Measuring floral resource availability for insect pollinators in temperate grasslands – a review

Running title: Measuring floral resource availability

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Abstract 1. The relationship between pollinators and flowering plants plays a crucial role in the function of terrestrial ecosystems. Although pollinators use floral nectar and pollen as food resources, no general methodology for floral resource availability estimates exists.

2. We provide a brief review on floral resource sampling methods frequently used in pollination studies. We focus on how representative vegetation samples are both spatially and temporally, and how these are constrained by sampling effort.

3. We selected field studies investigating flowering plant abundance for insect pollinators, in temperate grasslands. We categorised the reviewed studies according to aims, sampling units and count variables used and provide a descriptive summary on methodology. We also searched for

trade-offs between different aspects of sampling investment.

4. We reviewed 159 pollination studies. We found large methodological differences, and vegetation sampling was presented in many studies insufficiently. Sampling covered a small proportion (median: 0.69%) of the study sites, with long intervals (median: 30 days), and most studies lasted only a few years. The most often used count variables were indirect proxies of floral resources. We found negative relationships in some of the different aspects of sampling, e.g. the proportion of site covered with sampling decreased with increasing site area.

5. By tailoring sampling methods to specific research questions, research effort should be optimally allocated to obtain proper spatio-temporal resolution and data coverage. We suggest guidelines to design sampling, e.g. to increase coverage and frequency. We think that further field work on optimising sampling techniques is mandatory.

Key words: plant-animal interactions, flower, food-resource estimate, nectar resources, insect pollinated plants, vegetation sampling methods

Introduction

The relationship between pollinators and flowering plants plays a crucial role in maintaining most terrestrial ecosystems. Recently, there has been an increasing interest in pollination studies manifested in intensive research on a potential pollination crisis and consequent harvest fall (Potts *et al.*, 2010; but see Ghazoul, 2005), human impacts and the effect of climate change on pollinator communities (Benadi *et al.*, 2014; Petanidou *et al.*, 2014), and understanding pollinator foraging strategies (Goulson, 1999). The number of studies investigating plant-pollinator relationships at the community level and at the landscape scale is also increasing (Hegland & Totland, 2005; Henry *et al.*, 2012) and understanding entire plant-pollinator networks received special attention (Burkle et al. 2013). Several important and yet not sufficiently understood key problems concerning the conservation of plant-pollinator systems, such as sampling floral resource availability for pollinators, were identified by Dicks *et al.*, (2013).

Pollinators feed on nectar, pollen and oils produced by flowers (Goulson, 1999). Quality, quantity and production rates are highly changing by plant species, time of the day, age of flowers and competitors' consumption (Nicolson *et al.*, 2007). Resources offered to pollinators are advertised via many flower traits, but some flowers may deceive pollinators providing no reward (Goulson, 1999; Nicolson *et al.*, 2007). Pollinator abundance, diversity and resource-visit frequency are influenced by the number of floral resource species, quantity and density of flowers and the amount and quality of food in flowers, being the strongest factors structuring pollinator communities (Potts *et al.*, 2004; Dennis, 2010). In animal-pollinated species, the length of the flowering period, seed production and plant population dynamics depend on pollination (Nicolson *et al.*, 2007). Components falling out from complex plant-pollinator networks, due to local extinction, or temporal mismatches in plant-pollinator phenologies caused by differential effects of global change drivers such as climate change, or habitat loss and degradation (Burkle *et al.*, 2013), might have severe impacts on a given community. To investigate such potential impacts on a network requires reliable sampling methodology. Therefore, estimates of resource availability are essential in order to understand such ecological interactions and to establish restoration management (Dennis, 2010).

Botanists and zoologists study pollinators and flowering plants from different perspectives and use a wide range of methods in pollination research (Goulson, 1999; Bosch *et al.*, 2009). Some pollinator studies investigate flower availability superficially, by using only, for instance, species richness of flowering plants (Kitahara *et al.*, 2008), while some studies even neglect it completely and often conclude floral resource availability from indirect proxies such as consumption rates (Bakowski & Boron, 2005), pollen distribution in honey (Aronne *et al.*, 2012), or pollinators' pollen load (Hinners & Hjelmroos-Koski, 2009). Apparently, no generally used methodology exists to estimate floral resource availability, and many studies neglect standard vegetation sampling protocols (Elzinga *et al.*, 1998; Gibson, 2002). For instance, the suitability of counting flowers or sampling nectar was debated as early as the beginning of the 1980s (Tepedino & Stanton, 1981; Zimmerman & Pleasants, 1982; Tepedino & Stanton, 1982). Recommendations on how to measure floral resource availability for pollinators are still scarce (Zimmerman & Pleasants, 1982; Tepedino & Stanton, 1982; Frankl *et al.*, 2005; Hegland *et al.*, 2010).

We think that three important decisions have to be made before choosing an appropriate method to investigate pollinator food resource sampling. First, one has to choose the *focus* of the study: whether to investigate a single plant species and all its pollinators (Thompson, 2001), or a single pollinator species and all its flower resources (Rusterholz & Erhardt, 2000), or the entire pollination network (Junker *et al.*, 2013). Second, the *spatial and temporal scale* of the study has to be adjusted to the often wide array of foraging ranges and life cycles of focal pollinators (Osborne *et al.*, 2008; Dennis, 2010). The spatio-temporal resource distributions are also various including high seasonal and annual variation (Alarcón *et al.*, 2008; Kubo *et al.*, 2008; Dennis, 2010). These imply the necessity of frequent sampling and long-term studies, ranged from the level of individual flowers through patches to biotopes and landscapes (Hatfield & Lebuhn, 2007; Westphal *et al.*, 2008). Third, to define the unit of the *count variables* (i.e. count units to estimate flower resource amounts available for pollinators) insect perception should be taken into account (Kearns & Inouye, 1993). Plants have rather different body plans and inflorescence structures, and their pollinators are not less various in morphology, timing and foraging range, and these two parts have to match. Flowers therefore, may be perceived very differently by different pollinators, since insects use various cues to navigate at different spatial scales and use a wide range of sensory systems (Dauber *et al.*, 2010;

Dennis, 2010, Clarke *et al.*, 2013). Count variables can be nectar and pollen amount, counts of single flowers or inflorescences, the number of flowering shoots, or the number of single-species flower patches, and may vary depending on the pollinators investigated.

In the light of these three points and that the suitability of different methods depends on the specific research question, it is clear why generally used sampling methods are not available for investigating so complex systems. In this paper, we aim to review the methodology of estimating food availability for insect pollinators in temperate grasslands. We focus on how representative vegetation samples in pollination studies are both spatially and temporally, and how these are constrained by sampling effort. We also highlight challenges in estimating floral resource availability.

Data and methods

Our aim was to review research papers focusing on the relationships between resource availability for insect pollinators and pollinator abundance, diversity or flower preferences. We searched for papers upon four groups of search terms (i) “bee”, “bee fly”, “bumblebee”, “butterfly”, “hoverfly”, “moth”, “pollinator”, “visitor”, “wasp”; (ii) “diversity”, “foraging”, “feeding”, “network”, “preference”; (iii) “floral”, “flower”, “nectar”, “pollen” and (iv) “availability”, “resources”, and we used “and” operator between groups and “or” operator between keywords within groups. We used the databases *ISI Web of Science* (www.webofknowledge.com) and *Scopus* (www.scopus.com), accessed 08 Nov 2015. We selected field studies investigating flowering plant abundance, aimed at insect pollinators only, and carried out in the temperate climate zone. We excluded publications focusing only on a single or very few plant species, or mainly on flowering shrubs and trees, because the latter requires rather different sampling methods (references of the reviewed studies: Appendix S1).

We categorised the reviewed studies according to (i) aims, (ii) the sampling units and (iii) the count variables used, and (iv) whether estimates on the amount of nectar or pollen were applied. We refer to sampling units as "quadrat" in the broad sense, i.e. quadrat is a more or less equal sided sample area (Gibson, 2002) in all cases when authors used the terms "quadrat", "square", "circle" or "plot". Transects were elongated sampling units. We extracted information on sampling unit shape, as well as length and width of the sampling unit if it was quadrat or transect. Count variables (e.g. the number of flowers or visual floral display) were measured either with (i) rough estimates, such as ranks, and green cover or flower cover estimates, in all cases where flowering shoots, or inflorescences were not accurately counted, hereafter referred to as *categorical estimates*, or with (ii) *direct counts* of all shoots or other types of counted units within a sampling unit. Furthermore, we extracted the following numerical data from the articles: number of study sites; site area; number of sampling units per site per sampling event; area of sampling units; length, width and radius of sampling units; sampling interval; length of the study in years and the number of pollinator species (raw data: Appendix S2). We calculated mean values of these variables, if more than one values were given per study. Furthermore, we calculated sampling unit length:width ratio in case of rectangle-shaped sampling units, the total area of sampling per event, and the proportion of the site covered by sampling, if data were available (Appendix S2).

We present descriptive statistics by giving median, minimum and maximum values, and showing boxplots with individual data points. We investigated relationships between the temporal and spatial resolution of the studies to detect potential trade-offs in research investment. We expected trade-offs between the area of study sites and the number of sites; the proportion of the site covered by sampling and the number of sites; the number of sampling units and the area of study sites (we analysed studies using quadrats or transects pooled with all other studies as well as separately); the area and the number of sampling units; the proportion of the site covered by sampling and the area of study sites, sampling interval and the number of study sites; sampling interval and site area; sampling interval and the proportion of the site covered by sampling. We provide Kendall's *tau* correlation coefficients for rank data and *p*-values corrected for multiple comparisons with the method of Benjamini & Hochberg (1995). We also expected that categorical estimates require less research effort than direct counts, thus using categorical estimates allows sampling a larger total area than when direct counts are used. We tested this assumption with Mood's median test. We analysed all data in the R statistical environment (R Core Team, 2015).

Results

We found 159 studies published in 1981–2015 with the aims of estimating “pollinator population size or diversity” in 104 (66.0%) cases, “flower preferences” in 42 (26.6%) cases, both in 8 (5.1%) and “other” in 4 (2.5%) studies (raw data: Appendix S2, descriptive statistics: Fig. 1.). One study (Miller-Struttmann *et al.*, 2015) was based on two different historic datasets using different methodologies, and we analysed these data as if they were coming from two independent studies. Authors investigated 1–665 (median: 20) pollinator species, in 1–216 (median: 16, Fig. 1.A.) study sites, with 8 m^2–125 km^2 (median: 10 ha, Fig. 1.B.) site area per study. We found rather different methods in the reviewed studies, i.e. the applied sampling units and count variables varied considerably. Vegetation sampling procedures, such as the spatial and temporal distribution of the sampling units in the study sites were not clearly described (note NA-s in Fig. 1.) or the reasons why a given method had been used remained unexplained in many studies. For example, 57 (35.8%) studies lacked information on site area and 66 (41.5%) studies lacked information necessary to compute the proportion of the study site covered with the sampling units.

Sampling units were quadrats (60.4%), transects (34.0%), the monitoring of the whole area

(5.0%), or point intercept technique in a single study. The shapes of the sampling units, we refer to as quadrats in the broad sense (Gibson, 2002), were squared quadrats (41.7 %), rectangles (10.4%) and circles (10.4%) (Table 1.). Transects were belt transects (rectangular) in most cases (87.0%) and line transects in a few cases (3.7%), and in the rest of the studies transect type was not specified (Table 1.). Sampling was carried out using only a few sampling units per site per sampling event (median: 5, Fig. 1.C.), with 20 m^2 of median unit area (quadrat: 2 m^2, transect: 250 m^2, Fig. 1.D., Table 1.). The median cover of the study site area was 0.69% (Fig. 1.E.). Count variables were flower unit (i.e. visual display) (28.8%), flower (24.4%), flowering shoot (13.5%), flower cover (12.8%), inflorescences (10.3%), green cover (7.7%), and frequency of flowering shoots (1.9%). The measures of count variables were *categorical estimates* in 36.5% and *direct counts* in 61.6% of the studies. Nectar or pollen amounts were estimated in 8.8% of the studies with any method, although nectar amount was the count variable only in a single study (0.64%). Studies were one (63.9%), two (20.9%), three (8.2%), or four (4.4%) years long, only one lasted five, two lasted six and yet another nine years. Most sites were sampled with low frequency (median sampling interval: 30 days) during the study period and many of these used calendar time intervals, e.g. weekly, monthly, or annual sampling (Fig. 1.F.).

We found negative relationships in some of the different aspects of sampling. The area of study sites was slightly smaller if the number of sites were larger (tau = –0.20, P = 0.007, n = 102, Fig. 2.A.). The proportion of the area covered by sampling was not related to the number of sites (tau = –0.02, P = 0.794, n = 93, Fig. 2.B.). We did not find a relationship between the number of sampling units and the area of the study site, if we analysed all types of sampling units pooled (tau = 0.13, P = 0.099, n = 99, Fig. 2.D.). Although we did not find a relationship when analysing transects only (tau = –0.14, P = 0.388, n = 27), we found that the larger was the area of the study site, the more quadrats were used (tau = 0.29, P = 0.003, n = 60) when quadrats were analysed separately. With smaller sampling unit area, the number of units increased (tau = –0.47, P < 0.001, n = 99, Fig.

2.G.), although the proportion of the whole area covered by sampling significantly decreased with site area (*tau* = –0.45, $P < 0.001$, $n = 93$, Fig. 2.E.). Sampling interval increased with the number of sites (*tau* = 0.32, $P < 0.001$, $n = 148$, Fig. 2.C.), but was neither related to site area (*tau* = 0.06, $P = 0.478$, $n = 95$, Fig. 2.F.) nor to the proportion of the site area covered with sampling units (*tau* = –0.10, $P = 0.267$, $n = 86$, Fig. 2.H.). Researchers using categorical estimates sampled significantly larger total sampling areas during a single sampling event (median = 4500 m^2, $n = 49$) than those using direct counts (median = 446 m^2, $n = 90$; Mood's median test: $P < 0.001$).

Discussion

We found rather different methods applied to estimate food resource availability for pollinators in the reviewed studies. All variables characterising sampling strategies showed an extreme scatter (Fig. 1.). We found no general methodology, and many studies neglected or did not refer to existing vegetation sampling protocols (Elzinga *et al.*, 1998; Gibson, 2002). Some important details were not described in many cases, and the reasons why the given methods had been used were rarely explained. The lack of detailed description of methodology was also found by Mortelliti *et al.*, (2010) who reviewed studies of habitat quality. They concluded that this lack of information hinders carrying out meta-analyses (Mortelliti *et al.*, 2010). In addition, such practice makes reproducibility impossible. We suggest that the role of size and spatio-temporal heterogeneity of study sites were underestimated in many of the reviewed papers, as in the vast majority of studies in the field of ecology (Mortelliti *et al.*, 2010). Most of the reviewed pollinator studies did not carefully design resource availability sampling relative to the problem's complexity.

Many studies aim to primarily sample important and/or abundant plant and pollinator species (Hegland *et al.*, 2010), although rare species might also play an important role in maintaining specialised pollinators (Bosch *et al.*, 2009). We suggest that not only the rare, but even abundant species can be overlooked if flowers are highly aggregated in space, especially if only a small proportion of the entire area is thoroughly sampled, e.g. when using quadrats or transects. Furthermore, various methods may detect different floral species with different probabilities. Based on the fact that the number of flowering plant species was usually positively correlated with pollinator species richness (Ebeling *et al.*, 2008), some studies used only species lists, i.e. presence-absence data, to predict floral resource availability (Kitahara *et al.*, 2008). However, we agree with Hegland & Boeke (2006) that species lists alone are not appropriate estimates of floral resource availability: some quantitative estimates such as flower abundance are recommended.

Data on foraging ranges of some pollinators (Osborne *et al.*, 2008; Dennis, 2010) imply that the design of vegetation sampling was not representative in many of the reviewed studies, e.g. due to low spatial coverage. Pollinator home range as well as floral species phenology and its consequences for spatio-temporal variation in resource availability must also be taken into account to delineate study site and determine sampling methods for resource availability and resource use at the same scale. For instance, using the same sampling units may help to find the link between resource availability and resource use (Rusterholz & Erhardt, 2000; Hegland & Totland, 2005). Natural biotopes in the temperate zone are highly heterogeneous and many flowers are aggregated (Elzinga *et al.*, 1998; Hatfield & Lebuhn, 2007), and the spatial heterogeneity also influences the minimum number of sampling units required. If spatial coverage of sampling is low, then many species will be estimated with large bias (Hegland *et al.*, 2010). Unfortunately, we did not find recommendations on the proportion of the study site covered to sample floral resources. Compared to the median 0.69% cover for the reviewed studies, for an accurate estimate in a field study, we should have covered about 6.3 ± 3.6% [mean ± SD] of a 0.6 ha Central European colline meadow, estimated by Kupper's and Hafner's method (Kupper & Hafner, 1989, modified by El*zinga* et al., 1998; Sz*igeti* et al., unpublished). Insufficient quadrat cover yields biased data especially on rare and clumped species. On the one hand, clumped species can be sampled with less bias if quadrat sizes are increased or their shape varied, e.g. from square to elongated rectangle (Elzinga *et al.*, 1998). On the other hand, quadrat size should be maximum 2m × 2m, because small flowers in a larger quadrat can hardly be detected without stepping in (Kearns & Inouye, 1993). Long and narrow sampling units may overcome this problem (Elzinga *et al.*, 1998). We found a large scatter in the shape of sampling units (Table 1.). Although shape may resolve sampling difficulties for aggregated plants generally, we found no arguments on why a specific shape was used, except in those cases when the same transects were used for pollinator and plant sampling. The median 2 m belt width indicates that most researchers follow Kearns & Inouye's (1993) recommendation, although the large range shows that still many authors use belt widths within which detectability might vary severely.

Kearns & Inouye (1993) needed 12 hours for counting the number of flowers in 25 2m × 2m quadrats. The research effort necessary for a thorough quadrat sampling may also depend on the type of the count variables, not only on species richness and biotope heterogeneity. Nevertheless, in homogeneous biotopes such as agricultural plots, even a smaller number of sampling units may be sufficient.

Determining the *count variable*, the unit of resource availability, is also difficult. A count variable should estimate the feeding unit of the pollinator (Kearns & Inouye, 1993) and take into account how pollinators find their food resources (Goulson, 1999; Dauber *et al.*, 2010). Both count variables and feeding units may be specific to both plant and pollinator species and to the aim of the study. The most frequently used count variables were those simple to estimate, such as the number of flowers or flower area, and only a handful of studies investigated resource value (pollen or nectar amount) for different plant species or referred to other studies assessing resource values. We found direct measures of nectar-resource values only in a single study (Potts *et al.*, 2004). Although the ultimate goal to assess resource availability would be to obtain estimates on sugar and amino acid contents of nectar and pollen (Zimmermann & Pleasants, 1982), considering that food amount depends on species, site, individual, weather etc. (Tepedino & Stanton, 1982; Nicolson *et al.*, 2007), such estimates are rarely feasible. For example, collecting nectar in a sufficient amount for measurements is either complicated and labour-intensive, or hardly feasible at all for many flower species (Tepedino & Stanton, 1982; Morrant *et al.*, 2009). Hegland & Totland (2005) argued for using proxies, because the number of flowers and flower size were related to nectar amount in several studies. This relationship has been demonstrated mostly within species or families (Stanton & Preston, 1988; Galetto & Bernardello, 2004), albeit very few studies are available for investigating communities; some found similar relationships (Potts *et al.*, 2004; Torné-Noguera *et al.*, 2014), while others did not (Wäckers, 2004). In contrast, counting flowers may yield rather imprecise estimates for food availability (Benadi *et al.*, 2014), although even the flower patch may be a valid count variable if the project targets the landscape scale (Henry *et al.*, 2012). Pollinators prefer dense patches to minimise the costs of search (Hegland & Totland, 2005) and may use patches as sensory cues to find food resources rather than individual flowers or inflorescences (Goulson, 1999; Dauber *et al.*, 2010). However, traits such as flower size, colour or scent may directly indicate rewards available for flower visitors in a specific flower (Nicolson *et al.*, 2007) and visitors may use such cues when selecting flowers within a close distance (Weiss, 1991). Flower unit (visual display) may be a reasonably good choice, but the definition is not clear in all cases. For instance, Tepedino & Stanton (1981) counted flowers and inflorescences, depending on floral type and/or species, but did not define them as flower units. Rotenberry (1990) gave a definition for floral visual display and considered flowers, heads, or stems as unity, and emphasised that these were selected to match closely the flower visitor's view. Other authors use similar, albeit slightly different definitions, and some emphasise that the unit was defined so as pollinators should walk and not fly when foraging (Woodcock, 2014), rather than by the visual cues perceived from a distance (Cowgill, 1993; Hegland & Totland 2005). These approaches led to similar categories, although these categories may be difficult to apply at least for some plant species. Indeed, definitions are based more on examples than on rigorous descriptions of the categories due to the extreme variability of floral body plans.

In a few studies, besides using a count variable simple to estimate, e.g. number of shoots, floral traits such as the number of flowers per stems, flower dimensions or nectar amounts were also measured for a couple of individuals in several species. Then the measurements of these floral traits were extrapolated to the entire sample (e.g. Hegland & Totland 2005). This method may yield much more accurate estimates on food availability than using solely proxies such as flower units.

Plant-pollinator interactions are changing rapidly over the flowering and pollinator flight period in natural circumstances. Many pollinator studies focused on the temporal distribution of plant-pollinator interactions such as relationships between flowering phenology and pollinator floral resource choice (Bagella *et al.*, 2013; Benadi *et al.*, 2014; Petanidou *et al.*, 2014). This requires investigating temporal changes in species composition and flower density. Median resource sampling time was 30 days for the reviewed studies. In contrast, rapid changes of flowering were found over the season (Kubo *et al.*, 2008; Bagella *et al.*, 2013) or even during a day (Nicolson *et al.*, 2007; Fründ *et al.*, 2011), and these changes were partially due to the interactions between flowers and their insect visitors (Fründ *et al.*, 2011). Temporal changes should be taken into account when planning sampling frequency, since pollinators necessarily follow these changes (Goulson, 1999; Potts *et al.*, 2004; Kubo *et al.*, 2008). Furthermore, time elapsed between sampling events increased with the number of sites for the reviewed studies, indicating that sampling frequency was determined by research effort constraints. We argue that this typical trade-off between spatial and temporal representativeness could be overcome or its limitations could be reduced by combining different methods with either a high spatial or high temporal resolution. We suggest that recording presence-absence of flowering species in an entire meadow might detect some species that start blooming earlier than quadrats or transects, if these latter cover only a small proportion of the entire study area. In contrast, abundance estimates, e.g. by quadrat sampling, may be more suitable to estimate the change over time in relative densities across species, due to its higher resolution.

Sixty-four percent of the studies investigated a single year, thus being hardly representative of a plant community in the long run. Only four studies extended more than four years (Stefanescu, 1997; Alanen *et al.*, 2011; Petanidou *et al.*, 2014; Miller-Struttmann *et al.*, 2015). However, floral resource compositions vary considerably among years (Alarcón *et al.*, 2008), and we agree with Westphal *et al.* (2008) that one-year studies provide only a snapshot of plant-pollinator interactions.

In general, a trade-off emerges between spatio-temporal resolution and coverage of sampling. For example, although the number of sampling units increased with research area, the coverage of sampling decreased. Similarly, the effort invested in the temporal resolution of sampling decreased with the increasing size of the study site, although did not change with sampling unit size. Furthermore, direct counts involved smaller areas sampled, compared to the simpler categorical estimates, thus researchers have to decide on either using higher estimate accuracy or better spatial resolution. In contrast, we did not find relationships in all of the cases where we expected trade-offs among different aspects of research investment. We propose that many times researchers might overlook the necessary research investment in all the important aspects of the required sampling process when planning sampling protocols. Reasonably good estimates need labor-intensive and expensive methods, but research investment is always limited (Hegland *et al.*, 2010). Nevertheless, minimum criteria for sampling each component of a study should be defined.

Recommendations

Recommendations on sampling methods to estimate floral resource availability for pollinators are scarce (Frankl *et al.*, 2005; Hegland *et al.*, 2010), although a wide range of methods is described in the vegetation literature (Elzinga *et al.*, 1998; Gibson, 2002). Here we provide a few guidelines based upon the reviewed studies, that we think useful for estimating food resource availability for pollinators in temperate grasslands. We recommend that both quantity and quality as well as the spatio-temporal distribution of resources should be monitored when sampling floral resources. The selected sampling methods should be better adapted to the aim of the study, and to the complexity of the study system (spatial heterogeneity, seasonality, number and type of pollinator species etc; Kearns & Inouye, 1993; Hegland *et al.*, 2010). To investigate floral resource abundance, focal pollinators' feeding range in a specific area should be known. Rarity of important floral resources should be taken into account when choosing a sampling method.

Given the research question, one should decide how to allocate finite research effort into the spatio-temporal resolution and the coverage of sampling. In many cases, a high resolution is required in both spatial and temporal terms, or both sampling resolution and coverage must be sufficiently high to answer research questions. Using the same sampling units for pollinators and their food resources may help to find the link between resource availability and consumption. We argue that combining different methods that are appropriate to provide data with either high spatio-temporal resolution or coverage, is a reasonable approach. For instance quadrat or transect sampling could be completed with species lists on entire study sites, thus including information on all potential nectar resources. When quadrat sampling is not feasible or only limited efforts can be allocated to use this method, listing flowering species with a rough categorical abundance estimate, similar to the method used by Goulson & Darvill (2004), may serve as either complementary sampling or just a better option than the lack of abundance data. However, these methods considerably reduce accuracy compared to quadrat sampling. Furthermore, the presence of frequently visited, although rare floral sources may be noticed with the help of pollinator behaviour. However, using feeding rates as an estimate of resource availability is not a viable approach (Bakowski & Boron, 2005; Hinners & Hjelmroos-Koski, 2009; Aronne *et al.*, 2012). Although pollinators are certainly much better than scientists in finding floral resources, resource availability should be estimated independently of the consumers' visit frequency, because of their preferences.

Although the ultimate solution to estimate floral resource amounts would be directly measuring nectar and pollen, it is not feasible in many cases. Characterising flowers with such direct measures, and collecting larger samples on flower abundance could be a reasonably good compromise, especially when variability in nectar and pollen amounts is also taken into account. If direct measures on nectar or pollen amounts are not feasible, visual floral units from the pollinators perspective could be the appropriate count variable. We also recommend avoiding estimates based on green cover, since it is a very poor proxy of floral resource abundance for many plant species.

In contrast to Hegland *et al.* (2010), who found that only a few or even a single sampling event a year was sufficient for investigating key species in pollination networks, we recommend using shorter sampling intervals than used in most of the reviewed studies. The optimal sampling interval may vary among studies. We recommend adjusting it to the aims of the study, community structure and climate. We also recommend conducting long-term studies to lower the risk of distortion due to large annual variation in resource composition, abundance and consumption.

Remote sensing technologies, such as drones with high optical resolution (Bakó *et al.*, 2014) multi-spectral cameras (Peña-Barragán *et al.*, 2007), may change flower resource sampling in the near future. Several pollinator studies have already used remote sensing to estimate the amount of resource or habitat quality on the landscape scale (Osborne *et al.*, 2008; Henry *et al.*, 2012), or time-lapse photography to investigate flowering dynamics (Crimmins & Crimmins, 2008). However, such technologies have low spatial resolution for floral resource sampling (e.g. are unable to detect cryptic plants). Therefore, we think that traditional sampling methods should be further investigated to find efficient, widely usable methods to provide a sound methodological basis for understanding plant-pollinator interactions. We conclude that thoroughly planned field studies comparing sampling protocols at the community level, including remote sensing, and their appropriateness at different circumstances are still mandatory.

Contribution of authors

VS, JK, AK and JN designed the project. VS and JK collected data. VS performed and AK, JK, AH advised on data analyses. VS and JK wrote several drafts and all authors revised the final manuscript.

Supporting Information

Appendix S1. References of the reviewed studies.

Appendix S2. Raw data for the reviewed studies.

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Figure legends

Fig. 1. Distributions of the variables characterising sampling methods in the review. Boxplots show medians, lower and upper quartiles, whiskers include the entire range. Grey + symbols are the data points showing the proportion of data on the vertical axes. Horizontal axes are log10 scaled. NA-s are the number of papers lacking data.

Fig. 2. Relationships among different kinds of sampling investment. All axes are log10-scaled. The plus symbol represents a given study, except D) where *plus symbols* show transect, *squares* quadrats, the *triangle* point sampling and *circles* studies when the entire site was sampled.

Table 1. Shapes and sizes of common sampling units. We used “quadrat” in the broad sense of Gibson (2002), as a more or less equal sided sample unit denoting shapes “quadrat”, “square”, “circle” or “plot”. Transects were elongated sampling units.