1 **Measuring floral resource availability for insect pollinators in temperate**

2 **grasslands – a review**

3 Running title: Measuring floral resource availability

4

7

8 VIKTOR SZIGETI[1,2], ÁDÁM KŐRÖSI[2], ANDREA HARNOS[3], JÁNOS NAGY[4] and JÁNOS KIS[1]

9 [1]Institute for Biology, Szent István University, Budapest, Hungary, [2]MTA-ELTE-MTM Ecology

10 Research Group, Budapest, Hungary, [3]Department of Biomathematics and Informatics, Szent István

11 University, Budapest, Hungary, [4]Institute of Botany and Ecophysiology, Szent István University,

12 Gödöllő, Hungary

13

14 Correspondence: János Kis, Institute for Biology, Szent István University, Rottenbiller utca 50, H-

15 1077, Budapest, Hungary, Phone: +36 70 380 29 23, E-mail: jkis17@gmail.com

16

17 **Abstract** 1. The relationship between pollinators and flowering plants plays a crucial role in the

18 function of terrestrial ecosystems. Although pollinators use floral nectar and pollen as food

19 resources, no general methodology for floral resource availability estimates exists.

20   2. We provide a brief review on floral resource sampling methods frequently used in pollination

21 studies. We focus on how representative vegetation samples are both spatially and temporally, and

22 how these are constrained by sampling effort.

23   3. We selected field studies investigating flowering plant abundance for insect pollinators, in

24 temperate grasslands. We categorised the reviewed studies according to aims, sampling units and

25 count variables used and provide a descriptive summary on methodology. We also searched for

26   trade-offs between different aspects of sampling investment.

27   4. We reviewed 159 pollination studies. We found large methodological differences, and

28   vegetation sampling was presented in many studies insufficiently. Sampling covered a small

29   proportion (median: 0.69%) of the study sites, with long intervals (median: 30 days), and most

30   studies lasted only a few years. The most often used count variables were indirect proxies of floral

31   resources. We found negative relationships in some of the different aspects of sampling, e.g. the

32   proportion of site covered with sampling decreased with increasing site area.

33   5. By tailoring sampling methods to specific research questions, research effort should be

34   optimally allocated to obtain proper spatio-temporal resolution and data coverage. We suggest

35   guidelines to design sampling, e.g. to increase coverage and frequency. We think that further field

36   work on optimising sampling techniques is mandatory.

37

38

39   **Key words:** plant-animal interactions, flower, food-resource estimate, nectar resources, insect

40   pollinated plants, vegetation sampling methods

41

42

43   **Introduction**

44

45   The relationship between pollinators and flowering plants plays a crucial role in maintaining most

46   terrestrial ecosystems. Recently, there has been an increasing interest in pollination studies

47   manifested in intensive research on a potential pollination crisis and consequent harvest fall (Potts

48   *et al.*, 2010; but see Ghazoul, 2005), human impacts and the effect of climate change on pollinator

49   communities (Benadi *et al.*, 2014; Petanidou *et al.*, 2014), and understanding pollinator foraging

50   strategies (Goulson, 1999). The number of studies investigating plant-pollinator relationships at the

51 community level and at the landscape scale is also increasing (Hegland & Totland, 2005; Henry *et*

52 *al.*, 2012) and understanding entire plant-pollinator networks received special attention (Burkle et

53 al. 2013). Several important and yet not sufficiently understood key problems concerning the

54 conservation of plant-pollinator systems, such as sampling floral resource availability for

55 pollinators, were identified by Dicks *et al.*, (2013).

56   Pollinators feed on nectar, pollen and oils produced by flowers (Goulson, 1999). Quality,

57 quantity and production rates are highly changing by plant species, time of the day, age of flowers

58 and competitors' consumption (Nicolson *et al.*, 2007). Resources offered to pollinators are

59 advertised via many flower traits, but some flowers may deceive pollinators providing no reward

60 (Goulson, 1999; Nicolson *et al.*, 2007). Pollinator abundance, diversity and resource-visit frequency

61 are influenced by the number of floral resource species, quantity and density of flowers and the

62 amount and quality of food in flowers, being the strongest factors structuring pollinator

63 communities (Potts *et al.*, 2004; Dennis, 2010). In animal-pollinated species, the length of the

64 flowering period, seed production and plant population dynamics depend on pollination (Nicolson

65 *et al.*, 2007). Components falling out from complex plant-pollinator networks, due to local

66 extinction, or temporal mismatches in plant-pollinator phenologies caused by differential effects of

67 global change drivers such as climate change, or habitat loss and degradation (Burkle *et al.*, 2013),

68 might have severe impacts on a given community. To investigate such potential impacts on a

69 network requires reliable sampling methodology. Therefore, estimates of resource availability are

70 essential in order to understand such ecological interactions and to establish restoration

71 management (Dennis, 2010).

72   Botanists and zoologists study pollinators and flowering plants from different perspectives and

73 use a wide range of methods in pollination research (Goulson, 1999; Bosch *et al.*, 2009). Some

74 pollinator studies investigate flower availability superficially, by using only, for instance, species

75 richness of flowering plants (Kitahara *et al.*, 2008), while some studies even neglect it completely

76 and often conclude floral resource availability from indirect proxies such as consumption rates

77 (Bakowski & Boron, 2005), pollen distribution in honey (Aronne *et al.*, 2012), or pollinators' pollen

78 load (Hinners & Hjelmroos-Koski, 2009). Apparently, no generally used methodology exists to

79 estimate floral resource availability, and many studies neglect standard vegetation sampling

80 protocols (Elzinga *et al.*, 1998; Gibson, 2002). For instance, the suitability of counting flowers or

81 sampling nectar was debated as early as the beginning of the 1980s (Tepedino & Stanton, 1981;

82 Zimmerman & Pleasants, 1982; Tepedino & Stanton, 1982). Recommendations on how to measure

83 floral resource availability for pollinators are still scarce (Zimmerman & Pleasants, 1982; Tepedino

84 & Stanton, 1982; Frankl *et al.*, 2005; Hegland *et al.*, 2010).

85   We think that three important decisions have to be made before choosing an appropriate method

86 to investigate pollinator food resource sampling. First, one has to choose the *focus* of the study:

87 whether to investigate a single plant species and all its pollinators (Thompson, 2001), or a single

88 pollinator species and all its flower resources (Rusterholz & Erhardt, 2000), or the entire pollination

89 network (Junker *et al.*, 2013). Second, the *spatial and temporal scale* of the study has to be adjusted

90 to the often wide array of foraging ranges and life cycles of focal pollinators (Osborne *et al.*, 2008;

91 Dennis, 2010). The spatio-temporal resource distributions are also various including high seasonal

92 and annual variation (Alarcón *et al.*, 2008; Kubo *et al.*, 2008; Dennis, 2010). These imply the

93 necessity of frequent sampling and long-term studies, ranged from the level of individual flowers

94 through patches to biotopes and landscapes (Hatfield & Lebuhn, 2007; Westphal *et al.*, 2008).

95 Third, to define the unit of the *count variables* (i.e. count units to estimate flower resource amounts

96 available for pollinators) insect perception should be taken into account (Kearns & Inouye, 1993).

97 Plants have rather different body plans and inflorescence structures, and their pollinators are not

98 less various in morphology, timing and foraging range, and these two parts have to match. Flowers

99 therefore, may be perceived very differently by different pollinators, since insects use various cues

100 to navigate at different spatial scales and use a wide range of sensory systems (Dauber *et al.*, 2010;

101 Dennis, 2010, Clarke *et al.*, 2013). Count variables can be nectar and pollen amount, counts of

102 single flowers or inflorescences, the number of flowering shoots, or the number of single-species

103 flower patches, and may vary depending on the pollinators investigated.

104   In the light of these three points and that the suitability of different methods depends on the

105 specific research question, it is clear why generally used sampling methods are not available for

106 investigating so complex systems. In this paper, we aim to review the methodology of estimating

107 food availability for insect pollinators in temperate grasslands. We focus on how representative

108 vegetation samples in pollination studies are both spatially and temporally, and how these are

109 constrained by sampling effort. We also highlight challenges in estimating floral resource

110 availability.

111

112

113 **Data and methods**

114

115   Our aim was to review research papers focusing on the relationships between resource

116 availability for insect pollinators and pollinator abundance, diversity or flower preferences. We

117 searched for papers upon four groups of search terms (i) "bee", "bee fly", "bumblebee", "butterfly",

118 "hoverfly", "moth", "pollinator", "visitor", "wasp"; (ii) "diversity", "foraging", "feeding",

119 "network", "preference"; (iii) "floral", "flower", "nectar", "pollen" and (iv) "availability",

120 "resources", and we used "and" operator between groups and "or" operator between keywords

121 within groups. We used the databases *ISI Web of Science* (www.webofknowledge.com) and *Scopus*

122 (www.scopus.com), accessed 08 Nov 2015. We selected field studies investigating flowering plant

123 abundance, aimed at insect pollinators only, and carried out in the temperate climate zone. We

124 excluded publications focusing only on a single or very few plant species, or mainly on flowering

125 shrubs and trees, because the latter requires rather different sampling methods (references of the

126 reviewed studies: Appendix S1).

127   We categorised the reviewed studies according to (i) aims, (ii) the sampling units and (iii) the

128 count variables used, and (iv) whether estimates on the amount of nectar or pollen were applied. We

129 refer to sampling units as "quadrat" in the broad sense, i.e. quadrat is a more or less equal sided

130 sample area (Gibson, 2002) in all cases when authors used the terms "quadrat", "square", "circle" or

131 "plot". Transects were elongated sampling units. We extracted information on sampling unit shape,

132 as well as length and width of the sampling unit if it was quadrat or transect. Count variables (e.g.

133 the number of flowers or visual floral display) were measured either with (i) rough estimates, such

134 as ranks, and green cover or flower cover estimates, in all cases where flowering shoots, or

135 inflorescences were not accurately counted, hereafter referred to as *categorical estimates*, or with

136 (ii) *direct counts* of all shoots or other types of counted units within a sampling unit. Furthermore,

137 we extracted the following numerical data from the articles: number of study sites; site area;

138 number of sampling units per site per sampling event; area of sampling units; length, width and

139 radius of sampling units; sampling interval; length of the study in years and the number of

140 pollinator species (raw data: Appendix S2). We calculated mean values of these variables, if more

141 than one values were given per study. Furthermore, we calculated sampling unit length:width ratio

142 in case of rectangle-shaped sampling units, the total area of sampling per event, and the proportion

143 of the site covered by sampling, if data were available (Appendix S2).

144   We present descriptive statistics by giving median, minimum and maximum values, and showing

145 boxplots with individual data points. We investigated relationships between the temporal and spatial

146 resolution of the studies to detect potential trade-offs in research investment. We expected trade-offs

147 between the area of study sites and the number of sites; the proportion of the site covered by

148 sampling and the number of sites; the number of sampling units and the area of study sites (we

149 analysed studies using quadrats or transects pooled with all other studies as well as separately); the

150 area and the number of sampling units; the proportion of the site covered by sampling and the area

151 of study sites, sampling interval and the number of study sites; sampling interval and site area;

152 sampling interval and the proportion of the site covered by sampling. We provide Kendall's *tau*

153 correlation coefficients for rank data and *p*-values corrected for multiple comparisons with the

154 method of Benjamini & Hochberg (1995). We also expected that categorical estimates require less

155 research effort than direct counts, thus using categorical estimates allows sampling a larger total

156 area than when direct counts are used. We tested this assumption with Mood's median test. We

157 analysed all data in the R statistical environment (R Core Team, 2015).

158

159

160 **Results**

161

162 We found 159 studies published in 1981–2015 with the aims of estimating "pollinator population

163 size or diversity" in 104 (66.0%) cases, "flower preferences" in 42 (26.6%) cases, both in 8 (5.1%)

164 and "other" in 4 (2.5%) studies (raw data: Appendix S2, descriptive statistics: Fig. 1.). One study

165 (Miller-Struttmann *et al.*, 2015) was based on two different historic datasets using different

166 methodologies, and we analysed these data as if they were coming from two independent studies.

167 Authors investigated 1–665 (median: 20) pollinator species, in 1–216 (median: 16, Fig. 1.A.) study

168 sites, with 8 m^2–125 km^2 (median: 10 ha, Fig. 1.B.) site area per study. We found rather different

169 methods in the reviewed studies, i.e. the applied sampling units and count variables varied

170 considerably. Vegetation sampling procedures, such as the spatial and temporal distribution of the

171 sampling units in the study sites were not clearly described (note NA-s in Fig. 1.) or the reasons

172 why a given method had been used remained unexplained in many studies. For example, 57

173 (35.8%) studies lacked information on site area and 66 (41.5%) studies lacked information

174 necessary to compute the proportion of the study site covered with the sampling units.

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

176 (5.0%), or point intercept technique in a single study. The shapes of the sampling units, we refer to

177 as quadrats in the broad sense (Gibson, 2002), were squared quadrats (41.7 %), rectangles (10.4%)

178 and circles (10.4%) (Table 1.). Transects were belt transects (rectangular) in most cases (87.0%) and

179 line transects in a few cases (3.7%), and in the rest of the studies transect type was not specified

180 (Table 1.). Sampling was carried out using only a few sampling units per site per sampling event

181 (median: 5, Fig. 1.C.), with 20 m^2 of median unit area (quadrat: 2 m^2, transect: 250 m^2, Fig. 1.D.,

182 Table 1.). The median cover of the study site area was 0.69% (Fig. 1.E.). Count variables were

183 flower unit (i.e. visual display) (28.8%), flower (24.4%), flowering shoot (13.5%), flower cover

184 (12.8%), inflorescences (10.3%), green cover (7.7%), and frequency of flowering shoots (1.9%).

185 The measures of count variables were *categorical estimates* in 36.5% and *direct counts* in 61.6% of

186 the studies. Nectar or pollen amounts were estimated in 8.8% of the studies with any method,

187 although nectar amount was the count variable only in a single study (0.64%). Studies were one

188 (63.9%), two (20.9%), three (8.2%), or four (4.4%) years long, only one lasted five, two lasted six

189 and yet another nine years. Most sites were sampled with low frequency (median sampling interval:

190 30 days) during the study period and many of these used calendar time intervals, e.g. weekly,

191 monthly, or annual sampling (Fig. 1.F.).

192   We found negative relationships in some of the different aspects of sampling. The area of study

193 sites was slightly smaller if the number of sites were larger (*tau* = –0.20, *P* = 0.007, *n* = 102, Fig.

194 2.A.). The proportion of the area covered by sampling was not related to the number of sites (*tau* =

195 –0.02, *P* = 0.794, *n* = 93, Fig. 2.B.). We did not find a relationship between the number of sampling

196 units and the area of the study site, if we analysed all types of sampling units pooled (*tau* = 0.13, *P*

197 = 0.099, *n* = 99, Fig. 2.D.). Although we did not find a relationship when analysing transects only

198 (*tau* = –0.14, *P* = 0.388, *n* = 27), we found that the larger was the area of the study site, the more

199 quadrats were used (*tau* = 0.29, *P* = 0.003, *n* = 60) when quadrats were analysed separately. With

200 smaller sampling unit area, the number of units increased (*tau* = –0.47, *P* < 0.001, *n* = 99, Fig.

8

201 2.G.), although the proportion of the whole area covered by sampling significantly decreased with

202 site area ($tau = -0.45$, $P < 0.001$, $n = 93$, Fig. 2.E.). Sampling interval increased with the number of

203 sites ($tau = 0.32$, $P < 0.001$, $n = 148$, Fig. 2.C.), but was neither related to site area ($tau = 0.06$, $P =$

204 0.478, $n = 95$, Fig. 2.F.) nor to the proportion of the site area covered with sampling units ($tau = -$

205 0.10, $P = 0.267$, $n = 86$, Fig. 2.H.). Researchers using categorical estimates sampled significantly

206 larger total sampling areas during a single sampling event (median = 4500 m², $n = 49$) than those

207 using direct counts (median = 446 m², $n = 90$; Mood's median test: $P < 0.001$).

208

209

210 **Discussion**

211

212   We found rather different methods applied to estimate food resource availability for pollinators

213 in the reviewed studies. All variables characterising sampling strategies showed an extreme scatter

214 (Fig. 1.). We found no general methodology, and many studies neglected or did not refer to existing

215 vegetation sampling protocols (Elzinga *et al.*, 1998; Gibson, 2002). Some important details were

216 not described in many cases, and the reasons why the given methods had been used were rarely

217 explained. The lack of detailed description of methodology was also found by Mortelliti *et al.*,

218 (2010) who reviewed studies of habitat quality. They concluded that this lack of information hinders

219 carrying out meta-analyses (Mortelliti *et al.*, 2010). In addition, such practice makes reproducibility

220 impossible. We suggest that the role of size and spatio-temporal heterogeneity of study sites were

221 underestimated in many of the reviewed papers, as in the vast majority of studies in the field of

222 ecology (Mortelliti *et al.*, 2010). Most of the reviewed pollinator studies did not carefully design

223 resource availability sampling relative to the problem's complexity.

224   Many studies aim to primarily sample important and/or abundant plant and pollinator species

225 (Hegland *et al.*, 2010), although rare species might also play an important role in maintaining

226 specialised pollinators (Bosch *et al.*, 2009). We suggest that not only the rare, but even abundant

227 species can be overlooked if flowers are highly aggregated in space, especially if only a small

228 proportion of the entire area is thoroughly sampled, e.g. when using quadrats or transects.

229 Furthermore, various methods may detect different floral species with different probabilities. Based

230 on the fact that the number of flowering plant species was usually positively correlated with

231 pollinator species richness (Ebeling *et al.*, 2008), some studies used only species lists, i.e. presence-

232 absence data, to predict floral resource availability (Kitahara *et al.*, 2008). However, we agree with

233 Hegland & Boeke (2006) that species lists alone are not appropriate estimates of floral resource

234 availability: some quantitative estimates such as flower abundance are recommended.

235   Data on foraging ranges of some pollinators (Osborne *et al.*, 2008; Dennis, 2010) imply that the

236 design of vegetation sampling was not representative in many of the reviewed studies, e.g. due to

237 low spatial coverage. Pollinator home range as well as floral species phenology and its

238 consequences for spatio-temporal variation in resource availability must also be taken into account

239 to delineate study site and determine sampling methods for resource availability and resource use at

240 the same scale. For instance, using the same sampling units may help to find the link between

241 resource availability and resource use (Rusterholz & Erhardt, 2000; Hegland & Totland, 2005).

242 Natural biotopes in the temperate zone are highly heterogeneous and many flowers are aggregated

243 (Elzinga *et al.*, 1998; Hatfield & Lebuhn, 2007), and the spatial heterogeneity also influences the

244 minimum number of sampling units required. If spatial coverage of sampling is low, then many

245 species will be estimated with large bias (Hegland *et al.*, 2010). Unfortunately, we did not find

246 recommendations on the proportion of the study site covered to sample floral resources. Compared

247 to the median 0.69% cover for the reviewed studies, for an accurate estimate in a field study, we

248 should have covered about $6.3 \pm 3.6\%$ [mean $\pm$ SD] of a 0.6 ha Central European colline meadow,

249 estimated by Kupper's and Hafner's method (Kupper & Hafner, 1989, modified by El*zinga* et al.,

250 1998; S*zigeti* et al., unpublished). Insufficient quadrat cover yields biased data especially on rare

251 and clumped species. On the one hand, clumped species can be sampled with less bias if quadrat

252 sizes are increased or their shape varied, e.g. from square to elongated rectangle (Elzinga *et al.*,

253 1998). On the other hand, quadrat size should be maximum 2m × 2m, because small flowers in a

254 larger quadrat can hardly be detected without stepping in (Kearns & Inouye, 1993). Long and

255 narrow sampling units may overcome this problem (Elzinga *et al.*, 1998). We found a large scatter

256 in the shape of sampling units (Table 1.). Although shape may resolve sampling difficulties for

257 aggregated plants generally, we found no arguments on why a specific shape was used, except in

258 those cases when the same transects were used for pollinator and plant sampling. The median 2 m

259 belt width indicates that most researchers follow Kearns & Inouye's (1993) recommendation,

260 although the large range shows that still many authors use belt widths within which detectability

261 might vary severely.

262   Kearns & Inouye (1993) needed 12 hours for counting the number of flowers in 25 2m × 2m

263 quadrats. The research effort necessary for a thorough quadrat sampling may also depend on the

264 type of the count variables, not only on species richness and biotope heterogeneity. Nevertheless, in

265 homogeneous biotopes such as agricultural plots, even a smaller number of sampling units may be

266 sufficient.

267   Determining the *count variable*, the unit of resource availability, is also difficult. A count

268 variable should estimate the feeding unit of the pollinator (Kearns & Inouye, 1993) and take into

269 account how pollinators find their food resources (Goulson, 1999; Dauber *et al.*, 2010). Both count

270 variables and feeding units may be specific to both plant and pollinator species and to the aim of the

271 study. The most frequently used count variables were those simple to estimate, such as the number

272 of flowers or flower area, and only a handful of studies investigated resource value (pollen or nectar

273 amount) for different plant species or referred to other studies assessing resource values. We found

274 direct measures of nectar-resource values only in a single study (Potts *et al.*, 2004). Although the

275 ultimate goal to assess resource availability would be to obtain estimates on sugar and amino acid

276   contents of nectar and pollen (Zimmermann & Pleasants, 1982), considering that food amount

277   depends on species, site, individual, weather etc. (Tepedino & Stanton, 1982; Nicolson *et al.*, 2007),

278   such estimates are rarely feasible. For example, collecting nectar in a sufficient amount for

279   measurements is either complicated and labour-intensive, or hardly feasible at all for many flower

280   species (Tepedino & Stanton, 1982; Morrant *et al.*, 2009). Hegland & Totland (2005) argued for

281   using proxies, because the number of flowers and flower size were related to nectar amount in

282   several studies. This relationship has been demonstrated mostly within species or families (Stanton

283   & Preston, 1988; Galetto & Bernardello, 2004), albeit very few studies are available for

284   investigating communities; some found similar relationships (Potts *et al.*, 2004; Torné-Noguera *et*

285   *al.*, 2014), while others did not (Wäckers, 2004). In contrast, counting flowers may yield rather

286   imprecise estimates for food availability (Benadi *et al.*, 2014), although even the flower patch may

287   be a valid count variable if the project targets the landscape scale (Henry *et al.*, 2012). Pollinators

288   prefer dense patches to minimise the costs of search (Hegland & Totland, 2005) and may use

289   patches as sensory cues to find food resources rather than individual flowers or inflorescences

290   (Goulson, 1999; Dauber *et al.*, 2010). However, traits such as flower size, colour or scent may

291   directly indicate rewards available for flower visitors in a specific flower (Nicolson *et al.*, 2007) and

292   visitors may use such cues when selecting flowers within a close distance (Weiss, 1991). Flower

293   unit (visual display) may be a reasonably good choice, but the definition is not clear in all cases. For

294   instance, Tepedino & Stanton (1981) counted flowers and inflorescences, depending on floral type

295   and/or species, but did not define them as flower units. Rotenberry (1990) gave a definition for

296   floral visual display and considered flowers, heads, or stems as unity, and emphasised that these

297   were selected to match closely the flower visitor's view. Other authors use similar, albeit slightly

298   different definitions, and some emphasise that the unit was defined so as pollinators should walk

299   and not fly when foraging (Woodcock, 2014), rather than by the visual cues perceived from a

300   distance (Cowgill, 1993; Hegland & Totland 2005). These approaches led to similar categories,

301 although these categories may be difficult to apply at least for some plant species. Indeed,

302 definitions are based more on examples than on rigorous descriptions of the categories due to the

303 extreme variability of floral body plans.

304   In a few studies, besides using a count variable simple to estimate, e.g. number of shoots, floral

305 traits such as the number of flowers per stems, flower dimensions or nectar amounts were also

306 measured for a couple of individuals in several species. Then the measurements of these floral traits

307 were extrapolated to the entire sample (e.g. Hegland & Totland 2005). This method may yield much

308 more accurate estimates on food availability than using solely proxies such as flower units.

309   Plant-pollinator interactions are changing rapidly over the flowering and pollinator flight period

310 in natural circumstances. Many pollinator studies focused on the temporal distribution of plant-

311 pollinator interactions such as relationships between flowering phenology and pollinator floral

312 resource choice (Bagella *et al.*, 2013; Benadi *et al.*, 2014; Petanidou *et al.*, 2014). This requires

313 investigating temporal changes in species composition and flower density. Median resource

314 sampling time was 30 days for the reviewed studies. In contrast, rapid changes of flowering were

315 found over the season (Kubo *et al.*, 2008; Bagella *et al.*, 2013) or even during a day (Nicolson *et al.*,

316 2007; Fründ *et al.*, 2011), and these changes were partially due to the interactions between flowers

317 and their insect visitors (Fründ *et al.*, 2011). Temporal changes should be taken into account when

318 planning sampling frequency, since pollinators necessarily follow these changes (Goulson, 1999;

319 Potts *et al.*, 2004; Kubo *et al.*, 2008). Furthermore, time elapsed between sampling events increased

320 with the number of sites for the reviewed studies, indicating that sampling frequency was

321 determined by research effort constraints. We argue that this typical trade-off between spatial and

322 temporal representativeness could be overcome or its limitations could be reduced by combining

323 different methods with either a high spatial or high temporal resolution. We suggest that recording

324 presence-absence of flowering species in an entire meadow might detect some species that start

325 blooming earlier than quadrats or transects, if these latter cover only a small proportion of the entire

326 study area. In contrast, abundance estimates, e.g. by quadrat sampling, may be more suitable to

327 estimate the change over time in relative densities across species, due to its higher resolution.

328   Sixty-four percent of the studies investigated a single year, thus being hardly representative of a

329 plant community in the long run. Only four studies extended more than four years (Stefanescu,

330 1997; Alanen *et al.*, 2011; Petanidou *et al.*, 2014; Miller-Struttmann *et al.*, 2015). However, floral

331 resource compositions vary considerably among years (Alarcón *et al.*, 2008), and we agree with

332 Westphal *et al.* (2008) that one-year studies provide only a snapshot of plant-pollinator interactions.

333   In general, a trade-off emerges between spatio-temporal resolution and coverage of sampling.

334 For example, although the number of sampling units increased with research area, the coverage of

335 sampling decreased. Similarly, the effort invested in the temporal resolution of sampling decreased

336 with the increasing size of the study site, although did not change with sampling unit size.

337 Furthermore, direct counts involved smaller areas sampled, compared to the simpler categorical

338 estimates, thus researchers have to decide on either using higher estimate accuracy or better spatial

339 resolution. In contrast, we did not find relationships in all of the cases where we expected trade-offs

340 among different aspects of research investment. We propose that many times researchers might

341 overlook the necessary research investment in all the important aspects of the required sampling

342 process when planning sampling protocols. Reasonably good estimates need labor-intensive and

343 expensive methods, but research investment is always limited (Hegland *et al.*, 2010). Nevertheless,

344 minimum criteria for sampling each component of a study should be defined.

345

346

347 **Recommendations**

348

349   Recommendations on sampling methods to estimate floral resource availability for pollinators

350 are scarce (Frankl *et al.*, 2005; Hegland *et al.*, 2010), although a wide range of methods is described

351 in the vegetation literature (Elzinga *et al.*, 1998; Gibson, 2002). Here we provide a few guidelines

352 based upon the reviewed studies, that we think useful for estimating food resource availability for

353 pollinators in temperate grasslands. We recommend that both quantity and quality as well as the

354 spatio-temporal distribution of resources should be monitored when sampling floral resources. The

355 selected sampling methods should be better adapted to the aim of the study, and to the complexity

356 of the study system (spatial heterogeneity, seasonality, number and type of pollinator species etc;

357 Kearns & Inouye, 1993; Hegland *et al.*, 2010). To investigate floral resource abundance, focal

358 pollinators' feeding range in a specific area should be known. Rarity of important floral resources

359 should be taken into account when choosing a sampling method.

360   Given the research question, one should decide how to allocate finite research effort into the

361 spatio-temporal resolution and the coverage of sampling. In many cases, a high resolution is

362 required in both spatial and temporal terms, or both sampling resolution and coverage must be

363 sufficiently high to answer research questions. Using the same sampling units for pollinators and

364 their food resources may help to find the link between resource availability and consumption. We

365 argue that combining different methods that are appropriate to provide data with either high spatio-

366 temporal resolution or coverage, is a reasonable approach. For instance quadrat or transect sampling

367 could be completed with species lists on entire study sites, thus including information on all

368 potential nectar resources. When quadrat sampling is not feasible or only limited efforts can be

369 allocated to use this method, listing flowering species with a rough categorical abundance estimate,

370 similar to the method used by Goulson & Darvill (2004), may serve as either complementary

371 sampling or just a better option than the lack of abundance data. However, these methods

372 considerably reduce accuracy compared to quadrat sampling. Furthermore, the presence of

373 frequently visited, although rare floral sources may be noticed with the help of pollinator behaviour.

374 However, using feeding rates as an estimate of resource availability is not a viable approach

375 (Bakowski & Boron, 2005; Hinners & Hjelmroos-Koski, 2009; Aronne *et al.*, 2012). Although

15

376 pollinators are certainly much better than scientists in finding floral resources, resource availability

377 should be estimated independently of the consumers' visit frequency, because of their preferences.

378   Although the ultimate solution to estimate floral resource amounts would be directly measuring

379 nectar and pollen, it is not feasible in many cases. Characterising flowers with such direct measures,

380 and collecting larger samples on flower abundance could be a reasonably good compromise,

381 especially when variability in nectar and pollen amounts is also taken into account. If direct

382 measures on nectar or pollen amounts are not feasible, visual floral units from the pollinators

383 perspective could be the appropriate count variable. We also recommend avoiding estimates based

384 on green cover, since it is a very poor proxy of floral resource abundance for many plant species.

385   In contrast to Hegland *et al.* (2010), who found that only a few or even a single sampling event a

386 year was sufficient for investigating key species in pollination networks, we recommend using

387 shorter sampling intervals than used in most of the reviewed studies. The optimal sampling interval

388 may vary among studies. We recommend adjusting it to the aims of the study, community structure

389 and climate. We also recommend conducting long-term studies to lower the risk of distortion due to

390 large annual variation in resource composition, abundance and consumption.

391   Remote sensing technologies, such as drones with high optical resolution (Bakó *et al.*, 2014)

392 multi-spectral cameras (Peña-Barragán *et al.*, 2007), may change flower resource sampling in the

393 near future. Several pollinator studies have already used remote sensing to estimate the amount of

394 resource or habitat quality on the landscape scale (Osborne *et al.*, 2008; Henry *et al.*, 2012), or time-

395 lapse photography to investigate flowering dynamics (Crimmins & Crimmins, 2008). However,

396 such technologies have low spatial resolution for floral resource sampling (e.g. are unable to detect

397 cryptic plants). Therefore, we think that traditional sampling methods should be further investigated

398 to find efficient, widely usable methods to provide a sound methodological basis for understanding

399 plant-pollinator interactions. We conclude that thoroughly planned field studies comparing sampling

400 protocols at the community level, including remote sensing, and their appropriateness at different

16

401 circumstances are still mandatory.

402

403

404 **Contribution of authors**

405 VS, JK, AK and JN designed the project. VS and JK collected data. VS performed and AK, JK, AH

406 advised on data analyses. VS and JK wrote several drafts and all authors revised the final

407 manuscript.

408

409

410 **Supporting Information**

411 Appendix S1. References of the reviewed studies.

412 Appendix S2. Raw data for the reviewed studies.

413

414

415 **References**

416

417 Alanen, E.-L., Hyvönen, T., Lindgren, S., Härmä, O. & Kuussaari, M. (2011) Differential responses

418   of bumblebees and diurnal Lepidoptera to vegetation succession in long-term set-aside. *Journal*

419   *of Applied Ecology*, **48**, 1251–1259.

420 Alarcón, R., Waser, N. & Ollerton, J. (2008) Year-to-year variation in the topology of a plant–

421   pollinator interaction network. *Oikos*, **117**, 1796–1807.

422 Aronne, G., Giovanetti, M., Guarracino, M.R. & de Micco, V. (2012) Foraging rules of flower

423   selection applied by colonies of Apis mellifera: ranking and associations of floral sources.

424   *Functional Ecology*, **26**, 1186–1196.

425 Bagella, S., Satta, A., Floris, I., Caria, M.C., Rossetti, I. & Podani, J. (2013) Effects of plant

426   community composition and flowering phenology on honeybee foraging in Mediterranean sylvo-

427   pastoral systems. *Applied Vegetation Science*, **16**, 689–697.

428   Bakó, G., Tolnai, M. & Takács, Á. (2014) Introduction and testing of a monitoring and colony-

429   mapping method for waterbird populations that uses high-speed and ultra-detailed aerial remote.

430   *Sensors*, **14**, 12828–12846.

431   Bakowski, M. & Boron, M. (2005) Flower visitation patterns of some species of Lycaenidae

432   (Lepidoptera). *Biological Letters*, **42**, 13–19.

433   Benadi, G., Hovestadt, T., Poethke, H.-J. & Blüthgen, N. (2014) Specialization and phenological

434   synchrony of plant-pollinator interactions along an altitudinal gradient. *Journal of Animal*

435   *Ecology*, **83**, 639–650.

436   Benjamini, Y. & Hochberg, Y. (1995) Controlling the false discovery rate: a practical and powerful

437   approach to multiple testing. *Journal of the Royal Statistical Society. Series B (Methodological)*,

438   **57**, 289–300.

439   Bosch, J., González, A.M.M., Rodrigo, A. & Navarro, D. (2009) Plant-pollinator networks: adding

440   the pollinator's perspective. *Ecology Letters*, **12**, 409–419.

441   Burkle, L., Marlin, J. & Knight, T. (2013) Plant-pollinator interactions over 120 years: loss of

442   species, co-occurrence, and function. *Science*, **339**, 1611–1615.

443   Clarke, D., Whitney, H., Sutton, G. & Robert, D. (2013) Detection and learning of floral electric

444   fields by bumblebees. *Science*, **340**, 66–69.

445   Crimmins, M. & Crimmins, T. (2008) Monitoring plant phenology using digital repeat photography.

446   *Environmental Management*, **41**, 949–958.

447   Cowgill, S.E., Wratten, S.D. & Sotherton, N.W. (1993) The selective use of floral resources by the

448   hoverfly Episyrphus balteatus (Diptera: Syrphidae) on farmland. *Annals of Applied Biology*, **122**,

449   223–231.

450   Dauber, J., Biesmeijer, J.C., Gabriel, D., Kunin, W.E., Lamborn, E., Meyer, B., *et al.* (2010) Effects

451   of patch size and density on flower visitation and seed set of wild plants: a pan-European

452   approach. *Journal of Ecology*, **98**, 188–196.

453   Dennis, R.L.H. (2010) *A Resource-Based Habitat View for Conservation: Butterflies in the British*

454   *Landscape*. Wiley-Blackwell, Oxford.

455   Dicks, L. V, Abrahams, A., Atkinson, J., Biesmeijer, J., Bourn, N., Brown, C., *et al.* (2013)

456   Identifying key knowledge needs for evidence-based conservation of wild insect pollinators: a

457   collaborative cross-sectoral exercise. *Insect Conservation and Diversity*, **6**, 435–446.

458   Ebeling, A., Klein, A.-M., Schumacher, J., Weisser, W.W. & Tscharntke, T. (2008) How does plant

459   richness affect pollinator richness and temporal stability of flower visits? *Oikos*, **117**, 1808–1815.

460   Elzinga, C.L., Salzer, D.W. & Willoughby, J.W. (1998) *Measuring & Monitoring Plant Populations*.

461   U.S. Dept. of the Interior, Bureau of Land Management, Denver.

462   Frankl, R., Wanning, S. & Braun, R. (2005) Quantitative floral phenology at the landscape scale: Is

463   a comparative spatio-temporal description of "flowering landscapes" possible? *Journal for*

464   *Nature Conservation*, **13**, 219–229.

465   Fründ, J., Dormann, C.F. & Tscharntke, T. (2011) Linné's floral clock is slow without pollinators -

466   flower closure and plant-pollinator interaction webs. *Ecology Letters*, **14**, 896–904.

467   Galetto, L. & Bernardello, G. (2004) Floral nectaries, nectar production dynamics and chemical

468   composition in six Ipomoea species (Convolvulaceae) in relation to pollinators. *Annals of*

469   *Botany*, **94**, 269–280.

470   Ghazoul, J. (2005) Buzziness as usual? Questioning the global pollination crisis. *Trends in ecology*

471   *& evolution*, **20**, 367–73.

472   Gibson, D. (2002) *Methods in Comparative Plant Population Ecology*. Oxford University Press,

473   Oxford.

474   Goulson, D. (1999) Foraging strategies of insects for gathering nectar and pollen, and implications

475   for plant ecology and evolution. *Perspectives in Plant Ecology, Evolution and Systematics*, **2**,

476   185–209.

477   Goulson, D. & Darvill, B. (2004) Niche overlap and diet breadth in bumblebees; are rare species

478   more specialized in their choice of flowers? *Apidologie*, **35**, 55–63.

479   Hatfield, R. & Lebuhn, G. (2007) Patch and landscape factors shape community assemblage of

480   bumble bees, Bombus spp. (Hymenoptera: Apidae), in montane meadows. *Biological*

481   *Conservation*, **139**, 150–158.

482   Hegland, S.J. & Boeke, L. (2006) Relationships between the density and diversity of floral

483   resources and flower visitor activity in a temperate grassland community. *Ecological*

484   *Entomology*, **31**, 532–538.

485   Hegland, S.J., Dunne, J., Nielsen, A. & Memmott, J. (2010) How to monitor ecological

486   communities cost-efficiently: The example of plant–pollinator networks. *Biological*

487   *Conservation*, **143**, 2092–2101.

488   Hegland, S.J. & Totland, Ø. (2005) Relationships between species' floral traits and pollinator

489   visitation in a temperate grassland. *Oecologia*, **145**, 586–594.

490   Henry, M., Fröchen, M., Maillet-Mezeray, J., Breyne, E., Allier, F., Odoux, J.-F., *et al.* (2012)

491   Spatial autocorrelation in honeybee foraging activity reveals optimal focus scale for predicting

492   agro-environmental scheme efficiency. *Ecological Modelling*, **225**, 103–114.

493   Hinners, S. & Hjelmroos-Koski, M. (2009) Receptiveness of foraging wild bees to exotic landscape

494   elements. *The American Midland Naturalist*, 253–265.

495   Junker, R.R., Blüthgen, N., Brehm, T., Binkenstein, J., Paulus, J., Martin Schaefer, H., *et al.* (2013)

496   Specialization on traits as basis for the niche-breadth of flower visitors and as structuring

497   mechanism of ecological networks. *Functional Ecology*, **27**, 329–341.

498   Kearns, C.A. & Inouye, D.W. (1993) *Techniques for Pollination Biologists*. University Press of

499   Colorado, Niwot.

500   Kitahara, M., Yumoto, M. & Kobayashi, T. (2008) Relationship of butterfly diversity with nectar

plant species richness in and around the Aokigahara primary woodland of Mount Fuji, central Japan. *Biodiversity and Conservation*, **17**, 2713–2734.

Kubo, M., Kobayashi, T., Kitahara, M. & Hayashi, A. (2008) Seasonal fluctuations in butterflies and nectar resources in a semi-natural grassland near Mt. Fuji, central Japan. *Biodiversity and Conservation*, **18**, 229–246.

Kupper, L. & Hafner, K. (1989) How appropriate are popular sample size formulas? *The American Statistician*, **43**, 101–105.

Miller-Struttmann, N., Geib, J., Franklin, J.D., Kevan, P.G., Holdo, R.M., Ebert-May, D., *et al.* (2015) Functional mismatch in a bumble bee pollination mutualism under climate change. *Science*, **349**, 75–78.

Morrant, D.S., Schumann, R. & Petit, S. (2009) Field methods for sampling and storing nectar from flowers with low nectar volumes. *Annals of Botany*, **103**, 533–542.

Mortelliti, A., Amori, G. & Boitani, L. (2010) The role of habitat quality in fragmented landscapes: a conceptual overview and prospectus for future research. *Oecologia*, **163**, 535–547.

Nicolson, S.W., Nepi, M. & Pacini, E. (2007) *Nectaries and Nectar*. Springer, Dordrecht.

Osborne, J.L., Martin, A.P., Carreck, N.L., Swain, J.L., Knight, M.E., Goulson, D., *et al.* (2008) Bumblebee flight distances in relation to the forage landscape. *The Journal of animal ecology*, **77**, 406–415.

Peña-Barragán, J., López-Granados, F., Jurado-Expósito, M. & García-Torres, L. (2007) Mapping Ridolfia segetum patches in sunflower crop using remote sensing. *Weed Research*, **47**, 164–172.

Petanidou, T., Kallimanis, A.S., Sgardelis, S.P., Mazaris, A.D., Pantis, J.D. & Waser, N.M. (2014) Variable flowering phenology and pollinator use in a community suggest future phenological mismatch. *Acta Oecologica*, **59**, 104–111.

Potts, S., Biesmeijer, J. & Kremen, C. (2010) Global pollinator declines: trends, impacts and drivers. *Trends in Ecology & Evolution*, **25**, 345–353.

526 Potts, S., Vulliamy, B., Roberts, S., O'Toole, C., Dafni, A., Ne'eman, G., *et al.* (2004) Nectar

527   resource diversity organises flower-visitor community structure. *Entomologia Experimentalis et*

528   *Applicata*, **113**, 103–107.

529 R Core Team. (2015) R: A language and environment for statistical computing, R Foundation for

530   Statistical Computing, Vienna, Austria.

531 Rotenberry, J.T. (1990) Variable floral phenology: temporal resource heterogeneity and its

532   implication for flower visitors. *Holarctic Ecology*, **13**, 1–10.

533 Rusterholz, H.P. & Erhardt, A. (2000) Can nectar properties explain sex-specific flower preferences

534   in the Adonis Blue butterfly Lysandra bellargus? *Ecological Entomology*, **25**, 81–90.

535 Stanton, M.L. & Preston, R.E. (1988) Ecological consequences and phenotypic correlates of petal

536   size variation in wild radish, Raphanus sativus (Brassicaceae). *American Journal of Botany*, **75**,

537   528–539.

538 Stefanescu, C. (1997) Migration patterns and feeding resources of the Painted Lady butterfly,

539   Cynthia cardui (L.)(Lepidoptera, Nymphalidae) in the northeast of the Iberian peninsula.

540   *Miscel·lània Zoològica*, **20**, 31–48.

541 Tepedino, V. & Stanton, N. (1981) Diversity and competition in bee-plant communities on short-

542   grass prairie. *Oikos*, **36**, 35–44.

543 Tepedino, V. & Stanton, N. (1982) Estimating floral resources and flower visitors in studies of

544   pollinator-plant communities. *Oikos*, **38**, 384–386.

545 Thompson, J. (2001) How do visitation patterns vary among pollinators in relation to floral display

546   and floral design in a generalist pollination system? *Oecologia*, **126**, 386–394.

547 Torné-Noguera, A., Rodrigo, A., Arnan, X., Osorio, S., Barril-Graells, H., Rocha-Filho, L.C. da, *et*

548   *al.* (2014) Determinants of spatial distribution in a bee community: nesting resources, flower

549   resources, and body size. *PloS One*, **9**, e97255.

550 Wäckers, F. (2004) Assessing the suitability of flowering herbs as parasitoid food sources: flower

551   attractiveness and nectar accessibility. *Biological Control*, **29**, 307–314.

552 Weiss, M.R. (1991) Floral colour changes as cues for pollinators. *Nature*, **354**, 227–229.

553 Westphal, C., Bommarco, R., Carré, G., Lamborn, E., Morison, N., Petanidou, T., *et al.* (2008)

554   Measuring bee diversity in different European habitats and biogeographical regions. *Ecological*

555   *Monographs*, **78**, 653–671.

556 Woodcock, B.A., Savage, J., Bullock, J.M., Nowakowski, M., Orr, R., Tallowin, J.R.B., *et al.*

557   (2014) Enhancing floral resources for pollinators in productive agricultural grasslands.

558   *Biological Conservation*, **171**, 44–51.

559 Zimmerman, M. & Pleasants, J. (1982) Competition among pollinators: quantification of available

560   resources. *Oikos*, **38**, 381–383.

561

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.