,

we randomly established four study sites in actively managed olive groves and another four in phrygana habitats. Locations of the study sites are given in Table S1 (supplementary material).

Sampling methods

We used six different methods for bee sampling, following the methodology and sampling design described in Westphal et al. (2008). Sampling took place between March and July 2004. Each study site was sampled ten times using each method except for pan traps, which were deployed on six occasions (see supplementary material Table S2 for dates of sampling). Windy and rainy days were avoided for all samplings. The sampling methods were applied as follows:

- (1) *Observation plots (OP)* In each study site ten rectangular 1 × 2 m observation plots were established in a random location. Observations at each plot lasted 6 min. During the observation period, every bee that visited a flower was collected or recorded if it was possible to identify it on the wing. The order in which the plots were observed during an observation round was randomized to avoid temporal bias. Five field workers conducted the sampling of the observation plots. Observation plots, together with standardized (ST) and variable transect walks (VT) (see below) were conducted between 8:00 am and 5:00 pm.
- (2) *Standardized transect walks (ST)* A permanently marked 250-m-long and 4-m-wide corridor was divided into ten 25-m-long subunits and used for the standardized transect walks at each site. Each subunit was surveyed for 5 min during which all bees visiting flowers were collected, for a total of 50 min sampling time spent throughout the entire standardized transect in each round of observations. Four field workers conducted the standardized transect walks.
- (3) *Variable transect walks (VT)* To cover better the spatially heterogeneous distribution of floral resources, thereby increasing the probability of collecting a higher proportion of the bee species present, we established an additional 1-ha plot adjacent to the area where the other sampling methods were undertaken [see Fig. 2 in Westphal et al. (2008) for a detailed description of the setup of each study plot]. In the VT plot surveyors were allowed to search actively for bees throughout the plot by slowly walking around for 30 min. Five field workers conducted the variable transect walks.
- (4) *Pan traps (PT)* In each study site, 15 pan traps were set up in five clusters separated by 15 m. Each cluster contained three UV-bright pan traps, colored in white, yellow, and blue. These colors accounted for different color preferences among bee species, increasing the trap clusters' efficiency in attracting

bees (Leong and Thorp 1999; Toler et al. 2005). Each trap was filled with 400 ml of water and a few drops of liquid dishwashing detergent. The pan traps were mounted on a pole at the vegetation height, and left active for 48 h during six rounds of sampling throughout the season. Collected bees were temporarily stored in 70% alcohol before they were pinned for identification.

- (5 and 6) *Trap nests (TN)* In each study site ten poles were mounted containing five trap nests each. Two of the trap nests contained ca. 150 stems of common reed (*Phragmites australis*) internodes each, being 15–20 cm in length with diameters ranging between 2 and 10 mm (TNr; Gathmann et al. 1994). The remaining three trap nests contained paper tubes of distinct diameters, 6.5, 8, and 10 mm, respectively (TNp) and they were provided by the Oxford Bee Company (CJ WildBird Foods, Shrewsbury, UK). The trap nests were set out between March 20th and 23rd 2004 and were collected between 2nd and 4th August of the same year. Tubes with nests were stored at 4°C for at least 3 months after which temperature was increased hatched adults were pinned and identified to species.

Species identification

All specimens were identified to species, either in the field or later in the lab. About 2% of the specimens could not be identified (mainly due to damage) and were not included in the analysis. Collected specimens are held mainly at the *Melissotheque of the Aegean* (Laboratory of Biogeography and Ecology, Department of Geography, University of the Aegean, Greece) and a small number are housed by taxonomists that helped with the bee taxonomy (see Acknowledgments).

Data analyses

Similarities in species composition between study sites and sampling methods

To quantify the similarity of species assemblages found in different study sites and with different sampling methods we made pairwise comparisons using the Chao-Sørensen abundance-based estimator (Chao et al. 2005), a similarity estimator that has comparably low bias for rare species and small sample sizes (Chao et al. 2005, 2006). We calculated pair-wise similarities of the species assemblages found in each study site pooled over all sampling methods as well as for each sampling method separately using EstimateS 8.0.0 (Colwell 2005). Based on the estimated similarity values resulting from

comparisons of pairs of study sites (data from all sampling methods included) we calculated mean similarity (\pm SE). These estimates were used to assess whether the similarity in species assemblages were the same when sites of similar habitat was compared as when sites from different habitats were compared. To assess the complementarity of the methods we estimated the similarities among the species assemblages collected by the six different methods within each site. To analyze whether similarities in the bee species assemblages differed between habitat types and among the sampling methods, we used a two-way ANOVA in R 2.7.0 (R Development Core Team 2008). We performed nestedness analysis on the species assemblages found at each of the study sites to assess whether the species composition collected with the less efficient methods consisted of proper subsets of the species detected with the more efficient methods (Atmar and Patterson 1993). For the nestedness analysis, we used the software Aninhado 3.0.3 (Guimarães and Guimarães 2006). To assess whether the nestedness temperatures were lower than what could be expected by chance alone we generated 100 species distributions with null model Ce (null model 2 *sensu* Bascompte et al. 2003). Our test statistic was the probability that a random replicate would display an equal or lower nestedness value.

Dominance of the most common species in the two habitat types

To assess whether the dominance of the most common species varied between the two habitat types we used one-tailed *t* tests. Percentage of the individuals caught belonging to the particular species, pooled over all sampling methods, was used to describe the dominance of the species in each study site. The percentage values were arcsin square root transformed prior to analyses.

Differences between habitats and efficiency of methods

To assess the effectiveness of the different methods in sampling the bee communities and to compare the effectiveness of different methods in the two habitat types we used linear mixed-effect models (lme; Pinheiro and Bates 2000) using the nlme package (Version 3.1-86) in R 2.7.0 (R Development Core Team 2008). In the models we included sampling method and habitat type as fixed factors; study site was included as random factor. We used the number of species and individuals detected and the sample coverage of each method as response variables. The sample coverage was calculated as the number of species caught by a particular method divided by the total number of species caught by all methods combined within a study site. We also used the Abundance-based Coverage Estimator (ACE) (Magurran 2004) based on cross-section samples from each study site (see section below on “**Cross-section samples**”). The ACE estimates the number of species that

might have been detected with a particular method if sufficient sampling effort had been applied, e.g., covering a large enough area or conducting enough sampling events. ACE values were calculated with EstimateS 8.0.0 (Colwell 2005). Number of species and individuals, ACE values and sample coverage values were all log-transformed, $\log(n + 1)$, to achieve homoscedasticity.

Cross-section samples

Mediterranean habitats display large seasonal variations in climatic conditions, and thus we expected the bee species community to change dramatically through the season. To minimize the seasonal effect in species turnover when calculating ACE and the expected number of species (see below) we divided our samples into cross sections containing sampling events spanning the entire season. To assess bee species richness in the entire study community and in each habitat type separately, we used the pooled species assemblages over the entire season and all methods for each study site as cross-section samples (Krauss et al. 2003; Westphal et al. 2008). When assessing the effectiveness of the different sampling methods we used different cross sections for each method. For the observation plots, as a cross section we used all samples collected in a particular 1×2 m observation plot throughout the season, giving a total of ten cross-section samples in each study site. For the standardized transect walks we used all samples from each 5-min interval pooled over the entire season as cross-section samples; for instance, all bees collected during the first 5 min of all standardized transect walks conducted in a study site comprised one cross-section sample. For the pan traps, we used all bees caught in a particular pan trap cluster as a cross-section sample, giving a total of five cross sections in each study site. For the trap nests, we used each pair of trap nests with reed internodes and each cluster of three trap nests with paper tubes as one cross-section sample, because the trap nests were applied throughout the entire season. The variable transect walks could not be divided into cross sections covering the entire season. We therefore used each separate 50-min transect walk within each study site as a cross-section sample, with the potential bias that may be imposed by species that are active for only a limited time of the season.

Correlations between different sampling methods and total species richness

A sampling method can be considered a reasonable means for assessing the relative species richness of an area if its ability to sample species richness is highly correlated with the actual species richness found by all methods combined (our best estimate of the actual richness at the site). In order to assess whether there was a significant correlation between the number of species sampled by a particular method and the total number of

species found at each site we used correlation tests in R 2.7.0 (R Development Core Team 2008). As a measure of correlation, we report Pearson's product-moment correlation coefficient (R).

Subunit-based rarefaction curves

The sampling effort needed to detect a representative fraction of the bee species found at a study site was estimated with rarefaction curves based on cross-section samples. Based on the cross-section samples we calculated the expected number of species with EstimateS (Colwell 2005). The expected number of species (called Mao Tau in EstimateS) is calculated using the analytical formulas from Colwell et al. (2004). We calculated Mao Tau for an increasing number of cross sections for each sampling method in each study site and divided the estimated number of species (Mao Tau) by the total number of species detected in the particular study site. Hence, the rarefaction curves provide information on the number of cross-section samples needed to obtain a certain level of sampling coverage.

Collector bias

Differences in experience and knowledge as to where and how to collect bees can make bee collectors vary in their efficiency and effectiveness during censuses such as transect walks, thereby inducing collector-related bias in the sampling. Based on data from the two types of transect walks (ST and VT) we used two separate simple ANOVAs to analyze whether particular collectors caught more species or individuals than others. Our models included number of species or individuals caught per transect walk as response variables and collector

identity as predictor variable. The numbers of species and individuals were log-transformed, $\log(n + 1)$, to achieve homoscedasticity. Five collectors undertook all but one variable transect walk, while four collectors undertook all but two standardized transect walks. The three transect walks not conducted by the main collectors were excluded from these analyses. Each collector sampled between six and eight sites consequently covering both habitat types. Separate Chi-square tests showed that none of the bee collectors conducted a significantly higher proportion of a particular type of transect walk (VT or ST) in any of the habitat types (all p values >0.1). Except for the collector conducting variable transect walks, who was involved in round 9 and 10 only, the bee collectors conducted their sampling between five and nine out of the ten rounds and there was no seasonal bias for any of them.

Results

Overall bee species richness in the study system

We collected a total of 7,697 individual bees representing 267 species from 36 different genera (Table S3, supplementary material). There were large variations among study sites in the numbers of individual bees collected (range 525–1,535), the number of species (66–132), and the number of genera (18–26). The highest average number of individuals, species and genera were found in the managed olive groves, although the differences between the two habitat types were not statistically significant (for individuals $t_{5,95} = 0.74$, $p = 0.49$; species $t_{4,95} = 0.86$, $p = 0.43$; and genera $t_{4,81} = 1.18$, $p = 0.29$; Table 1, see also Table S2, Supplementary material, for details on the

Table 1 Number of individuals, species, and genera of bees collected in each habitat type separately and pooled over all habitats and study sites

	Habitats		
	All study sites ($n = 8$)	Olive grove ($n = 4$)	Phrygana ($n = 4$)
Individuals	7,697 (962.3 \pm 120.9)	4,217 (1,054.3 \pm 285.0)	3,480 (870.0 \pm 168.2)
Species	267 (105.3 \pm 6.8)	221 (111.3 \pm 7.2)	203 (99.3 \pm 11.9)
Genera	36 (22.4 \pm 1.0)	35 (23.5 \pm 1.0)	30 (21.3 \pm 1.7)
Method	Expected number of species (ACE)		
PT	214.9	147.7	194.8
VT	237.8	180.0 ^a	196.6 ^a
ST	252.8 ^a	216.7 ^a	193.2 ^a
OP	102.1	69.0	53.3
TNr	31.3	29.2	18.0
TNp	12.5	14.0 ^a	7.4

Total numbers are followed by the average per study site and standard error; n is the number of study sites. The expected number of species based on the Abundance Based Coverage Estimator (ACE) for the entire study system and the two habitat types separately, based on samples collected by the different methods; variable transect walks (VT), standardized transect walks (ST), pan traps (PT), observation plots (OP), trap nests with reed internodes (TNr), trap nests with paper tubes (TNp). In the analysis, each cross-section sample contained all bees collected with a particular method at a particular site

^aThe estimator Chao 1 is used in stead of the ACE (recommended by EstimateS)

number of individuals caught in each method each round of sampling).

The abundance-based coverage estimator (ACE), based on the species assemblages at each study site, showed that by including a higher number of study sites, and applying sufficient sampling effort in space while keeping the sampling effort constant within each site, the number of species expected to be found was 276 in the olive groves, 246 in the phrygana habitat, and 309 in the entire study system. There was a marked difference in the expected number of species (ACE) in the two habitats and in the entire study system depending on the sampling method applied (Table 1).

Bee species richness in managed olive groves and in phrygana

Similarity of bee species assemblages collected in the two habitats

We found that the similarity in bee species assemblages between sites was generally high with Chao-Sørensen index values in the range of 0.773–1.000. Average similarity was higher when comparing study sites of the same habitat type than when comparing study sites from different habitats types. Highest average similarity was

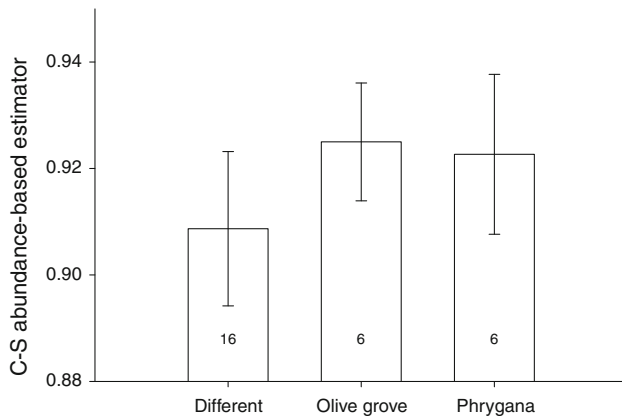


Fig. 1 Similarity (Chao-Sørensen abundance-based estimator) in bee species assemblages among study sites of the same habitat type (olive groves, phrygana) or different habitat types (phrygana compared to olive grove; different). Error bars show SE. Numbers at the column feet show the number of site pairs included in the analysis of the particular site combination

found among olive grove sites (Fig. 1), but the difference in average similarity among the three groups of similarity values (viz. among: sites in different habitats, sites in olive groves, and sites in phrygana) was not statistically significant ($F_{2,25} = 0.36$, $p = 0.70$).

The most common species

Only 17 species were found to be shared by all eight study sites. They included species with relatively low abundance (e.g., *Andrena cinereophila* with a total of 24 individuals in all eight sites; *Eucera punctulata* with 29 individuals; and *Nomada rubricollis* with 32 individuals) and species with the highest abundances recorded in the study. The two most common species were *Apis mellifera* and *Lasioglossum marginatum* with 2,054 and 1,507 individuals, accounting for the 26.7 and 19.6% of the total number of sampled individuals, respectively (Table S3, Supplementary material). The distribution of these two species was clearly partitioned between the two habitat types. *A. mellifera* was more abundant in the phrygana (mean 37.7% of the sampled individuals vs. 16.6% in the olive groves), whereas *L. marginatum* was more abundant in the olive groves (mean 24.7% of the sampled individuals vs. 7.0% in the phrygana). The differences in abundance between habitats were however, not significant (*A. mellifera*: $t_{4.87} = -1.64$, $p = 0.082$, *L. marginatum*: $t_{3.30} = 1.77$, $p = 0.084$).

Unique species in each habitat type

A high proportion (41%) of the bee species collected was found in only one of the habitat types (110 species). Forty-six species were found only in the semi-natural phrygana habitat, while 64 were only in managed olive groves. The number of individuals of the species caught exclusively in one habitat type was small (Table 2). Fifty-eight species occurred as singletons (Table S3). When including only species of which five or more individuals were caught, the number of species caught in one habitat only was reduced to nine (four in olive groves and five in phrygana).

Effectiveness of the different sampling methods

The different sampling methods recorded differing numbers of bee individuals, species, and genera (Table 3;

Table 2 A description of the unique species to a habitat type, i.e., species caught exclusively in olive groves or in phrygana

Habitat type	No. of unique species	Mean no. of individuals of unique species \pm SE	Maximum no. of individuals of unique species	No. of singletons	No. of doubletons
Olive groves	64	1.78 \pm 0.16	7	36	20
Phrygana	46	2.35 \pm 0.27	8	22	8

The number of unique species is given together with the average number of individuals of the unique species; SE is standard error. The maximal number of individuals of a unique species caught is also reported. Singletons and doubletons refer to the number of unique species represented by one or two individuals, respectively

Fig. 3). Pooled over all study sites the variable transect walks (VT) yielded the highest number of species, followed by standardized transect walks (ST) and pan traps (PT). However, the pan traps caught the highest average number of species per site, followed by standardized and variable transect walks (Table 3). Ninety-two bee species, i.e., 34.5% of all species caught in the study, were collected exclusively with one sampling method (Table 3). The three most effective methods (viz. PT, VT, and ST) collected high numbers of unique species, while out of the 23 species collected in trap nests (TN), four (17%) were unique to this sampling method (Table 3). However, including only species of which five or more individuals were caught reduced the number of species caught in one sampling method only to four (three in PT and one in TNr).

Out of the total 267 bee species collected in this study, 112 (42%) were not collected by pan traps. Of these, 66 species were found in variable transect walks and 62 in standardized transect walks. When considering only those species for which five or more individuals were caught, 23 (17%) of the species were not collected in PT while 19 and 20 of these were found in VT and ST, respectively.

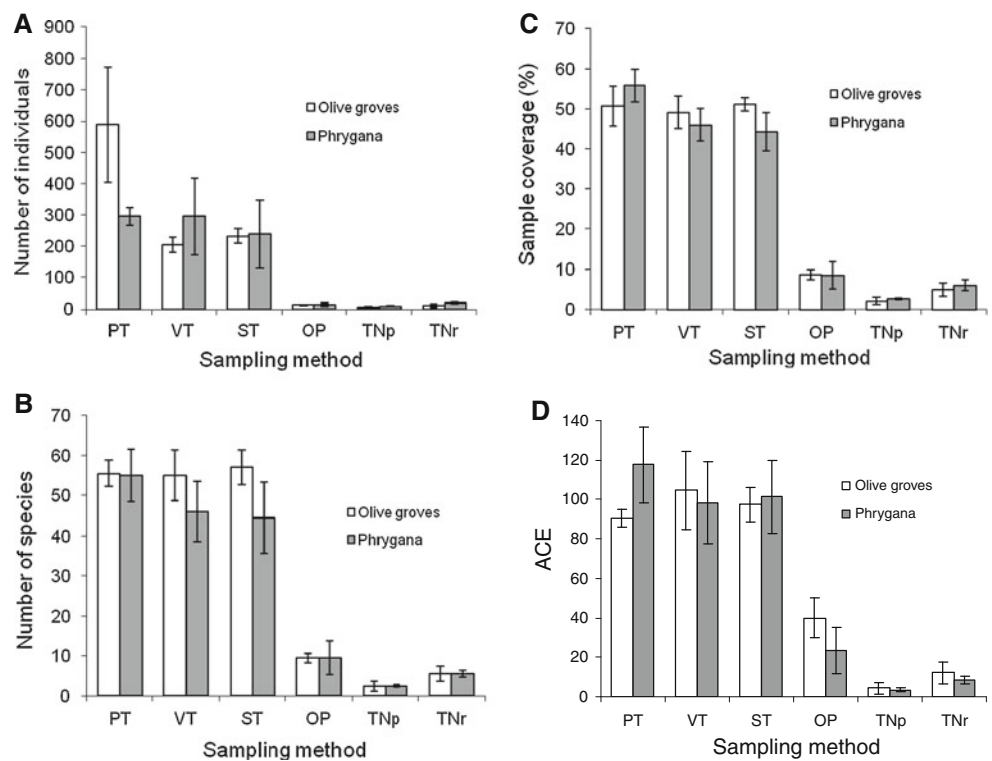
The effectiveness in estimating bee species richness varied among of the sampling methods, but the effectiveness of the methods did not vary between the two habitat types studied (Number of individuals: Method $F_{5,30} = 54.29$, $p < 0.0001$, Habitat $F_{1,6} = 0.20$, $p = 0.67$, Method \times Habitat $F_{5,30} = 1.39$, $p = 0.26$; Number of species: Method $F_{5,30} = 65.88$, $p < 0.0001$, Habitat $F_{1,6} = 0.17$, $p = 0.69$,

Table 3 Description of the bee species richness detected with the different tested methods

	Individuals	Species	Genera	Unique species
VT	1993 (249.1 \pm 60.3)	181 (50.5 \pm 4.9)	32 (17.1 \pm 1.0)	32
ST	1885 (235.6 \pm 51.9)	174 (50.8 \pm 5.1)	32 (16.0 \pm 0.8)	25
PT	3533 (441.3 \pm 102.8)	164 (55.3 \pm 3.4)	27 (14.5 \pm 1.0)	30
OP	103 (12.9 \pm 3.0)	56 (9.5 \pm 2.0)	19 (6.6 \pm 1.3)	2
TNr	125 (15.6 \pm 3.2)	22 (5.5 \pm 1.0)	9 (2.9 \pm 0.5)	3
TNp	58 (7.3 \pm 1.6)	10 (2.5 \pm 0.6)	5 (1.6 \pm 0.4)	0
TN	183 (22.9 \pm 3.4)	23 (6.4 \pm 0.9)	9 (3.4 \pm 0.5)	4
All methods	7697	267	36	92

See legend to Table 1 for abbreviations. TN abbreviate the two types of trap nests combined. Values are: total numbers pooled over all study sites (average number per study site \pm standard error). In all cases $n = 8$ study sites. The “Unique species” column gives the number of species collected exclusively with a particular method

Fig. 2 Effectiveness of the different sampling methods in the study habitats, estimated as the average number of individuals (a) and species (b) caught, the sample coverage (c) and the ACE (d) value. Values are averages over the four study sites in each habitat type \pm SE. Abbreviations are as in Table 3



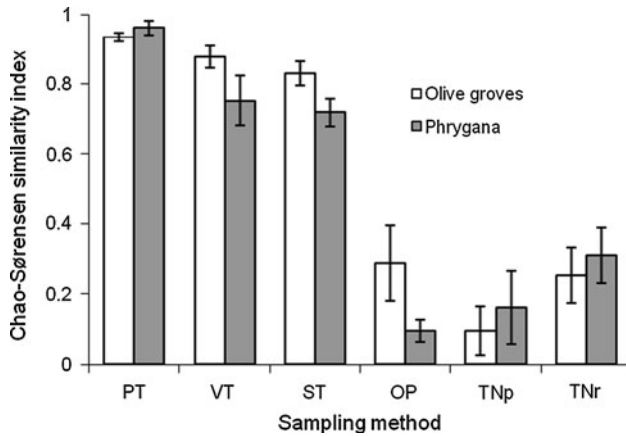


Fig. 3 Similarities (calculated as abundance-based Chao-Sørensen indices) among the bee assemblages sampled with the different methods within this study. Values are averages of all pairwise similarity indices over all four study sites in each habitat type ($n = 6$ similarity indices per method and habitat type; error bars show SE)

Table 4 Similarities, given as Chao-Sørensen abundance-based estimator values, in the bee species assemblages collected with the most efficient sampling methods

Methods compared	Olive groves	Phrygana
Variable transect: standardized transect	0.919 ± 0.037	0.938 ± 0.077
Variable transect: pan traps	0.865 ± 0.053	0.852 ± 0.092
Standardized transect: pan traps	0.869 ± 0.041	0.869 ± 0.103

Average values ± SE are taken from separate calculations from four study sites within each habitat type ($n = 4$ study sites for all comparisons)

Method × Habitat $F_{5,30} = 0.48$, $p = 0.79$; Sample coverage: Method $F_{5,30} = 71.30$, $p < 0.0001$, Habitat $F_{1,6} = 0.10$, $p = 0.77$, Method × Habitat $F_{5,30} = 0.53$, $p = 0.75$; Abundance-based coverage estimator (ACE): Method $F_{5,30} = 42.00$, $p < 0.0001$, Habitat $F_{1,6} = 0.20$, $p = 0.67$, Method × Habitat $F_{5,30} = 1.02$, $p = 0.42$). The three methods PT, VT, and ST caught more species than OP, TNp and TNr (Fig. 2).

Between habitat similarities of samples collected with the same method

Based on the Chao-Sørensen abundance-based estimator we quantified the similarity between all pairs of samples collected with the same method within the four study sites of a habitat type. Using a two-way ANOVA we found that the similarity of samples varied among the different sampling methods but that there was no difference in similarities between the two habitat types (Method $F_{5,60} = 63.11$, $p < 0.0001$, Habitat $F_{1,6} = 1.60$, $p = 0.21$, Method × Habitat $F_{5,60} = 1.45$, $p = 0.22$).

The samples collected with pan traps showed the highest similarity among sites (Fig. 3). Two separate

t tests showed that the average similarity among samples collected with pan traps was significantly higher than among the samples collected by the other two effective methods (VT $t_{12,82} = 3.10$, $p = 0.0086$ and ST $t_{14,38} = 5.41$, $p < 0.001$). The species richness recorded by the least effective methods (OP, TNr, and TNp) were more dissimilar among sites (i.e., showed lower similarity values) than those recorded by the more effective methods (Fig. 3).

Similarities of samples collected with different methods

The highest similarity values were found among the samples collected by VT and ST in both habitat types, indicating that these methods demonstrate low complementarity (Table 4). The similarity values between PT and the two transect methods were lower, though still relatively high. Nestedness analysis revealed that the species assemblages caught with the different methods were highly nested for the entire study system and for the two habitats separately (all $p < 0.01$). However, the order of the methods regarding which methods species assemblage was nested within which varied among the three datasets. For the entire study system species caught in pan traps (PT) represented a subset of the species caught in standardized transects (ST) that in turn represented a subset of the species caught in variable transects (VT). In olive groves, PT was a subset of VT that in turn was a subset of ST and in phrygana ST was a subset of VT that in turn was a subset of PT.

Correlations between different sampling methods and total species richness

The number of species collected by VT, ST, and OP were positively correlated with the total number of species collected in the same study sites (Table 5); while the passive sampling methods (PT and TN) were not. Due to the low number of replicates (four study sites in each habitat type), analysis at the level of each habitat type was not possible.

Sampling effort needed by each method to detect 50% of the total species richness

We used subunit-based rarefaction curves to identify the sampling effort (number of cross section samples) needed to detect an arbitrary selected proportion (50% sample coverage) of the expected number of species estimated for a site (Westphal et al. 2008). Sample coverage was calculated as the number of expected species (Mao Tau), based on the cross section samples from a given method, divided by the total number of species detected by all methods combined at a site. In both habitat types the PT reached 50% sample coverage within the sampling effort applied (Fig. 4). It seems, however, that the full extent of sampling effort applied

was necessary in order to achieve 50% sample coverage [including all five pan trap clusters as cross sections; for olive groves 51.0%, for phrygana 56.2% (Fig. 4)]. In the olive groves the ST achieved higher sample coverage than the VT, while both methods achieved approximately the same coverage in the phrygana habitat (Fig. 4). Sample coverage of 51.2% was reached after 50 min of ST in the olive grove habitat. The VT and the

ST in the phrygana habitat did not reach 50% sample coverage within the sampling effort applied. The rarefaction curves for PT and the two transect methods were quite steep in both habitats and showed no sign of leveling off within the sampling effort applied. By extrapolating the rarefaction curves in Fig. 4, it appears that the transect methods would have reached 50% sample coverage with the addition of a minor increase in sampling effort: one extra 30-min variable transect in the phrygana habitat; two extra variable transects in the olive groves; and three extra 5-min cross sections of standardized transect walk in the phrygana. OP reached only a low sample coverage within the sampling effort applied (8.5% in the olive groves, and 6.8% in the phrygana habitat) and the TN revealed even lower sample coverage (< 6%) (Fig. 4). The rarefaction curves for OP and TN were less steep than those of the three other methods. Despite this, OP and TN increased towards the full extent of the sampling effort applied. However, to reach sample coverage of 50% the sampling effort would need to be increased greatly. Note that the sampling effort tested varies substantially among the

Table 5 Correlations between the number of bee species collected by a sampling method versus the total number of species sampled in the same study site using all methods ($n = 8$ study sites)

Method	R	t_6	p value
Variable transect (VT)	0.8539	4.0192	0.0070
Standardized transect (ST)	0.8952	4.9225	0.0027
Observation plots (OP)	0.8054	3.3282	0.0158
Pan traps (PT)	0.4822	1.3482	0.2263
Trap nests paper (TNp)	0.4578	1.2613	0.2540
Trap nests reed (TNr)	-0.1370	-0.3387	0.7464

Pearson's product moment correlation coefficients (R) are given as the measure of correlation for all sampling methods explored

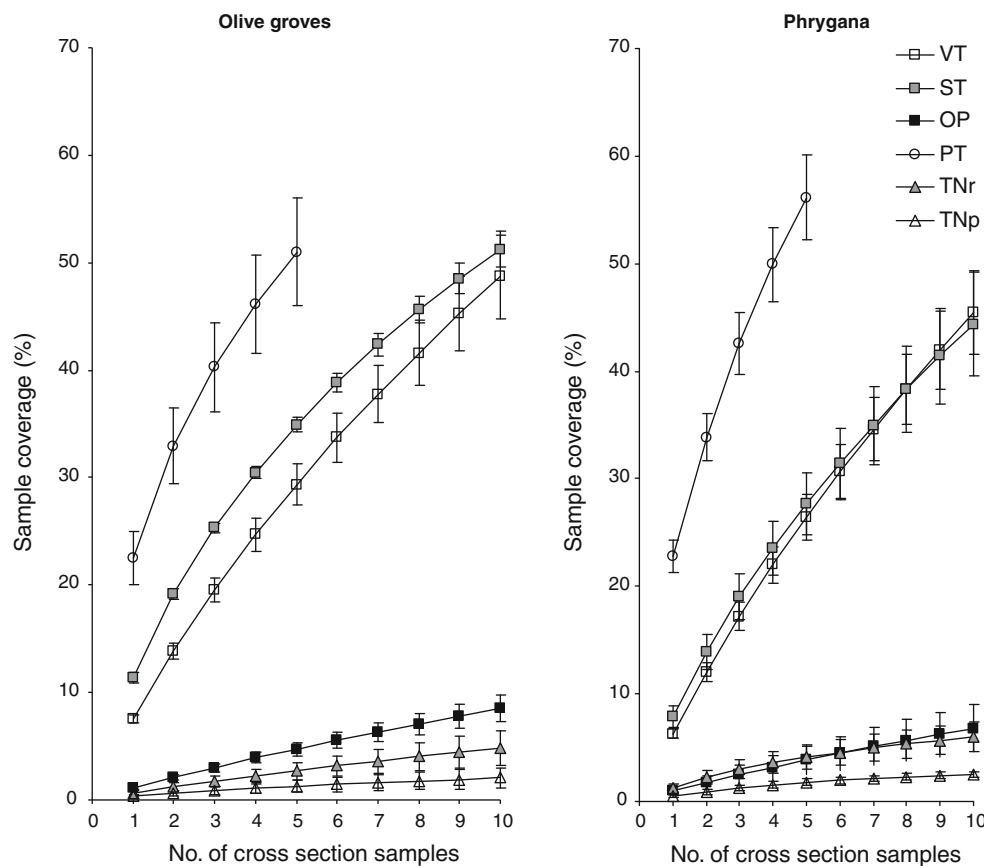


Fig. 4 Subunit-based rarefaction curves for the six different sampling methods employed in the managed olive groves ($n = 4$ sites) and in the phrygana ($n = 4$ sites). The sample coverage for an increasing number of rarefied cross-section samples is given. Sample coverage is calculated for each method as the expected number of species (Mao Tau) divided by the total number of species recorded by all methods within a site multiplied by 100.

Values are means over four study sites \pm SE. Each cross-section sample represents the cumulative species numbers found during all surveys within a particular subunit, i.e., specific observation plot, pan trap cluster, 5-min intervals of a transect walk. The rarefaction curves for variable transects are based on entire 30-min samples, as the variable transects could not be divided into subunits

methods and must be accounted for in the interpretation of the results. For example, PT cross-section sample consisted of three colored bowls, left out for 48 h. Each OP cross-section sample is based on 60-min (10×6 min) observations of a 1×2 observation plot. Each VT cross section is based on 30-min variable transect surveys conducted in a 1-ha area, while each cross section of a ST is based on 50-min (10×5 min) surveying a 100 m² area. This might explain why the ST walks seem to provide higher sample coverage at a given number of cross-section samples, as compared to VT.

Collector biases associated with the two types of transect walks (ST and VT)

There were differences among the field workers as to their effectiveness in sampling bees. For the ST, the average number of species collected during the 50-min sampling varied from 3.6 to 13.4 and the number of individuals from 18.2 to 29.1 for the four bee collectors involved. For the VT the average number of species collected during 30-min varied between 4.8 and 12.7, whereas the number of individuals varied from 12.0 to 68.5 among the five bee collectors involved in the study. Two two-way ANOVAs showed significant differences among the collectors in how many species and individuals they collected per transect walk, but that there was no difference in the number of individuals caught between the two types of transect walks (Number of species: Field worker $F_{4,152} = 9.66$, $p < 0.0001$, Type of transect walk $F_{1,5} = 2.18$, $p = 0.14$, Field worker \times Type of transect walk $F_{4,152} = 3.45$, $p = 0.0099$; Number of individuals: Field worker $F_{4,152} = 5.51$, $p = 0.0004$, Type of transect walk $F_{1,5} = 2.78$, $p = 0.098$, Field worker \times Type of transect walk $F_{4,152} = 2.99$, $p = 0.020$). Significant interaction terms indicated that the number of species and individuals collected by each field worker depended on the type of transect walk, but not in a consistent way.

Discussion

Bee species richness in Mediterranean habitats

The sampling of two Mediterranean habitat types conducted at eight study sites revealed a total of 267 bee species. Of the bees collected, 11 species have not previously been described (Table S3). The species-richness in managed olive groves (221 bee species) was similar to that in semi-natural phrygana (203 species), a pattern previously found by Potts et al. 2006 in the same habitats on the island. Our results confirm the importance of the Mediterranean region as a hotspot for bee diversity (Michener 2007). In a similar study carried out in eight habitat types (32 study sites) spread over five European countries, and using the same methodology, Westphal et al. (2008) found a total of 278 bee species with most

Table 6 Bee species richness in different habitats and regions sampled with similar methods and intensity

Habitat type	Country	No. of bee species
Mediterranean		
Phrygana (scrub)	Greece	203
Traditionally managed olive groves	Greece	221
Temperate grasslands		
Semi-dry pasture	Sweden	73
Wet meadow	Poland	99
Chalk grassland	UK	70
Calcareous grassland	Germany	122
Agricultural lands		
Buckwheat	Poland	46
Oilseed rape	UK	26
Oilseed rape	Germany	27
Cantaloupe	France	104

The Mediterranean habitats are from this study, the rest are from Westphal et al. (2008)

species caught in calcareous grassland in Germany (122 bee species), whereas agricultural fields comprised a much poorer bee fauna (Table 6). The latter finding was not surprising given the comparatively lower floral diversity and general lack of nesting sites in the agricultural fields (annual monocultures of buckwheat, oilseed rape, and cantaloupe) and the reduced sampling period due to the limited flowering season of these monocultures. Thus, our olive groves, which contain a wide variety of plants flowering throughout the season, are comparable to the semi-natural habitats of Westphal et al. (2008); this is reflected in their comparable bee diversities. The bee species richness in the agricultural fields of Avignon (France), at the margins of the Mediterranean garrigue, was also found to be more diverse compared to crops from more northern European regions (Table 6; Westphal et al. 2008).

Bee species richness in managed olive groves and phrygana

While they found a lower absolute bee species richness per site compared to this study, Potts et al. (2006) found significantly higher bee species richness in managed olive groves than in newly burned forest habitats (i.e., phrygana). There are probably two main reasons why bee species richness in managed olive groves is similar, or even higher, than in semi-natural phrygana habitats. First, traditionally managed olive groves, such as those included in this study, contain a high diversity of wild flower resources compared to other managed agricultural habitats (e.g., Westphal et al. 2008). This is because the management of olive groves, mainly through light plowing applied occasionally (i.e., once every 2–3 years), facilitates the growth of annual flowering plants, thus keeping the grove floor open. Indeed, previous studies have shown that abandoned olive groves, where

management was no longer applied, contained lower bee species richness than managed olive groves, probably due to the lower amount and diversity of floral resources they contained (Potts et al. 2003b, 2006). Second, habitat quality for bee communities also depends on the availability of nesting resources such as nesting sites and nest building material (Potts et al. 2005). The availability of bare ground after plowing, together with the existence of rocky crevices, stone fences, stonewall supported terraces for agricultural use and other manmade structures make the olive groves a heterogeneous habitat containing a high diversity of potential bee nesting sites (Petanidou and Ellis 1996; Potts et al. 2005). Potts et al. (2005) showed that the availability of nesting resources, in particular the amount of bare ground and suitable nesting cavities, were important factors in explaining variations in bee species richness.

Bee species associated with olive groves and phrygana

Of all the bee species collected, 41% were unique to one habitat type only (64 species in olive groves and 46 species in phrygana out of a total of 267 species). However, the number of individuals of these unique species was low (e.g., 58 singletons and 28 doubletons; Table S3) and only nine species including more than five individuals were found in one habitat only. This suggests that the rareness of the species may be the reason for them to be found exclusively in one of the habitats. Though we cannot rule out a specificity of certain groups of bees for a particular habitat, especially considering that the two most frequently collected species in our study showed contrasting dominance in the two habitat types. Honeybees were more frequently caught in the phrygana than in olive grove habitats, though the difference only tended towards significance. This is in accordance with the ancient observations by Plato that phrygana habitats “only afford sustenance to bees” (meaning “honeybees”; Critias by Plato, written 360 B.C.; <http://classics.mit.edu/Plato/critias.html>). We believe that the higher density of honeybees observed in our semi-natural habitat to be driven by two factors: First, the open nature of the Phrygana habitats might facilitate the attractiveness of flowers as they can be more easily detected by the honeybees. Mass-flowering as seen in certain entomophilous crops (e.g., Westphal et al. 2008) is absent in olive groves. However, certain plant species temporarily dominate the floral resource pool in the phrygana (e.g., *Asphodelus*, *Thymus* and *Cistus* spp.) thereby attracting numerous honeybee individuals (Petanidou; personal observation). Second, though we do not hold data on bee keeping in the landscapes surrounding the study sites, we acknowledge that proximity to beekeepers must be an important factor explaining variations in local abundance of honeybees. We know that beekeepers try to locate their hives in open and flower-rich habitats such as phrygana and avoid doing so in relatively risky areas such as olive

groves (due to insecticide spaying against *Dacus oleae*; Petanidou, personal observations).

The second most common species (*Lasioglossum marginatum*) were more frequently caught in olive groves, though the difference was only marginally significant. The higher abundance of these bees in olive groves might be due to the availability of bare ground, which can be a limiting resource for this ground-nesting species (Potts et al. 2005). The plowing of the olive groves is done in spring and generates large amounts of bare soil, an important nesting resource for *Lasioglossum* bees (Potts et al. 2005). As plowing is done at most biannually, *L. marginatum* should also benefit from this management regime even though the queens can survive in the nest for several years (Packer 1991).

Efficient methods for assessing bee species richness in the Mediterranean

Westphal et al. (2008) suggest that pan traps are the single most efficient method for estimating bee species richness in long-term and large-scale monitoring programs based on their effectiveness and low sampling bias. Our results show that this is also the case in species-rich communities such as the Mediterranean. Pan traps were the only method that reached, on average, 50% sample coverage per study site for our two habitats. This is, however, much lower than the >80% sample coverage found across the European regions included in the study of Westphal et al. (2008). When pooling data collected over all eight study sites by each of the different methods, we found that the numbers of species caught by both variable and standardized transect walks were higher than in pan traps. As a consequence, bee species collections by pan traps were more similar among sites than the collections sampled by transect walks. This suggests that pan traps collect a more or less consistent fraction of the bee fauna, regardless of the spatial heterogeneity in species composition among sites. As a result, the spatial variation in bee species composition between sites and communities may not be revealed when using only pan traps as a sampling method. The above conclusion is also supported by the finding that the expected number of species found if sampling a sufficient number of study sites was higher for the two types of transects than for pan traps (Table 1). Nestedness analysis revealed that the species assemblages collected with the different methods were highly significantly nested. However, the ordering of the methods as to the number of species caught varied depending on the dataset analyzed, i.e., what method-specific species assemblages were nested within which others. This further highlights the importance of including more than one method when conducting bee species inventories.

A high number of species were not collected by pan traps. One reason for this might be the insufficiency of our arbitrary choice of five pan trap clusters as our full

sampling effort with this method. Also, the reduced number of sampling rounds conducted (six as compared to ten for the other methods) might have an effect, though we expect this to be less important, as the rounds were distributed throughout the full extent of the season and lasted 48 h each round. Indeed, the steepness of the curves in Fig. 4, illustrating the relationship between sampling effort and sample coverage, indicate that the sample coverage of pan traps would have increased dramatically if higher sampling effort had been applied, i.e., using a larger number of pan trap clusters. However, pan traps collected, on average, the highest number bee species and the species composition of the pan trap samples were similar to the samples that were collected during transect walks. We did not however, find any correlation between the bee species richness collected in pan traps and the total sampled bee species richness at a site. A closer inspection of the data revealed that the lack of correlation was mainly due to the relationship between the total bee species richness and the richness collected in pan traps at one particular site. At this site the total bee species richness was highest (132 species) while the bee species collected in pan traps was the second lowest (48). The low number of samples (8) makes the correlation analysis very sensitive to outliers and consequently, removing this site from the analyses resulted in a significant positive correlation ($r = 0.788$, $p = 0.035$, $n = 7$).

There was a significant difference in the number of species and individuals caught by different bee collectors during standardized transect walks, but there was no significant difference between the two types of transect walks. However, a significant interaction term in the models indicates that the difference in performance of the bee collectors was not consistent between the two methods i.e., that the collectors catching the highest number of species in variable transects did not catch the highest numbers in standardized transects. This is somewhat surprising as we would expect the variable transects to be more prone to bias related to the skills of the bee collectors, while the standardized transects would show less difference in the number of individuals and species caught among the collectors. This shows that the effectiveness of transect walks is highly dependent on the person doing the bee collecting. Indeed, the experience of the field workers involved in this study was highly variable. Training of field workers to optimize their effectiveness and to reduce collector-related sampling bias is therefore of utmost importance if census methods are to be included in monitoring schemes (Westphal et al. 2008).

We found that the observation plots inadequately sampled the overall species richness, and the trap nests proved to be the least efficient method. In total 23 species were collected in trap nests, while only four species (1.5% of the total bee species richness) were collected exclusively with reed internode and paper tube trap nests. The sample coverage of trap nests reached low values compared to the most efficient methods (pan

traps and transect walks). This is not surprising as this method only collects cavity nesting species, a group usually representing only a small fraction of the entire bee fauna. Despite this limitation, trap nests can be a good and unbiased indicator for assessing relative changes in bee species richness of this particular guild. Observation plots collected 56 species in total, but only two unique species (0.75% of the total bee species richness). The full sampling effort of observation plots included 10 cross sections covering 2 m² each. Observing all flower-visiting bees in flower rich communities is difficult and the probability of not being able to observe all visits is therefore high. Increasing the area covered by each observation plot would therefore most probably not detect more species unless additional bee collectors are involved in each observation. We therefore suggest increasing the number of observations and/or the time spent during each observation to improve the estimates of bee species richness given by the observation plots. Trap nests and observation plots showed no correlation with the total bee species richness and had low sample coverage. These results are in accordance with Westphal et al. (2008) and suggest a limited value of these methods as bee diversity indicators in large-scale survey or monitoring programs especially in species-rich communities such as the Mediterranean. However, observation plots were the only method able to generate quantitative plant–pollinator networks and should be used if this is the aim of the study. The limited number of bee species caught with this particular method as well as the sampling biases associated with it suggests however, that interpretations should be done with caution.

The sample coverage associated with the tested methods (rarefaction curves) reached much lower values in our study than in Westphal et al. (2008) within the sampling effort applied. This shows that even the most effective methods, i.e., pan traps, variable and standardized transect walks, have a lower absolute effectiveness when applied in bee-rich Mediterranean landscapes. Consequently, when monitoring species-rich habitats, a much higher sampling effort is needed in order to reach the same relative level of coverage as in northern and central European grasslands.

We agree with Westphal et al. (2008), that pan traps are the most efficient and unbiased method for sampling bee species richness, even in areas of high bee species diversity. However, the sample coverage reported for pan traps in our study suggest that to obtain a more reliable estimate of the relative bee species richness at a site, comparable to those found in Westphal et al. (2008), requires at least ten pan trap clusters. The high number of species not caught in pan traps, and the contrasting biases associated with the different methods, suggest that if resources allow, transect walks should be included in survey and monitoring programs aiming at detecting the total bee species richness in species-rich communities such as the Mediterranean. The value of proper training of bee collectors as well as the importance of skilled bee taxonomists to determine the

identity of the numerous specimens is critical, especially in areas with large, diverse and poorly studied bee communities.

Conclusions

Mediterranean communities, here represented by the Greek island of Lesvos, have high bee species richness as compared to other biogeographic regions. We found high bee species richness in both managed olive groves and in semi-natural phrygana habitats. Pan traps proved to be the single best method for sampling bee species in both olive groves and phrygana. However, the high number of species not found in pan traps suggests that other methods, in particular transect walks, should be included in bee surveys and monitoring programs, if the aim is to detect as many species as possible. This may not only be true for the Mediterranean, but also other communities with high bee species richness.

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