



Review

Pollination in Agroecosystems: A Review of the Conceptual Framework with a View to Sound Monitoring

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Abstract: The pollination ecology in agroecosystems tackles a landscape in which plants and pollinators need to adjust, or be adjusted, to human intervention. A valid, widely applied approach is to regard pollination as a link between specific plants and their pollinators. However, recent evidence has added landscape features for a wider ecological perspective. Are we going in the right direction? Are existing methods providing pollinator monitoring tools suitable for understanding agroecosystems? In Italy, we needed to address these questions to respond to government pressure to implement pollinator monitoring in agroecosystems. We therefore surveyed the literature, grouped methods and findings, and evaluated approaches. We selected studies that may contain directions and tools directly linked to pollinators and agroecosystems. Our analysis revealed four main paths that must come together at some point: (i) the research question perspective, (ii) the advances of landscape analysis, (iii) the role of vegetation, and (iv) the gaps in our knowledge of pollinators taxonomy and behavior. An important conclusion is that the pollinator scale is alarmingly disregarded. Debate continues about what features to include in pollinator monitoring and the appropriate level of detail: we suggest that the pollinator scale should be the main driver.

Keywords: bees; pollinators; vegetation; agroecosystems; landscape analysis



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1. Introduction

The goal of this outline is to summarize the methods currently used in solving research questions related to pollinators and pollination services, with a special focus on agricultural landscapes and the Mediterranean region. Our interest in the topic arises from an awareness of gaps in pollinator monitoring in our country, Italy, and the pressing need to define a reproducible procedure to solve a set of difficulties. We anticipate problems with the following aspects: geomorphological complexity ranging from plains to hills to mountains, rocky shores, sandy beaches, and islands (Sicily and Sardinia) with their intrinsic variability; the variety of cultivations and practices of plain, hill and mountain farms that form a gradient from intensive to traditional agriculture; the Mediterranean hotspot of plant and pollinator biodiversity; and political organization, with a central government that assigns agricultural management and subsidies to the regions (i.e., dispersed recipient stakeholders with different priorities).

The Mediterranean region is a heterogeneous macro-region, and the seaboard countries are in a very special situation. Geographically, these countries share the influence of a climate mitigated by the sea, which favors biological diversification (e.g., many endemic plant species [1]). Economically, they rely mainly on agriculture consisting of farms that have remained relatively small [2]. Traditionally, Mediterranean countries have developed in the context of a complex and variable environment that has given rise to outstanding and appreciated high nature value farmlands [3] and the Mediterranean diet [4]. Consequently, any conservation action devoted to sustaining this peculiar environment will

have remarkable spillovers across different areas, including human health and economic stability. Unfortunately, pollinators are not yet a top priority, despite their high diversity in the Mediterranean (especially bees [5]), and our dependence on them for the yield of most European cultivated species [6,7]. Moreover, a well-accepted landscape-wide sampling methodology for studies on pollinators is still lacking, even if recent efforts have endeavored to boost the validity of grid-based sampling for field data collection [8]. When addressing the benefits of pollination for yield, a fact often overlooked is that pollination is intrinsically linked to plant physiology and may, therefore, change in relation to resource availability [9]. Estimation of the spatial distribution of some important ecological features across a landscape is also controversial, with attempts to integrate spatial resolutions from existing databases [10]. Evidence suggests that local policies and agricultural practices may be the drivers of stable crop production at a local scale [11].

Here, we present some examples illustrating the main approaches emerging from a wide survey of the leading international literature on pollination ecology. These approaches can be considered independently in the context of similar situations and jointly to gain a wider perspective.

2. Methods

Our literature search was conducted in two main steps. First, we conducted systematic searches of relevant keywords and phrases with and without Boolean operators. The main search terms were “monitoring”, “pollinator”, “bees”, “wild bees”, “landscape”, “agriculture”, “transect”, “plot”. We searched three library databases—Web of Science, Mendeley, and Google Scholar—for articles, reviews, and books, skipping citations. Although this may not identify all relevant publications, it selects key publications in which authors have prioritized these terms as core to their content.

Secondly, for selected papers especially relevant to our study, we also surveyed all articles citing these papers. After reviewing the content of several, it was clear that, in many cases, they only contained marginal information related to our topics. These papers were, therefore, discarded. We finally organized the information of the remaining papers into four main approaches based on research questions, landscape ecology, vegetation and pollinators. Of the 147 documents originally screened, we chose those most likely to shed light on our main concern: how to tackle the monitoring of pollinators in a Mediterranean country. Less than 15% of the papers selected were published before 2010: we did not intentionally skip older papers, but we only found multidisciplinary approaches suitable for our purposes, including plants, pollinators and landscape ecology, in the more recent literature.

3. Leading Role of the Research Question Perspective

The perspective is like a wave gathering and mingling information from a complex situation. It is fundamental to sort out why data are gathered in a certain way and to what extent they can be generalized. When considering bees (Figure 1, starting on the left side), we need to bear in mind that they have two main requirements. The first is a rich foraging area. The foraging area is characterized by the variety of food sources in it, which also vary in availability and distance from the nest. There are selective pressures on these food sources, such as farming practices and cultivars but also which bee population is foraging on them at any given moment. The second important requirement for bees is a suitable nesting area. The characteristics of this area may vary from bare soil to trees and need to cope with parasite pressure and compositional complexity. These two requirements (foraging and nesting areas) may not overlap but need to be at a close distance: a healthy bee population cannot survive in the absence of either. Inversely, when considering crop pollination (Figure 1, starting on the right side) pollination is addressed from a landscape perspective, where soil use and field margins are important. In agricultural landscapes, managed pollinators (honeybees, bumblebees and *Osmia* spp.) are frequently used and can

upset competition and the survival of local wild bee populations. Interest in the importance of wild bee population availability has only recently begun to increase.



Figure 1. Research perspectives. Opposite but complementary perspectives of research topics addressing pollination in agroecosystems (Section 3). Although the waves of factors may proceed by different paths (green and orange in the figure), the factors themselves are part of a more general context and are placed according to the perspective adopted in a given study.

Scientific studies usually have a research question pertaining to which different approaches are possible. The data that we collect depend on the research perspective: interest in crop pollination is not the same as interest in bee pollination. Whatever the perspective, we may need the same kind of data to answer our research question, e.g., the abundance of foraging insects, or farming practices, as shown by the arrows in Figure 1. Mayer et al. [12] organized research questions in the field of pollination ecology according to 14 subtopics, highlighting the scientific gaps to be filled. We discovered that even when the same topic is tackled (i.e., the importance of pollination as an ecosystem service for crops and orchards), opposite workflows are employed. Whatever the direction of the research question, there is clearly an overall contribution of information to a larger, comprehensive perspective: the complexity of plant–pollinator relationships in a (mostly agricultural) landscape. Whatever the initial perspective of a given study, gathering information on pollinators has to combine all findings. Future research should endeavor to assemble the results.

3.1. Investigating Pollination by Bees

Papers resulting from the search “pollination BY bees” focus on the abundance and diversity of pollinators, depending on the crops and orchards present, or the reproductive needs of a given bee species. The solitary polylectic species, *Osmia bicornis* L., was the target of the study of Holzschuh et al. [13] who studied oilseed rape fields to evaluate their influence on the abundance of this single bee species. The study used two landscape

scales with different distances to semi-natural ecosystems, fundamental as nesting sites for the bee. The presence of semi-natural habitats (nesting options) and landscape scales including agroecosystems (foraging options) are often drivers in studies addressing the distribution of bees, whether individual species [14–16] or pollinator assemblages [17,18]. In Holzschuh et al. [13], oilseed rape was the foraging unit of *Osmia bicornis*, and the authors distinguished mass-flowering crop fields on the basis of their proximity to semi-natural landscapes (calcareous grasslands) but not on the intrinsic characteristics of individual plants in the foraging or nesting area. Using the functional diversity of pollinators as a tool, Grass et al. [19] investigated the influence of two main drivers on plant reproductive output and the stability of plant–pollinator communities in Africa: loss of natural habitat and exotic plant invasions. Bee functional diversity was also considered in investigating wild bees as vicariants of honeybees in the pollination of 20 apple orchards in Canada [20]. The authors identified useful wild bee pollinators and discussed their needs in terms of landscape composition and configuration.

3.2. Investigating Pollination of Crops/Orchards

From the perspective of “pollination OF crops and orchards”, some colleagues investigated pollination results on the basis of the complexity (or absence) of a guild of pollinators. Other colleagues focused on the pollination service to crop fields and how it is impacted by landscape variability [21]. In a broad meta-analysis, Woodcock et al. [22] evaluated the relative importance of pollinator abundance and functional divergence for oilseed crop pollination, finding it in favor of the former. As expected, differences in farming practices influenced pollinators as well as the resulting ecosystem service. The results of Nicholson et al. [23] highlighted the simultaneous influence of local management and landscape pattern on the visitation rate, abundance, and species richness of native pollinators of blueberry. Tillage is another practice that may impact wild pollinators: untilled squash and pumpkin cultivations showed greater density of local, wild pollinators in the U.S.A. [24], while tillage influenced eusocial versus solitary wild bees differently in Austrian vineyards [25]. Steffan-Dewenter et al. [18], following the existing literature, split habitat into macro-categories (e.g., arable land; fallow fields and ruderal areas, including field margins; hedgerows, forest; rock habitats and vegetation along inshore waters). However, no precise plant species were indicated: plant assemblages were assumed to characterize given habitats, indicating that differences in pollinator diversity and abundance can be expected.

3.3. Integrating Pollinators and Plants

The evolutionary history of plant–animal interactions shows a complex two-way path in which the development of functional features allows each player to better exploit its counterpart. Clough et al. [26] conducted a large meta-analysis in which they considered plants on the basis of species-specific traits, those related to their pollination syndrome and those responding to land-use change. If the scale of the study is a single plant species, the flower functional diversity as well as conspecific and heterospecific flower densities may be included in the research questions [27]. Flower–visitor interaction webs were investigated in 27 meadows with varying flower diversities in southern Germany [28]. The authors found overall flexibility in flower preferences in the different meadows and dynamic resource partitioning among pollinators, including considerable specialization and complementarity between species. Increasing plant functional diversity has been considered a way to support pollinator abundance and diversity by enriching agricultural landscape contexts with flower strips. However, limits have been detected in the role of plant functional diversity at different scales [29].

Summing up the last three subsections, the importance of the research question in leading data gathering is illustrated by the above examples. Our results underline that studying bees implies investigating their environment, which is directly linked to bee biology. This is confirmed in the study of individual bee species as well as bee functional groups. Likewise, the study of crop pollination needs to include variables directly linked

to the crops themselves, indirectly influencing the pollination service carried out at each site. Both kinds of studies underline the importance of a local perspective in relation to the landscape in which the species live. We also need to bear in mind the evolutionary paths of plant–pollinator interactions, which may fill gaps and/or settle misunderstandings in currently observed pollination events.

4. Addressing the Increasing Power of Landscape Analysis

The manner of describing a site has certainly become less intuitive with the progress of landscape ecology, especially in studies related to pollinators and agroecosystems. In an ideal study, the choice of meaningful and appropriate spatial units is predefined and logical. However, knowledge gaps, field data collection, and administrative–private boundaries may make the perfect choice difficult. We grouped spatial unit categorization under three definitions, which appear in different studies with different levels of descriptive detail (Figure 2). The first spatial unit is that of the study area (SA). The SA describes where the site of the study is on a very large scale and gives a prompt picture of the geographic and/or ecological landscape in question. SA descriptions often include climatic/edaphic parameters. A more detailed description can be obtained with specific spatial units (SSUs), i.e., the field site(s) where the actual sampling is done. The SSU has various components and possible links between them, as explained below. Finally, sometimes a posteriori analyses imply the use of different advanced spatial units (ASUs). These may be enclosed or somehow related to the SSU and its components. ASUs usually extend from SSUs in different directions at increasing distances. This is the spatial level most closely linked to the landscape ecology analysis. Figure 2 summarizes the complexity of the landscape analysis that emerged from our search. It includes some of the examples that are explained in more detail in the following sections.

4.1. Study Area [SA] and Specific Spatial Unit [SSU]

The concept of the study area (SA) may be as wide as a continent, a region, a valley, or a city, with or without its surroundings. The concept of the specific spatial unit (SSU) may involve various components and/or their mutual relationships. In the meta-analysis of Clough et al. [26], regarding the abundance and richness of insect-pollinated grassland plants, the SSUs were semi-natural grasslands and the SAs containing them were five European countries, i.e., part of a continent. In Brittain et al. [14], a study of pollination services by wind and insects, the study area was two counties of northern California and the SSUs were 23 almond orchards where the authors selected field sites (individual trees). In Fortel et al. [30], a study of pollinator communities and urbanization, the study area was the urban community of Grand Lyon (516 km²) where three SSUs were established on the basis of an impervious surface gradient (<30%, 30–70%, >70%), each with eight replicates.

Specific spatial units often coincide with a specific environment. Hopfenmüller et al. [31] focused on the calcareous grasslands of Upper Franconia. Their SSUs were 23 grasslands in which the plots (the field sites) contained attractive nesting sites and food sources, and were spaced more than 1 km apart to avoid overlap of bee communities. However, an SSU could also include more than one defined landscape element/habitat. Morandin et al. [32] tested the edges of tomato fields in California: managed under restoration programs, and unmanaged with perennial weeds. Their SSUs combined matched managed and unmanaged field sites, surrounded by at least 85% intensively managed crops in a 1500 m radius, and the two field sites were 1–3 km apart so as not to sample overlapping pollinator communities. The links or relationships between different field sites in a study are an important topic. Boundaries of SSUs need to be clearly defined, since they are not supposed to be crossed by the pollinator population investigated. Boundary size can nevertheless vary widely: Franzén et al. [33] considered entire islands as SSUs with their boundaries clearly defined by the sea. Otherwise, the most frequent distance is related to pollinator flying ability. Riedinger et al. [34] considered a 2 km radius to investigate the succession of early–late flowering crops in the sustenance of local pollinators. The same

distance was assumed in a study investigating green areas in urban contexts [30], and in another focusing on semi-natural grasslands [35].

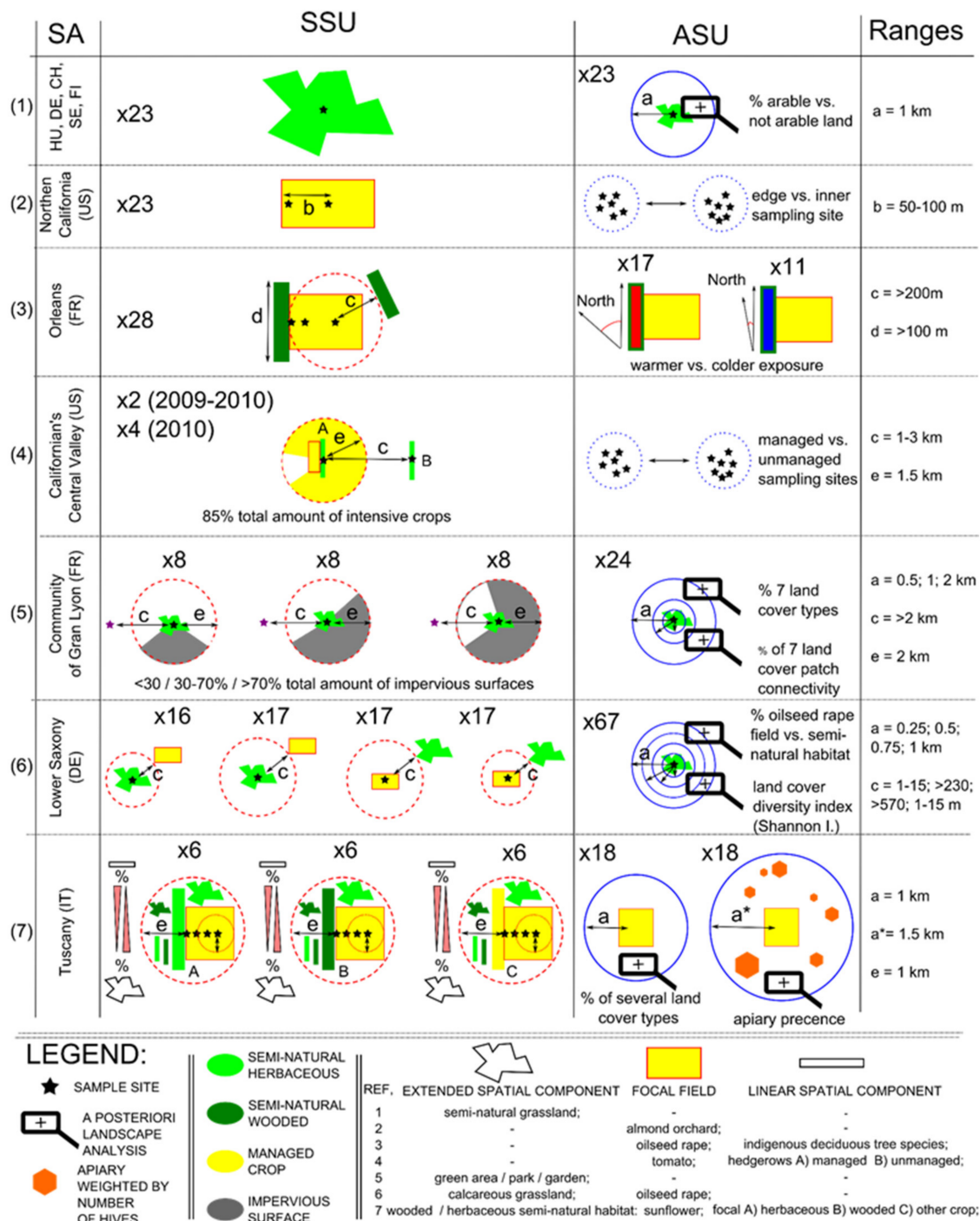


Figure 2. The landscape ecology approach. Innovation in landscape ecology enables generalization and introduces new definitions for methodological approaches (Section 4). We grouped them according to three spatial units: the study area (SA), the specific spatial unit (SSU), the advanced spatial unit (ASU). Level of detail can vary but can be resumed and compared according to spatial context. The examples were inspired by (1) [26]; (2) [14]; (3) [21]; (4) [32]; (5) [30]; (6) [13]; (7) [36].

In studies concerning agricultural landscapes, the SSU is often a complex environment made up of crop fields and semi-natural habitats. Bailey et al. [21] defined a SSU of oilseed rape fields adjacent to forests at least 100 m in length. Field sites were selected in oilseed

rape fields at increasing distances from the adjacent forest edge, and at least 200 m distant from other nearby forests. Thus, the SSUs contained specific components (oilseed rape field and forest) and their components had a certain spatial setting. Similarly, Holzschuh et al. [37] defined SSUs as grassland patches at a certain distance (1–15 m to 230–570 m) from oilseed rape fields with a minimum distance of 1 km between SSUs.

4.2. Advanced Spatial Units [ASUs]

The boundaries of ASUs mirror geographic, administrative or ecological/functional units. Some studies base them on a single parameter, such as a spatial gradient: inside or outside orchards [14], or cold/northern and warm/southern edge orientation [21]. Especially in agricultural contexts, ASU variability is considered to play a major role in insect-pollination performance. Senapathi et al. [38] considered radii of 5 and 10 km as the background landscape. Due to pollinator species-specificity, more than one distance can be considered: three ASUs with increasing radii of 500 m, 1000 m and 2000 m centered in field sites to investigate the urban gradient [30], or radii of 250, 500, 750, 1000 m [37]. However, defining boundaries based on objective landscape descriptors is not an easy task. A common landscape descriptive source for Europe is the Corine Land Cover inventory, which can be a good starting point, but has the flaw (when considering pollinator scale) of low, 25 ha, resolution. Nevertheless, it is useful to spot landscape simplification or agricultural intensification affecting the pollinator community. Clough et al. [26] used Corine Land Cover to obtain proxies for agricultural land-use intensity. To counterbalance the lack of power when dealing with pollinators, better spatial resolution may be obtained by ortho-rectified digital aerial photos as found elsewhere [31,37]. In other cases, new spatial categories can be defined to better match the research question, such as historical land cover classifications [38], or scores based on foraging and nesting potential for pollinators [39].

4.3. Predictive Power

Studies that lack the field data collection step but take an opposite path, endeavoring to predict pollinator presence through spatial models, are a special case. They mostly rely on previous findings, which are integrated into a newly developed matrix. To maintain spatial homogeneity, the landscape is separated into subplot grids that are spatially regular or include some thematic characters. Predictive models of presence support this type of study. Keil et al. [40] worked on the beta diversity of European birds, plants, butterflies, amphibians and reptiles, and on British plants, Catalanian birds and Finnish butterflies, integrating large, 50 km grids that had to be homogeneous for species presence (deduced from the climatic risk atlas of European butterflies), land cover patterns (defined by Corine Land Cover) and climatic conditions (average monthly precipitation and temperature). In another study, the same authors applied smaller, 1 km grids [41] over Great Britain to simulate pollinator presence, described by honeybee hive presence, wild bee potential presence (defined by species distribution), land cover, bio-climatic data, topography (slope, orientation), and pesticide risk (linked to crop presence).

In recent years, authors have applied different solutions to integrate landscape ecology into pollination studies: this is certainly a wise decision to foster future monitoring programs. The solutions so far adopted are well sustained by the research question and by local environmental features. However, standardizing the protocols for the application of landscape ecology may facilitate the identification of common trends in pollinator communities and should be a pre-requisite for monitoring programs. When dealing with pollinators, landscape interpretation may be trickier than expected due to the short range of most pollinator species; in Mediterranean areas, difficulties increase due to the greater complexity of the landscape. Use of different ASUs for *a posteriori* analysis is an auxiliary tool that may allow comparisons and the extraction of general patterns. The possibilities for investigating ASUs and applying grids and definitions to different existing spatial databases is almost infinite. However, a major gap is the scale of the existing databases that we can consult for landscape analysis. Many are based on grids much larger than any that

would be biologically meaningful for pollinators or skip information that may be crucial in the understanding of species occupancy.

5. Addressing the Neglected Vegetation

The landscape is composed of different features of which vegetation is certainly the most important in studies of pollination ecology. When we are concerned with a single crop or orchard, vegetation is usually depicted by descriptive lists of spontaneous flora near the cultivated area or at its margins. When the focus is a complex habitat composed of agricultural and semi-natural areas, vegetation can be described from maps or photographic records. When the study focuses on direct relationships of certain plant and pollinator species, other features are included, such as diversity, abundance, floral traits and plant reproductive success (Figure 3).

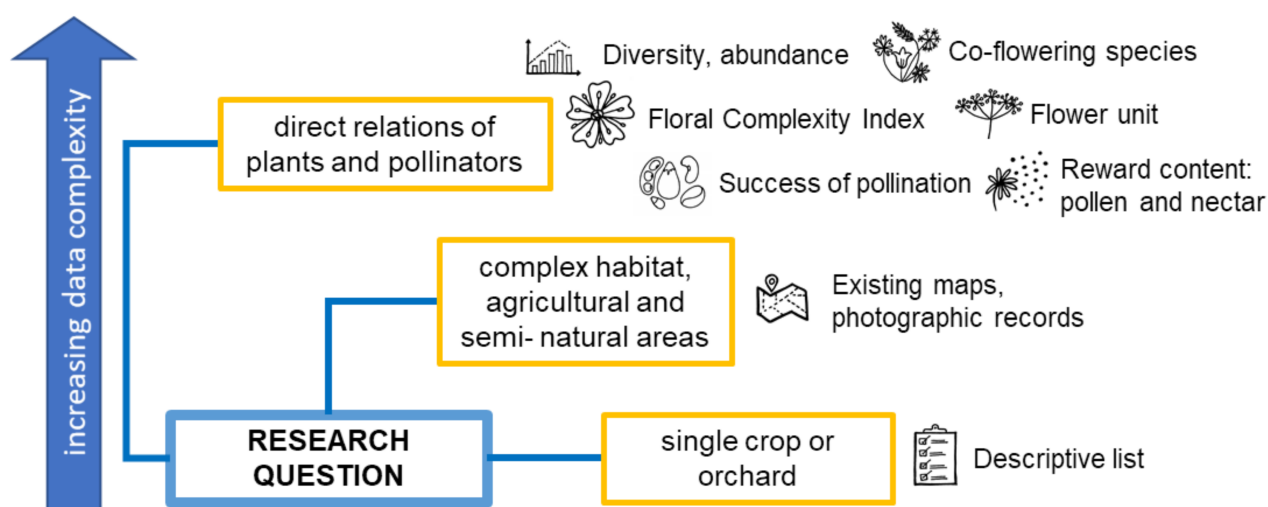


Figure 3. Levels of complexity of vegetation. The different ways of describing vegetation, with increasing levels of detail, as addressed in Section 5.

The distribution of vegetation on different scales is usually investigated through plots. Prior studies have been concerned with areas featuring pollinator flight buffers, indicated as the area that pollinators may cover between potential nesting and foraging sites. To investigate these areas, plots are placed along transects [42]; sometimes two types of plots are used concurrently [43]. The plot concept is traditionally linked to modern phytosociological surveys for which a statistical approach is used to combine data (plant species identification and abundance) from numerous plots to describe the vegetation type of hectare-wide areas. In Europe, common ranges of such plots are 4–25 m² for herbaceous types and 50–400 m² for scrublands and forests [44].

The vegetation of agricultural areas, on the other hand, consists of the content of individual farms, i.e., large, cultivated fields and their surroundings. Increasingly cultivated, worldwide, some insect-pollinated, crops have received more attention than others, for example, oilseed rape (reviewed in [45]), sunflower [36,46] and dioic watermelon [47,48]. In such cases, the precise cultivar may be indicated [49,50]. Otherwise, in studies addressing variability at different spatial scales, a list of species describes the surrounding environment. In these case studies, the relative distance of farms from semi-natural habitats [51,52] is of some importance. Similarly, the distance/isolation of urban contexts [53] or the contribution of managed field edges [32] are features that can contribute directly to understanding the pollinator population.

5.1. Plots for Pollination Studies

There is quite a large variability in plot size and shape and their relative arrangement when the aim is to distinguish vegetation (Figure 4). In studies addressing resource diver-

sity and its importance for pollinators at the landscape level, smaller plots are usually a better option. Frankie et al. [54] used 1.5×1.5 m plots in a Californian urban landscape, while another study selected five 2×5 m plots to evaluate the influence of urbanization in France [55] or 1×1 m plots in 17 private yards in Springfield [56]. Likewise, Californian researchers used 1×1 m plots to investigate bumblebees foraging in areas including extensive agriculture and moderate urbanization [57]. Fründ and colleagues [28] sampled flowers in 2×2 m plots arranged diagonally. They excluded plots with too few or no open flowers, identifying all the species (or species aggregate) in the remaining plots and calculating the total flowering area per species. This method is linked to entomological surveys and applied to vegetation for convenience in data comparison. Less often, researchers selected larger plots. Ebeling et al. [58] evaluated how plant diversity affects nesting site availability in Germany, assessing vegetation in 20×20 m plots. Nielsen et al. [59] selected patches of variable area in six areas representing four European biogeographic regions.

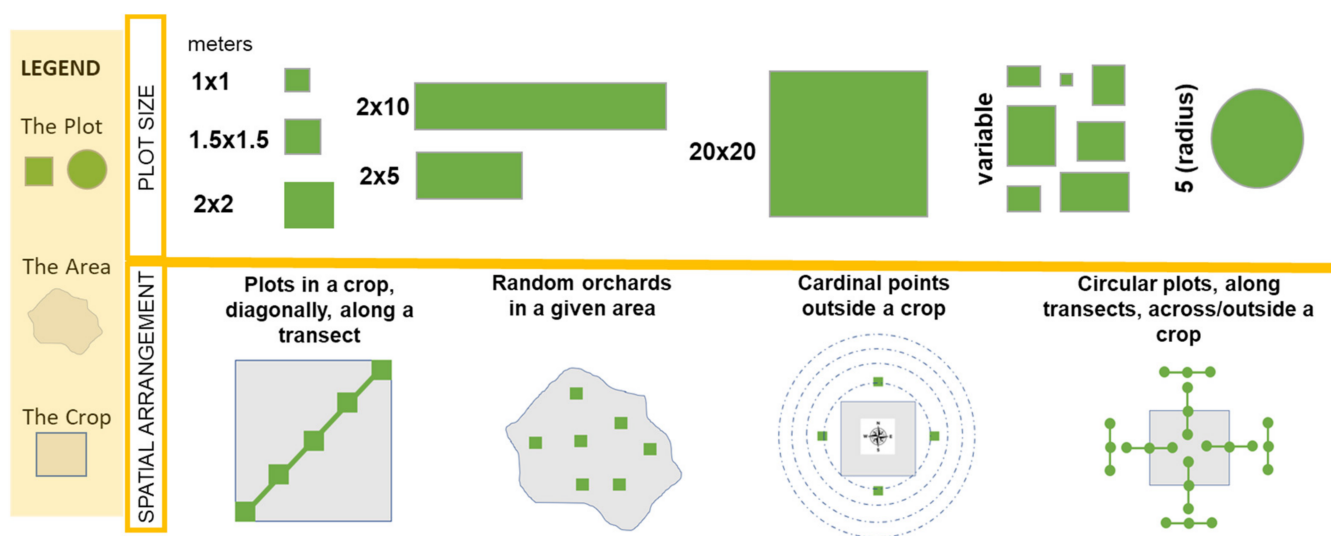


Figure 4. Plots: a space to describe vegetation. Complexity of plot size and spatial arrangements. Plot size coincided in some studies, e.g., 1×1 m [56,57]. Examples of plot arrangement were inspired by (1) [28]; (2) [56]; (3) [55]; (4) [57].

5.2. Flower Traits

Plots are also employed when studying plant–pollinator interactions, in which case, a given blooming plant species is the target. The anthesis period, from the first open flower observed on a given plant until no flowers with anthers remain, is the crucial interval [60,61]. We reviewed flower traits in the literature. Some authors solve the morphological variability between the solitary flower and inflorescence by identifying the pollination unit, grouping the compact inflorescences as a single unit (e.g., Asteraceae, [28,59,62]). Others use existing flower categories based on expected pollination syndromes, such as those proposed by Pellissier et al. [63]. Some authors classify flower traits, such as shape, depth, symmetry, corolla segmentation and functional reproductive units. Stefanaki et al. [64] assigned a weight to each variable to build a floral complexity index, finally linking plant vulnerability and pollinator relationship through floral complexity. Another trait often considered is reward content, especially that of nectar. Its production is measured in volume per flower, sugar, content and nectar concentration per flower and per plant, or by counting the number of open and empty flowers per plant [65]. Carvalheiro et al. [66] carried out a meta-analysis based on flower units (standardized to 1 cm^2), reward in terms of the amount of sugar in nectar, and phylogenetic proximity between plant species. Albor et al. [27] investigated the influence of co-flowering species on pollination success, using *Cakile edentula* as a sentinel species, at different spatial scales on sand dunes of the Yucatan Peninsula. In their 2×10 m plots (10 m apart), they recorded numerous traits for each species: corolla diameter and

width, flower height, degree of herkogamy, color, taxonomic diversity, flower density and abundance as number of flowers.

5.3. Pollination Success

When measuring the success of pollination, more information from the plant side is needed. This implies longer fieldwork since the outcome can only be determined at seed production time. Pollination success is especially important for products of economic importance. Indeed, many studies have sought an understanding of seeds set in specific varieties of products. Yamamoto et al. [67] measured pollination success as the proportion of open flowers and the number of yellow passion fruits by the pollinator guild. They also checked fruit sets 15 days after pollination, by observing pollen tube growth in pistils and estimating pollination quality through the percentage of ovules penetrated by pollen tubes. In another study, the total number of fruits and seeds and eggplant weight and length were indirect measures of the success of wild bee visits [68]. These authors also investigated the relationship between handling time in flowers of different ages (newly open, 1 day old, 2 days old, more than 2 days old) and the number of pollen grains remaining on the anther. Pollination success may be addressed in various ways, including fruit or seed set, variations for male and female plants, pollen limitation, competition and facilitation between plants, to cite some topics much discussed in the literature. However, when addressing pollination success in relation to pollination efficacy at larger scales, it may be difficult to pinpoint plant–pollinator disruption, as found by a Chinese study investigating legume pod content in herbarium specimens [69]. Although the study of seed sets may be crucial in certain contexts, there is still no way of applying it on a large scale and obtaining reliable information on real plant–pollinator interactions occurring in nature.

The monitoring of agroecosystems implies considering the cultivated crop/orchard and the wild vegetation surrounding it. The former is of economic interest and the latter is a repository of wild pollinators. Whatever the main research question, it should be borne in mind that there is always a precise relationship between a plant species and its flower visitors. Evaluating flower traits makes the study more complex and data collection more onerous. However, such data can also sort out preferential guilds of pollinators and reveal differences and similarities between otherwise very distant agroecosystems. Plots are a widely accepted method but have been employed without a common standard. The lack of standardized methods to define plot size and the spatial arrangement of replicates makes it difficult to compare the results of different studies. The monitoring of large areas that adopt the same method could help in selecting the most suitable reference unit for future use.

6. Addressing the Key Players: Pollinators

In temperate areas, especially the Mediterranean, bees are the best-known pollinators. They depend entirely on flower resources for adult maintenance and larval development and therefore, have an outright commitment to visiting flowers. However, other insect groups are considered good pollinators and are mentioned in this section. As many authors have pointed out [70–72], the insect groups considered to be the most effective pollinators are the Hymenoptera (especially bees, the Apoidea), the Diptera (especially hoverflies, Syrphidae) and the Lepidoptera. Species belonging to these three groups are often recorded interacting with flower reproductive parts and actively transferring pollen. Although effective pollen transfer while visiting a flower is the action to which the term *pollinator* is intended to refer, it does not always happen, and in some studies, all animals that visit flowers are considered pollinators, regardless of how effective they are in transferring pollen [73,74]. The study of pollinators (Figure 5) includes the field task of insect capture, and the laboratory task of specimen identification.

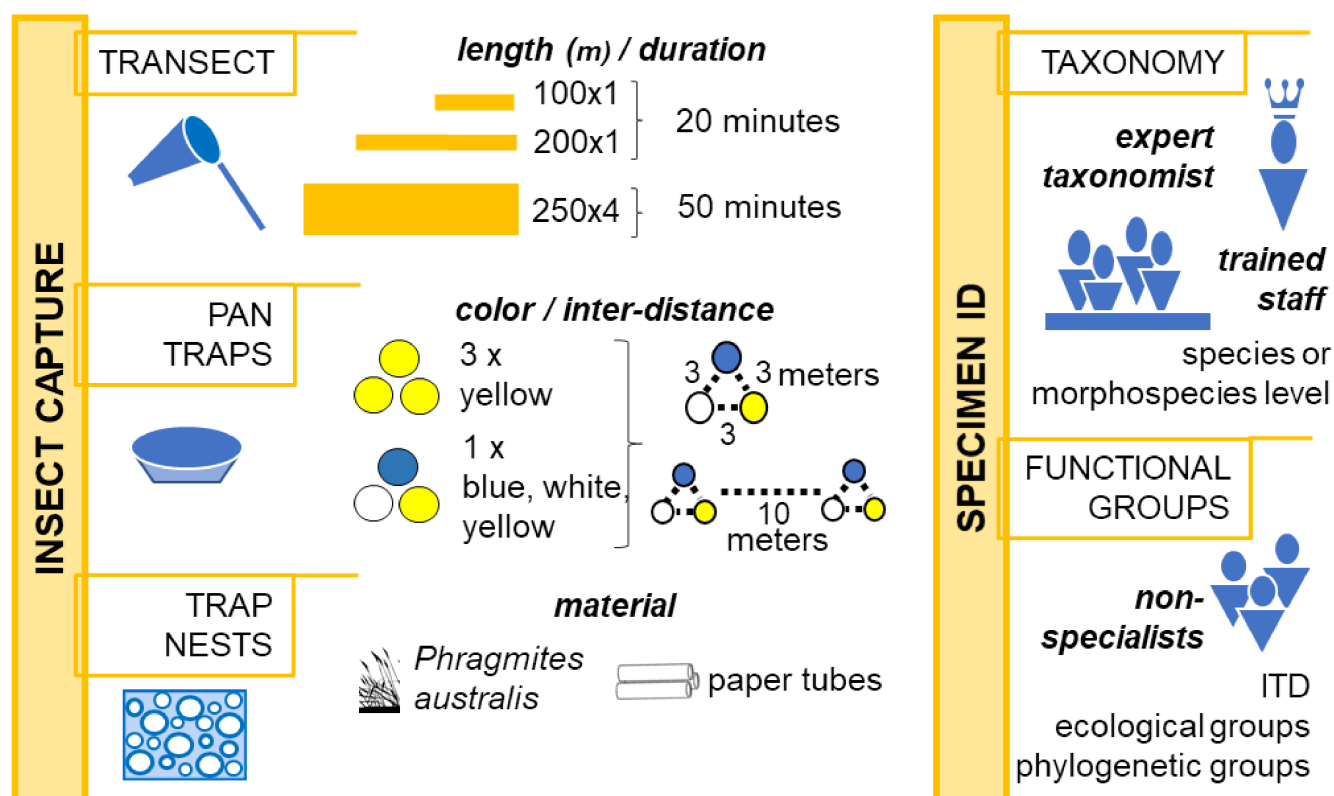


Figure 5. Study of pollinators. The two tasks for pollinator collection and specimen identification, as explained in Section 6.

6.1. Capture

The work of Westphal et al. [75] is a milestone paper with practical instructions for the capture of pollinators. The methods include walking transects, pan traps and observation plots in flowering patches. All have been widely used in recent decades. Walking transect ranges span from 100×1 m trails to be covered in 20 min [68], to 200×1 m in 20 min [76], and 250×4 m in 50 min [59]. Walking transects are usually defined as variable [31,76,77] when the position and direction of a transect are chosen randomly during the sampling, or standard-fixed when the same spot is visited in subsequent monitoring periods. However, the term “variable” is understood differently in Westphal et al. [75] and Nielsen et al. [59]: they both refer to a second transect walked in an area (1 ha) adjacent to the standard-fixed transect, where the collector can move freely among attractive flowering patches for 30 min. Specimens are usually captured with a sweep net, and the plant visited is normally recorded. The walking transect method may imply observer bias [78], prevented by concurrent use of UV-bright pan traps. However, pan traps too have their limits: they miss any information correlated with flowering vegetation and imply heavy killing. Their distribution in the field may also vary, for example, with regard to the color combination employed. Bailey et al. [21] and Sheffield et al. [79] only used yellow traps, while other studies used a combination of three colors (yellow, white and blue: [80–82]). Droege et al. [83] found a color-dependent taxonomic bias, with an underestimation of Colletidae and an overestimation of Halictidae. The traps are almost constantly placed in a 3×3 m grid, with a distance of 10 m between triplets [59,75,79,81,82]. In North America, some studies have used vane traps, which are similar to pan traps [84,85]. There is much debate about whether transects or pan traps are the best solution: most researchers suggest simultaneous use of both where possible [86–88], although they also agree that the increased effort does not seem to be rewarded by more reliable data. Some evidence seems to support a relatively low impact of killing procedures [89] on bee communities: bee populations are known to naturally fluctuate and this may obscure the real impact of bee capture during monitoring.

6.2. A Bottleneck: The Taxonomy

The neglected importance of taxonomy is explained well by Wägele et al. [90]. In some studies, the authors rank and group observed and/or collected insects because species determination is difficult. This problem is intensified by the overwhelming number of species that remain undescribed and arises more frequently in areas with high biodiversity and relatively few insect taxonomy specialists. However, determination to species level is very important for pollination studies. Many researchers indicate species or morphospecies [14,21,26,31,35,39]. Morphospecies is an artefact obtained by grouping all individuals similar to a given genus [90]. A morphospecies can be determined by trained staff on the basis of visual comparison of the main physical features of specimens.

Another way to group pollinators is based on ecological aspects. Functional groups are created according to characteristics related to the pollinator's behavior in the ecosystem. One example is inter-tegular distance (ITD), a proxy for body size [91] that is purported to reflect foraging range [52]. Zurbuchen et al. [92] advise that more research is needed: the maximum foraging ranges of most wild species are still unknown. ITD is measured with a digital caliper or an ocular micrometer [93]. Functional groups are more appropriate when investigating the population structure of a vegetation type or plant species, or the response of species to environmental changes. As pointed out by de Palma et al. [94], bees display a wide range of lifestyles, which can be assigned to ecological groups or guilds. These in themselves include further information on habitat and the bee community. Sheffield et al. [79] used kleptoparasite bees as indicator taxa for bee communities. These species play a stabilizing role and are therefore considered to represent the apex of a community. The authors surveyed bee communities in 19 sites, spanning from highly managed agroecosystems to unmanaged ones and natural habitats. They confirmed a positive relationship on richness and abundance of kleptoparasite species and ecological gradient. Hoverflies and butterflies also have ecological traits that can be used to group species. Examples are feeding strategies and the size of adults in the case of Syrphidae [35]; mobility and habitat specificity in the case of Lepidoptera [95].

6.3. Plant-Pollinator Interactions

Walking transects and pan traps are so far the most indicated techniques for investigating the pollinator community of a given area [59,80,82], but as mentioned, they prove to be poor for indicating plant–pollinator interactions and pollination efficiency. When the main goal is to study the interaction between plants and pollinators [87], observation plots in flowering patches are the most appropriate method. Records usually focus on the behavior of insects crossing plot borders. They include checking whether the visit promotes outcrossing, and recording the resource gathered (pollen and/or nectar) and the number of flowers visited in the patch [96,97]. It is customary to assign pollinators to functional groups, and to collect specimens for further identification in the laboratory. Trap nests [98] have been especially useful for citizen awareness of wild bees and for studies in urban environments [99,100]. Trap nests passively collect cavity-nesting bees. The materials employed to build them and the model may be [75] a box containing common reed (*Phragmites australis* (Cav.) Trin. ex Steud.) internodes with a diameter of 2–10 mm and a length of 15–20 cm, or paper tubes of different diameters. After a given survey period, the reed internodes and paper tubes are set aside for diapause (3 months at 4 °C), to later collect emerging adults for identification. In this case, the plant–pollinator interaction is investigated by examining the pollen provisioned inside the trap nests [101]. However, this method is only valid for a small sub-sample of bees with a certain nesting ecology [102].

Pollinators are known to be declining, yet the best method to monitor them involves killing some. A good monitoring plan requires a task force of taxonomy experts from the very beginning. Identification to the species level is fundamental for fostering knowledge of pollinator species distribution and abundance. Although acknowledging the landscape features of a site may suggest the types of pollinator living in the area, the lack of information on the limitations encountered by different bee species during their biological cycles

in similar ecological situations is still a bottleneck. This can sometimes be overcome by taxonomy [103] if the information is detailed enough. However, factors defining diversification in bees are still poorly understood: exploring behavioral traits may be crucial, as in the case of Megachilidae, which use foreign as well as conventional material to build nests [104]. In future, trends correlating functional traits, taxonomy and landscape features may help in understanding the composition of local populations of pollinators.

7. Discussion and Conclusions: Emerging Key Points

The current decrease in pollinator populations makes it extremely important to understand their requirements in terms of food and nesting sites in order to promote effective conservation measures. We may be losing crucial components of the environment before we have fully understood their value. Spatial resolution of ecological features is a very new line of research in recent studies. However, there are still gaps and pitfalls due to largely unknown local agricultural practices and limits posed by geographic/political boundaries. We aim to promote discussion of how attempts to generalize a pollinator monitoring program should remain in the context of the existing literature while also considering local/national circumstances (Table 1).

Table 1. Monitoring approaches. The main components of the present conceptual framework.

Conceptual Framework	the Research Question Perspective	Increasing Power of Landscape Analysis	the Neglected Vegetation	Pollinators
	usually has a one-way workflow	needs to select the appropriate spatial unit	is a key feature of the landscape and vital for pollinators	managed and wild bees are the best-known pollinators
Research Approaches	goal: pollination of crops (managed pollinators, soil use, field margins)	SA: the study area	crop or orchards + spontaneous flora	insect capture in the field
	goal: pollination by bees (nesting + foraging areas)	SSU: the specific spatial unit containing the field site(s)	complex habitat or direct plant-pollinator relations	specimen identification in the lab
	similar data irrespective of goal	ASU: advanced spatial unit for a <i>posteriori</i> analysis	plots + buffers flower traits	walking transects, pan traps and/or observation plots
Current/Future Expectations	assembling previous results irrespective of how they were gathered	predicting pollinators based on landscape features	standardizing plot size and spatial arrangement	increasing taxonomic identification and behavioral observations
	including the evolutionary history of pollinators and plants	including landscape analysis as a future pre-requisite for monitoring studies	reminding the precise relationship between a plant species and its flower visitors	correlating functional traits, taxonomy and landscape features

Pressing honeybee colony losses have led to moves to increase the number of hives in some European countries, though it is controversial whether honeybee abundance can affect wild bees and in what way. Very recent studies highlighted how honey bee presence may affect the wild bee population in New World countries where the honey bee is not native [105]. It could be more difficult to perceive the impact in the Old World, even if the problem is equally driven by human intervention on the total number of hives in an area. Herrera [106] predicted a negative influence of the constant increase in honeybees and confirmed this prediction on the basis of the existing literature: a gradual long-term replacement of wild bees by honeybees has begun and may consolidate, depending on future policy measures. However, since pollinators are (all) responsible for an important ecosystem service that influences the availability of food for human consumption, their value is also economic and closely linked to agriculture. As we demonstrated (Figure 1), questions related to pollinator conservation and their contribution to agroecosystems often converge on the aspects to address. Long-term monitoring projects should consider this convergence: certainly, more focus is needed on key biological features that interact directly

at the landscape scale of pollinators. Most recent applications of landscape analysis open a wide range of opportunities to investigate different spatial scales by combining globally available databases and applying or developing models and algorithms. We can count on biodiversity and conservation databases on a world scale, such as the Global Biodiversity Information Facility (GBIF) and the International Union for Conservation of Nature's Red List of Threatened Species (IUCN Red List). To map and evaluate landscapes, a multi-criteria metric approach is recommended, employing international- and/or national-scale databases that report variations in land use and additional data whenever available. However, various directions have been explored (Figure 2) and some trends seem to have emerged.

In our view, approaches based on the keywords *pollination* and *agriculture* include: (a) interpretation of the landscape composed of cropland/orchard as well as adjacent non-cultivated areas (field margins, forests, urban areas); (b) evaluation of cultivated plant species as well as nearby wild plants; and (c) consideration of our limited knowledge on pollinator species abundance, variability and interdependence, and the difficulty of gathering data on them. Monitoring should be dedicated to agroecosystems, while bearing variability due to farming practices in mind, as well as landscape complexity often typical of the Mediterranean area. Picón et al. [107] even found differences in a restricted group of wild bees colonizing trap nests: polycrop systems provided a variety of resources in space and time that increased the number of brood cells and individuals reaching the adult stage. It is important to highlight farming practices that favor environmentally sustainable production. In Mediterranean countries, this may translate into appreciating traditional practices and mosaic landscape composition. Numerous studies have highlighted the benefits of semi-natural habitats and proximity to grassland patches in a landscape context. Grassland proximity, in combination with an abundance of beehives, were the best indicators of pollinator communities [108]. Balzan et al. [109] confirmed the importance of rural landscapes, i.e., patches of semi-natural and agro-ecosystems, for the delivery of multiple ecosystem services. Dainese et al. [110] showed that hedgerows in a landscape rather than a local context can improve ecosystem services, including pollination, the hedgerows possibly working as ecological corridors between crop and non-crop areas. Landscape heterogeneity also resulted in a key element of plant species richness in field boundaries in Spain [111], possibly influencing local pollinator communities by a cascade effect.

A bottleneck of monitoring programs, soon to be addressed, is bee identification, especially in hotspots, such as the Mediterranean basin. Wild bees have recently been claiming more attention and an increasing number of studies are focusing on them. Regarding the problem of bee identification, positive expectations are based on genetic resolution, with the application (possibly widening in the near future) of barcoding technologies. Particularly critical for identifying understudied insect groups, genetic tools will help us achieve more accurate estimates of the true number of pollinating species, which is expected to be higher than currently thought [72]. However, an essential milestone is still the backup provided by taxonomy, which must be implemented without delay, especially in southern European countries.

Since wild bee flight ranges are relatively short, high bee diversity can be maintained even in small ecosystems whenever basic resources are available. Morrison et al. [112] found that variability at the landscape scale did not influence wild bee richness, diversity or evenness. Dorchin et al. [113] identified flower diversity and semi-natural habitats within 500 m as main drivers, and abundant unique bee assemblages (with high spatial and temporal turnover), even in the smallest habitat fragments. These results seem to indicate the need to take a different perspective on this important group of pollinators, more susceptible to local than to large landscape contexts. Present limited knowledge of pollinator species abundance, variability, community composition and structure are mainly linked to the difficulty of gathering consistent field data. A focus on wild bees and an effort to gather more species-specific data are certainly crucial for future conservation efforts, but also to maintain stable crop production. National bodies should enforce pollinator

monitoring, considering landscape scale and local priorities, in order to link the influence of local agronomic practices to the diversity and abundance of pollinators. Countries rich in bee species should foster species-specific ecological and behavioral studies, especially in the biodiverse Mediterranean basin, where the high diversity of pollinators and the ecosystem services they provide justify greater investments in their conservation.

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