Comparing two methods for estimating floral resource availability for insect pollinators in semi-natural habitats

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Abstract

Pollinator and flowering plant interactions play a critical role in maintaining most terrestrial ecosystems, including agroecosystems. Although estimates of floral resource availability are essential to understand plant-pollinator relationships, no generally accepted methodology exists to date. We compared two methods for sampling floral resources in a single meadow. About every three days, we recorded species lists of insect-pollinated plants with abundance
categories assigned to each species (hereafter referred to as scanning) and we counted the
flowering shoots in 36 2×2 m quadrats (hereafter quadrat sampling). These methods were
compared with respect to (i) the number of species detected, (ii) estimated floral resource
abundance, and (iii) temporal changes in flowering. With scanning, we found more potential
nectar-plant species and species were found earlier than with quadrat sampling. With the
latter, abundant species were found with higher probability than the scarce. Flower
abundances were correlated between the two methods. We predicted that a cover of 6.3±3.6%
should be used for an appropriate estimate of flower abundance in our study site, although the
optimal cover probably varies across different habitats. Furthermore, flower abundance
changed 6% per day compared to the flowering peak. Overall, scanning seems to be more
appropriate for detecting presence and the timing of species, while quadrats may provide
higher resolution for abundance estimates. Increased sampling coverage and frequency may
enhance research accuracy and using scanning and quadrat sampling simultaneously may help
to optimize research effort. We encourage further development of sampling protocols.

Résumé

Comparaison de deux méthodes pour estimer la disponibilité des ressources florales
pour les insectes pollinisateurs dans des milieux semi-naturels. Les interactions entre
pollinisateurs et plantes à fleurs jouent un rôle capital dans le maintien de la plupart des
écosystèmes terrestres, y compris les agroécosystèmes. Bien que des estimations de la
disponibilité des ressources florales soient essentielles pour comprendre les relations plantes-
pollinisateurs, aucune méthodologie largement acceptée n'existe actuellement. Nous avons
comparé deux méthodes pour échantillonner les ressources florales dans une prairie. Tous les
trois jours environ, nous avons établi des listes de plantes pollinisées par les insectes, avec des
catégories d'abondance pour chaque espèce (méthode appelée ici "balayage") et nous avons compté les tiges portant des fleurs dans 36 quadrats de 2 m × 2 m (méthode de l'échantillonnage par quadrant). Ces deux méthodes ont été comparées pour trois paramètres (1) le nombre d'espèces comptabilisées, (ii) l'abondance estimée de la ressource florale, et (iii) les variations temporelles de la floraison. Dans la méthode du "balayage", nous avons trouvé plus de plantes produisant potentiellement du nectar, et les espèces ont été détectées plus tôt qu'avec la méthode de l'échantillonnage par quadrat. Avec cette dernière méthode, les espèces abondantes ont été détectées avec une probabilité plus élevée que les espèces plus rares. L'abondance des fleurs était corrélée entre les deux méthodes. Nous avons prédit qu'une couverture de 6,3 ± 3,6 % de la surface pouvait être utilisée pour une estimation correcte de l'abondance des fleurs dans notre site d'étude, bien que la couverture optimale varie probablement selon les milieux. De plus, l'abondance en fleurs a changé de 6% par jour en comparaison au pic de floraison. Dans l'ensemble, la méthode du "balayage" semble être plus appropriée pour détecter la présence et la phénologie des espèces, alors que les quadrats peuvent permettre d'avoir une meilleure résolution dans les estimations d'abondance. Une hausse de la surface couverte par l'échantillonnage et de sa fréquence peut améliorer la précision des résultats et l'utilisation combinée des deux méthodes évoquées peut aider à optimiser les efforts de recherche. Nous encourageons vivement au développement de méthodes d'échantillonnage.

Key words: plant-animal interactions, flower food-resource estimate, nectar resources, vegetation sampling methods, scanning, quadrat
Introduction

Plant-pollinator interactions play a critical role in maintaining most terrestrial ecosystems, including agroecosystems. Pollinators consume floral nectar, pollen and oils, while sexual reproduction of plants depends on pollen transfer by pollinators (Goulson 1999; Nicolson et al. 2007; Patiny 2012). In natural circumstances, plant-pollinator interactions may rapidly change at the spatio-temporal scale (Kubo et al. 2008; Fründ et al. 2011; Bagella et al. 2013), and food resources can be highly aggregated in space or time (Elzinga et al. 1998; Hatfield & Lebuhn 2007). Floral food resource quality, quantity and production rates show a huge variation with plant species, time of the day, age of flowers and competitors' consumption (Galetto & Bernardello 2004; Nicolson et al. 2007; Hicks et al. 2016). The number of floral resource species, quantity and density of flowers and the amount of food in flowers at least partly determine pollinator abundance, diversity and resource-visit frequency, and are the strongest factors structuring pollinator communities (Potts et al. 2004; Iserbyt et al. 2008; Dennis 2010; Curtis et al. 2015). Floral resources offered to pollinators are advertised via different traits (Goulson 1999; Nicolson et al. 2007; Chartier et al. 2011), and insects use a wide range of sensory systems and various cues to navigate at different spatial scales, thus flowers may be perceived very differently by pollinators (Dennis 2010; Dauber et al. 2010; Clarke et al. 2013).

Most field studies reviewed by Szigeti et al. (2016) measure floral resource availability to investigate the relationship between resource availability and pollinator population size or diversity (e.g. Kovács-Hostyánszki et al. 2013); the relationship between resource availability and flower preferences (e.g. Goulson & Darvill 2004); the effect of resource availability on pollinator flight distance between floral patches (e.g. Wolf & Moritz 2008); or the effects of
temporal changes in floral composition on the structure of pollination networks (e.g. Bosch et al. 2009). Further studies investigated the link between pollinator conservation and floral resource availability (e.g. Roger et al. 2016), while others investigated long-term compositional changes and their impact on evolutionary processes (e.g. Miller-Struttmann et al. 2015) or developed methods for estimating floral resource availability (e.g. Frankl et al. 2005). In order to design sampling, the spatial and temporal scale of the study must be considered and this is ultimately derived from the research question. For example, rather different spatial scales are required if the research question targets foraging strategies such as learning resource cues (Laverty 1994; Chittka et al. 1997) or the diversity of the plant-pollinator networks at the landscape scale (Potts et al. 2006; Weiner et al. 2011). Working at different scales requires different allocation of sampling investment and resolution. Szigeti et al. (2016) suggested to specify three research approaches: the focus, the spatial and temporal scale of the study and the unit type of the count variables i.e. the count units to estimate flower resource amounts available for pollinators. Sampling depends on these three approaches, e.g. the spatio-temporal scale of measurement has to be adjusted to the foraging ranges and life cycles of focal pollinators (Szigeti et al. 2016). Furthermore, sampling design should also be adjusted to the type of the investigated biotopes, e.g. for homogeneous agricultural landscapes less sampling investment, i.e. smaller sampling cover is required than for heterogeneous semi-natural habitats. Taking all the factors influencing pollinator foraging into account is challenging and sampling protocols are rather difficult to design.

In order to understand the mechanisms of plant-pollinator interactions, or to establish restoration management, estimates of resource availability are mandatory (Dennis 2010). Sampling methods to estimate floral resource availability in the field are important key procedures of plant-pollinator studies (Dicks et al. 2013). Currently no generally accepted
methodology exists to estimate floral resource availability (Szigeti et al. 2016). Although there is a vast amount of studies investigating plant sampling protocols for botanical studies, including the comparison and evaluation of different methods (Walker 1970; Everson & Clarke 1987; Vittoz & Guisan 2007; Symstad et al. 2008), recommendations on how to measure floral resource availability for pollinators are scarce, and rather different methods have been applied to estimate resource availability (Frankl et al. 2005; Hegland et al. 2010; Hicks et al. 2016; Szigeti et al. 2016). Some studies neglect or do not refer to existing vegetation sampling protocols or the description of methodology is insufficient (Szigeti et al. 2016). Sampling floral resource availability is entirely missing in many pollinator studies, often only species lists, i.e. presence-absence data are recorded as an estimate (Kitahara et al. 2008), or resource availability is concluded from indirect proxies such as consumption rates (Bąkowski & Boroń 2005; Hinners & Hjelmroos-Koski 2009; Aronne et al. 2012). Further studies estimate the amount of floral resource, and the count variables can be the abundances of flowers as a proxy (Goulson & Darvill 2004; Kovács-Hostyánszki et al. 2013) or even nectar and pollen amounts (Potts et al. 2004; Hicks et al. 2016). Many pollination studies apply quadrat (sensu Gibson 2002) or transect sampling, commonly used by botanists for plant community studies (Elzinga et al. 1998; Gibson 2002; Bonham 2013), but these cover only a small proportion of the area of the study sites (Szigeti et al. 2016). Furthermore, different aspects of sampling, such as study site area and sampling coverage are traded off, reflecting to a limited research investment (Szigeti et al. 2016). These findings show that further field work on optimising sampling techniques, including traditional sampling methods is mandatory to find efficient and feasible methods to provide a sound basis for understanding plant-pollinator interactions.

We aimed to compare the benefits and pitfalls of two existing methods for sampling floral
We repeatedly scanned a single meadow to list insect-pollinated flowering plants and assigned an abundance category to each species. In parallel, we used repeated quadrat sampling in the meadow. Data collected with these methods were compared from the following aspects: (i) the number of species detected, since species richness is widely used as a proxy of floral resource availability, (ii) floral resource abundance, as abundance is an estimate for the importance of each species as a potential floral source, and (iii) temporal changes in flowering, estimated with first and peak flowering and the percent of daily changes in flowering.

Methods

Study site
We carried out field work in a 0.6 hectare meadow in the Visegrádi-hegység, Hungary (47°44'23"N, 19°03'33"E, at 300 m a.s.l.), between late April to early June in 2011–2013. Our study period was adjusted to the flight period of the Clouded Apollo butterfly *Parnassius mnemosyne* (L. 1758), since we were also interested in its nectar consumption patterns (Szigeti et al. *in prep.*). Therefore, our sampling period is limited from the point of view of other pollinators or flowering plants in general. The vegetation of this colline meadow is characterised as the Arrhenatheretalia association of the Pannonian floristic region. It is rich in insect-pollinated flowering plant species heterogeneously distributed in the meadow. It had previously been regularly mown once a year, but mowing had been abandoned at least for 20 years. The meadow is surrounded by a Turkey oak *Quercus cerris* (L. 1753) forest.

Sampling floral resource availability
We monitored the occurrence and the abundance of insect-pollinated flowering plants. We identified plant species according to Simon (1994). Sampling included scanning the entire field and using quadrats. Sampling was carried out by JK (scanning in all years) and VS (quadrats in all years and scanning in 2013), every three days on average, depending on weather (sampling dates: Tables 1 and 2), between 9:00-17:00h. We used pathways to avoid destroying the vegetation by trampling, and pathways were evenly scattered over the entire meadow (Figure 1).

First, we scanned insect-pollinated flowering plants by walking through the entire meadow along the pathways every three days in about one hour per sampling. We recorded a species list, and estimated the abundance for each species. Estimates were categories upon our overall impression of the meadow's vegetation during the one hour walk. We estimated the levels of flower abundance categories for each species only for open, non-wilted flowers on a rank scale for the entire meadow: 1: very scarce; 2: scarce; 3: more or less scarce; 4: more or less abundant; 5: abundant; 6: extremely abundant. We chose these categories because we felt that they could be reliably distinguished in the field and yielded the maximum achievable resolution. The definition and the used number of such categories may vary across studies. We aimed to use approximately equidistant categories. Similar sampling protocols were used in a few studies investigating food availability for insect pollinators (Stefanescu 1997; Goulson & Darvill 2004).

Second, we placed 36 2×2 m permanent quadrats more or less homogeneously in space, by distributing them along the pathways at random distances from the starting point of the respective path, placed 30 cm from the edge of the paths (Figure 1). We recorded plant abundance for each species by counting flowering shoots with open, non-wilted flowers every three days. Quadrats covered 2.4% of the total meadow area and we assessed ~20 quadrats per
hour. We investigated 36 quadrats because it required the time we could just fit in the
schedule of our other field studies such as observing pollinator behaviour. The 2×2 m quadrat
size is the minimal area generally used for mowed meadows (Lengyel et al. 2016) and the size
should not exceed 2×2 m, since small flowers in a larger quadrat can hardly be detected
without stepping in (Kearns & Inouye 1993).

**Number of species**

We compared the annual number of flowering species recorded with scanning and quadrat
sampling. We summed the number of species for each year and we presented the percent of
differences found with the two sampling procedures. We also calculated pseudoturnover, the
percentage of species overlooked by one observer or method but not the other, for both
methods for each year as well as for scanning between the two observers for 2013 (Nilsson &
Nilsson 1985; Morrison 2015). Pseudoturnover [%] = (A + B) / (N_a + N_b) × 100; where A and
B = the number of species recorded exclusively by one of the two observers or methods; N_a
and N_b = the total number of species recorded by each observer or method. We compared
annual presence-absence data between the two methods by computing Jaccard-indices
(Jaccard 1912). This index is a similarity coefficient showing the proportion of the species,
found by the two methods, to the number of available species. Furthermore, we applied a
binomial Generalized Linear Mixed model (Zuur et al. 2009; Bates et al. 2015) to investigate
if species detection per quadrat sampling event was affected by flower abundance, by using
the presence or absence of a floral species during an observation event as the response
variable. Flower abundance, estimated by scanning, was the explanatory variable, and we
included year and sampling event as nested random factors into the model to take the
dependence of observation events into consideration. We applied this model only for the
species detected by scanning at a given sampling event.

Floral resource abundance

For abundance analyses, we assigned zero to species abundance when a species was not recorded on a given sampling event, but it was detected at least once in a specific year. Furthermore, for quadrat sampling, we averaged the number of flowering shoots of each plant species across the quadrats for each sampling event. We calculated Kendall's correlation coefficients ($\tau$) between the mean number of flowering shoots and flower abundance categories provided by scanning.

We estimated the reliability of scanning between two recorders (JK & VS). We analysed flower abundance categories with squared weighted kappa (Graham & Jackson 1993). We give only kappa-values, omitting $p$-values, since our data points are not independent.

To see how many quadrats would be sufficient to properly estimate the amount of flowering shoots, i.e. flower density, we used the 36 quadrats as a pilot sampling, and we applied the method proposed by Kupper & Hafner (1989) following Elzinga et al. (1998): 

$$n = \left( \frac{(Z\alpha)^2 \times (s)^2}{B^2} \right)$$

where $n$ = uncorrected sample size estimate; $Z\alpha$ = Z-score from standard normal distribution corresponding to the desired confidence level ($Z = 1.96$ for 95% CI, $\alpha = 0.025$); $s =$ standard deviation; $B =$ the desired precision expressed as half of the maximum of the acceptable confidence interval width. We set $B$ equal to the mean, since our study site was patchy, providing large variation among quadrats, and we did not aim to reduce this variation because it characterises the entire study site. We corrected sample size (the number of quadrats) by using the correction table in Elzinga et al. (1998). We also estimated the sufficient proportion of the study site area covered with quadrats: we multiplied the estimated number of quadrats with 4 m$^2$ (area of one quadrat) and divided it by the area of the whole...
Temporal changes

We compared the days of first flowering and peak flowering between the two sampling methods using March dates (the number of days since 1st March). We smoothed species' flowering dynamics with a kernel smoother (bandwidth = 3; Wand 2013). We defined the date of first flowering of a species as the 1st day when the estimated flower abundance was higher than 5% of the species' maximum flower abundance. Peak flowering date was the day when the estimated value was the maximum. We excluded species that started flowering prior to sampling or that were likely to have peak flowering after the sampling period. We did not use the length of the flowering period and the last day of flowering, since field work was finished before some of the flowers would have started to wilt. We tested if the differences in the number of days between the two methods or the two observers were distinct from zero (one-sample exact Wilcoxon-signed-rank test). From kernel smoothed data, we calculated mean daily changes in the number of flowering shoots (quadrat) compared to its peak value for each species.

We analysed all data in the R statistical environment (R Core Team 2015), using the “sets” package (Meyer & Hornik 2009) for calculating Jaccard indices, the “lme4” (Bates et al. 2015) for generalized linear mixed models, the “irr” package (Gamer & Lemon 2012) for computing reliabilities, the “chron” package (James & Hornik 2013) for calculating March dates, the “KernSmooth” package (Wand 2013) for kernel smoothing, and the “coin” package (Hothorn et al. 2008) for the Wilcoxon-signed-rank test.
Results

Number of species

Scanning and quadrat sampling yielded different results finding insect-pollinated floral species, and this difference varied from year to year. With scanning, we found more species, but some species were found only by quadrat sampling. With the latter method, we found on average only 60.6% of species found by scanning (2011: 69.0%, quadrat: 40 / scanning: 58 species; 2012: 48.9%, 23/47; 2013: 63.8%, 44/69), while scanning allowed us to find on average 87.3% of the species found by quadrat sampling (2011: 90.9%, scanning: 40 / quadrat: 44 species; 2012: 79.3%, 23/29; 2013: 91.7%, 44/48). Two researchers found almost the same number of species by scanning (JK: 69 & VS: 73; 2013). Pseudoturnovers were 21.6% (2011); 39.5% (2012); 24.8% (2013) for the two methods, and 7.0% for scanning between the two observers in 2013. The Jaccard index comparing the two sampling methods for presence-absence data was on average 0.56 (2011: 0.65; 2012: 0.43; 2013: 0.60). The probability to detect a species by quadrat sampling significantly increased with increasing flower abundance (binomial model: exp(coefficients of flower abundance) is 2.34; $P < 0.001$; i.e. the odds that quadrat sampling finds a species increased 2.34 times with one category increase in flower abundance estimated with scanning).

Floral resource abundance

The number of flowering shoots (quadrat) and flower abundance categories (scanning) were correlated in most cases (2011: $\tau = 0.59$; 2012: $\tau = 0.49$; 2013: $\tau = 0.66$; Figure 2). Correlation varied within and between years (Table 1). The estimates of scanning were
reliable between the two observers ($\kappa = 0.82$; Figure 3). However, reliabilities varied during the sampling period (Table 2).

We predicted that $94 \pm 54$ (mean $\pm$ SD, averaged for all species), 11–161 (range) quadrats with a cover of $6.3 \pm 3.6\%$ (mean $\pm$ SD), 0.7–10.7\% (range) should be used for an appropriate estimate for the 0.6 ha study site.

**Temporal changes**

First flowers were detected earlier with scanning than quadrat sampling (mean $\pm$ SD difference in days, one-sample Wilcoxon-signed-rank test; 2011: $7.09 \pm 6.42$, $P < 0.001$; 2012: $6.17 \pm 3.24$, $P < 0.001$; 2013: $5.78 \pm 5.50$, $P < 0.001$). Scanning and quadrat sampling gave similar estimates for the date of peak flowering (2011: $-0.53 \pm 3.57$, $P = 0.781$; 2012: $-0.96 \pm 3.47$, $P = 0.363$; 2013: $1.83 \pm 6.24$, $P = 0.147$). By scanning, JK found first flowers slightly but significantly earlier than VS ($0.83 \pm 4.18$, $P = 0.007$), and both observers estimated peak flowering similarly ($-0.43 \pm 7.57$, $P = 0.749$). The number of flowering shoots changed $5.8 \pm 5.4\%$ per day [mean $\pm$ SD].

**Discussion**

**Number of species**

We found more potential floral resource species with scanning than with the quadrat method. Pseudoturnover and Jaccard-index values imply that the two methods found plant species with different chances. Pseudoturnovers between the two methods were much larger than between the two observers for scanning, and for scanning, the inter-observer error was
smaller than values found in published botanical studies (Vittoz & Guisan 2007; Symstad et al. 2008; Morrison 2015). Although we failed to detect a few species by scanning that were found by quadrat sampling, this may be due to (i) the difference between the focus of the two methods, i.e. with scanning, researchers may overlook tiny plant species covered with vegetation, and (ii) the difference between the sampling persons in their ability to distinguish similar species (Elzinga et al. 1998; Morrison 2015). The odds to find a species with quadrat sampling deteriorated with species rarity. We suggest that even abundant species could be overlooked with quadrat sampling, if plants are highly aggregated in space, especially if only a small proportion of the entire area is sampled thoroughly. Various methods may detect different species with different probabilities (Walker 1970; Everson & Clarke 1987; Vittoz & Guisan 2007). Since the number of flowering plant species are often increasing with pollinator species richness (Ebeling et al. 2008), some studies listed only the presence of species to predict floral resource availability (Kitahara et al. 2008). In contrast, we recommend using quantitative estimates such as flower abundance, because species lists alone are not suitable estimates of floral resource availability (Hegland & Boeke 2006). For estimating the number of species of potential floral resources for a study site, scanning the whole area, as we used in this study and similar to the method used by Goulson & Darvill (2004) or using belt transects (Carvell et al. 2007) are more appropriate than quadrat sampling. The presence of frequently visited, although rare floral sources may be noticed with the help of pollinator behaviour.

**Floral resource abundance**

Scanning and quadrat sampling yielded similar results, although with a large scatter. Similarly, standard botanical sampling procedures also provide different values for abundance.
The scanning method we used, as well as similar methods (e.g. Goulson & Darvill 2004), are subjective sampling techniques with low resolution and high coverage. They are fast and easy to apply at the landscape scale, no special skills or equipment are needed, except to be able to identify all flowering plants at the required taxon level (Elzinga et al. 1998). Although identifying all the plant species is a strong skill in some regions, this is necessary for most methods. Scanning yields more information than just listing presence/absence, since it provides a rough estimate on abundance, thus enables detecting phenological trends. Studies using similar estimates involved larger sampling areas than direct counts (Szigeti et al. 2016), thus researchers have to decide on either using higher estimate accuracy or higher spatial coverage. Due to higher coverage, scanning compared to quadrat sampling is less sensitive to measurement errors caused by habitat patchiness. Scanning was fairly reliable between the recording persons, although reliabilities varied during the sampling period. Reliability could be enhanced with experience. The qualitative measurement of abundance is usually influenced by personal bias (Bonham 2013), e.g. for many botanical methods, there are lots of differences between observers (Walker 1970; Vittoz & Guisan 2007) and this bias is expected to increase with the method's subjectivity (Morrison 2015). Some source of bias may come from ill-defined categories and lack of experience. An other important source of bias for this method is that the detectability of different flower species is different (Bonham 2013; Morrison 2015), e.g. tiny *Myosotis discolor* (Pers. 1798) flowers are less likely to be detected than the large flowers of *Inula hirta* (L. 1753). A further drawback of scanning is its inability to estimate data scatter per sampling event per species, since it yields only one data point for each species per sampling, although partitioning the trajectory scanned may refine this procedure and provide more accurate data.
Overall, we think that scanning yields a reasonably good estimate for abundance. With careful standardization of the sampling procedure, this method could be significantly improved. Carvell et al. (2007) associated estimated density ranks to rough count estimates, using the following ranking: 0 shoots = 0 rank; ≤ 25 shoots = 1; 26–200 shoots = 2 etc. We recommend using even-spaced or even-spaced logarithmic categories (Stefanescu 1997; Elzinga et al. 1998), because such data can be handled easier mathematically compared to uneven categories, and may considerably increase the reliability of scanning for a relative density estimate. The number of categories should be adapted to the study. Hahn & Scheuring (2003) claim that the best estimates can be achieved at an optimum number of categories, recommending e.g. ten categories. Although ten categories provide high resolution relative to the method's simplicity, this requires even-spaced sampling, especially for small datasets, since a variable with even-spaced categories may be used as a numerical covariate in statistical modeling. We recommend using scanning for rough-scale estimates when time-consuming quadrat sampling is not feasible (e.g. mapping good honey-plant meadows for beekeepers). Further investment may be required due to the difficulties to estimate the number of available flowers for species with different inflorescence structures, or nectar amount estimates.

Using quadrats to estimate flowering plant abundance provides much higher resolution with the cost of requiring much more research investment than scanning. For example, quadrat or transect sampling need a lot of key sampling design decisions, such as the arrangement of plots, boundary definitions, plot size and shape, counting unit, and estimate precision, and presumes a preliminary survey. Most of these are thoroughly investigated in the botanical methodology literature (Elzinga et al. 1998; Gibson 2002; Bonham 2013) and should be used for floral resource estimates as well. Among these, the estimate on the
necessary number of quadrats is important in our case. Insufficient quadrat cover yields biased data (Hicks et al. 2016) 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; Bonham 2013). Our results indicate that more quadrats should be applied than generally used in most case studies. Compared to the median 0.69% for 158 studies reviewed by (Szigeti et al. 2016), we covered 2.4% of our study site and this turned out to be insufficient to estimate the abundance of most species. Approximately 6.3% of our heterogeneous study site should have been covered for a reliable estimate, similar to the recommendations (5–10%) for standard botanical studies (Bonham 2013). We did not find other recommendations, but the appropriate cover is probably highly dependent on vegetation heterogeneity. Recommendations for different types of vegetation based upon thorough studies would be helpful to those having limited capacity to assess floral composition. However, the proper procedure is to run a pilot study to estimate sampling cover prior to sampling. In natural biotopes, flowers are often aggregated (Elzinga et al. 1998; Hatfield & Lebuhn 2007), thus the minimum number of quadrats required may be rather large. The formula of Kupper-Haffner's method implies, as well as Hegland et al. (2010) and Bonham (2013) suggest that the rare and/or aggregated species increase the number of sufficient quadrats considerably. Since different aspects of research investment are traded off (Szigeti et al. 2016), researchers need to find the overall optimum for achieving sufficiently high resolution and coverage simultaneously. Nevertheless, in homogeneous biotopes such as agricultural plots, especially in crops, even a smaller number of sampling units may be sufficient and quadrat sampling is recommended. Quadrat cover may also depend on the type of the count variables, not only on species richness and biotope heterogeneity. Choosing the appropriate count variables are essential,
since if the estimated nectar and pollen production of flower units are known, measuring
proxies could provide reliable data of the total food production of an entire meadow, e.g. the
number of flowers and flower size are related to nectar and/or pollen amount (Stanton &
Preston, 1988; Galetto & Bernardello 2004; Potts et al. 2004; Torné-Noguera et al. 2014;
Hicks et al. 2016; but Wäckers 2004). Researchers use various count variables to assess
resource availability (Szigeti et al. 2016), such as estimates on sugar and amino acid contents
of nectar and pollen (Zimmermann & Pleasants 1982; Kearns & Inouye, 1993), often using
proxies (Hegland & Totland 2005). Using a count variable simple to estimate, such as the
categorical estimates on the number of flowers, including floral traits like nectar and/or pollen
amounts were also measured for a couple of individuals in all plant species and the
measurements of these floral traits were extrapolated to study site (Hegland & Totland 2005;
Hicks et al. 2016). This provide more reliable estimates on food availability than using solely
proxies such as flower units, although the effective sampling of very small volumes of nectar
or pollen in a sufficient amount for measurements is complicated and labour-intensive

Walker (1970) found that with increasing time spent on a sampling event, precision
dropped, and the limit of reliable botanical sampling time was 4–5 h per day in a tropical
study. Kearns & Inouye (1993) took 12 hours for counting the number of flowers in 25 2×2 m
quadrats. We needed one hour to sample 20 quadrats for counting flowering shoots. We used
36 quadrats since we were time-constrained, and the sufficient quadrat estimates predicted on
average 94 quadrats necessary to use, i.e. about 5 hours of sampling in a 0.6 hectare meadow.
In contrast, scanning of our 0.6 ha meadow took one hour. Scanning might be accelerated and
used also for much larger meadows effectively. We suggest that using both quadrats and
scanning may help to optimise the trade-off between spatio-temporal resolution and sample
coverage for a given amount of research investment (Szigeti et al. 2016). Because quadrat sampling detects some species poorly and probably estimates the abundance with larger scatter for rare species, relative densities for the more or less abundant species can be estimated with quadrats whereas rare species can be detected with scanning.

**Temporal changes**

Scanning estimated the appearance of first flowers earlier than quadrat sampling, although there was a large variation among species. We found that recording presence-absence of flowering species in an entire meadow potentially detected most flowering species earlier than quadrats. Earlier detection with scanning is due to small quadrat cover relative to the spatial heterogeneity of the vegetation. Furthermore, one observer found first flowers slightly earlier than the other, and it may reflect how this method is influenced by field experience. Nevertheless, we did not find differences in detecting peak flowering either between the observers or the two methods. Investigating peak flowering might yield more robust results than first flowering dates (see also Miller-Rushing et al. 2008). Scanning in small meadows potentially detects all flowering species even in case of extreme species heterogeneity. In contrast, quadrat sampling is more appropriate to estimate the change over time in the relative densities across species, due to its higher resolution. In order to get better estimates for first, peak and last flowering dates, we recommend using kernel smoothing (Wand 2013), if sampling is carried out frequently enough for data imputation. The daily changes in flower abundances were 6% in our study, and it can be very different across various biotopes or seasons. Seasonal changes in flower abundances were also found by others (Kubo et al. 2008; Bagella et al. 2013; Hicks et al. 2016). Pollinators necessarily follow the changes in the temporal distribution of flowering phenology (Goulson 1999; Potts et al. 2004; Kubo et al.)
Even so, rapid changes in floral resources are overlooked in many studies (Szigeti et al. 2016), although this should be taken into account when planning sampling frequency. In contrast, many studies used >30 days for sampling intervals (Szigeti et al. 2016). Furthermore, time elapsed between sampling events often increases with the number of sites, indicating that sampling frequency is determined by research effort constraints (Szigeti et al. 2016). We argue that this typical trade-off between spatial and temporal representativeness could be overcome by combining different methods with either a high spatial or high temporal resolution.

We suggest that scanning presence-absence of flowering species in an entire meadow might detect some species to 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.

**Conclusion**

We compared two sampling methods to estimate floral resource availability. Our data were collected in one single meadow, thus conclusions have to be treated with caution. We found that scanning and quadrat sampling yielded more or less similar results for estimating abundance, although the two methods were different in detecting species. We suggest that increased sampling coverage and frequency may enhance research accuracy and using scanning and quadrat sampling simultaneously may help to optimize research effort. Although a large variety of methods for botanical sampling is available (Elzinga et al. 1998; Gibson 2002; Bonham 2013), there are no appropriate, standardized, widely used methods to measure pollinators' floral food resources, even if some recommendations are available in Frankl et al.
With this case study we would like to initiate further development of sampling protocols. Future studies should address evaluating several sampling protocols in order to find or develop more appropriate sampling techniques to estimate pollinators' floral resource availability.

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Table 1. Kendall’s tau for the estimates between scanning (daily ranks) and quadrat sampling (abundance) by year. We show March dates (the number of days since 1st March) of sampling events for both methods. Dates may be slightly different between the methods.

<table>
<thead>
<tr>
<th>Year</th>
<th>Scanning</th>
<th>Quadrat</th>
<th>Scanning</th>
<th>Quadrat</th>
<th>Scanning</th>
<th>Quadrat</th>
<th>Scanning</th>
<th>Quadrat</th>
</tr>
</thead>
<tbody>
<tr>
<td>2011</td>
<td>0.59</td>
<td>0.59</td>
<td>0.34</td>
<td>0.48</td>
<td>0.69</td>
<td>0.67</td>
<td>0.65</td>
<td>0.66</td>
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<tr>
<td>2012</td>
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<td>0.49</td>
<td>0.54</td>
<td>0.66</td>
<td>0.66</td>
<td>0.71</td>
<td>0.72</td>
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<tr>
<td>2013</td>
<td>0.54</td>
<td>0.56</td>
<td>0.55</td>
<td>0.55</td>
<td>0.64</td>
<td>0.64</td>
<td>0.57</td>
<td>0.65</td>
</tr>
</tbody>
</table>

Table 2. Reliabilities for flower abundance estimates between the two sampling persons, JK and VS: squared weighted kappa for categorical data. We show March dates (the number of days since 1st March) of sampling events for both recorders. Dates may be slightly different between recorders.

<table>
<thead>
<tr>
<th>Year</th>
<th>JK</th>
<th>VS</th>
<th>Kappa</th>
</tr>
</thead>
<tbody>
<tr>
<td>2013</td>
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<td>56</td>
<td>0.58</td>
</tr>
<tr>
<td></td>
<td>59</td>
<td>60</td>
<td>0.68</td>
</tr>
<tr>
<td></td>
<td>65</td>
<td>63</td>
<td>0.82</td>
</tr>
<tr>
<td></td>
<td>68</td>
<td>67</td>
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</tr>
<tr>
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<tr>
<td></td>
<td>100</td>
<td>100</td>
<td>0.82</td>
</tr>
</tbody>
</table>
Legends

**Figure 1.** Study site. White patches represent shrubs, forest or individual trees, dotted lines are the pathways, dark squares show quadrat positions.

**Figure 2.** Relationships between flower abundance categories (scanning) and the number of flowering shoots by years. Each dot represents one plant species at a single sampling event. Dots are jittered horizontally and vertically for better visualisation.

**Figure 3.** Scatter plot to visualize reliability for estimating flower abundance categories for the two observers for scanning. Each dot represents a plant species found at a specific sampling event. Dots are jittered horizontally and vertically for better visualisation.