

# *A method for the objective selection of landscape-scale study regions and sites at the national level*

Article

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## A method for the objective selection of landscape-scale study regions and sites at the national level

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Abstract:	<p>1) Ecological processes operating on large spatio-temporal scales are difficult to disentangle with traditional empirical approaches. Alternatively, researchers can take advantage of “natural” experiments, where experimental control is exercised by careful site selection. Recent advances in developing protocols for designing these “pseudo-experiments” commonly do not consider the selection of the focal region and predictor variables are usually restricted to two. Here we advance this type of site selection protocol to study the impact of multiple landscape scale factors on pollinator abundance and diversity across multiple regions.</p> <p>2) Using datasets of geographic and ecological variables with national coverage, we applied a novel hierarchical computation approach to select study sites that contrast as much as possible in four key variables, while attempting to maintain regional comparability and national representativeness. There were three main steps to the protocol: i) selection of six 100 km x 100 km regions that collectively provided land cover representative of the national land average, ii) mapping of potential sites into a multivariate space with axes representing four key factors potentially influencing insect pollinator abundance, and iii) applying a selection algorithm which maximised differences between the four key variables, while controlling for a set of external constraints.</p>

3) Validation data for the site selection metrics were recorded alongside the collection of data on pollinator populations during two field campaigns. While the accuracy of the metric estimates varied, the site selection succeeded in objectively identifying field sites that differed significantly in values for each of the four key variables. Between variable correlations were also reduced or eliminated, thus facilitating analysis of their separate effects.

4) This study has shown that national datasets can be used to objectively select randomised and replicated field sites within multiple regions and along multiple interacting gradients. Similar protocols could be used for studying a range of alternative research questions related to land use or other spatially explicit environmental variables, and to identify networks of field sites for other countries, regions, drivers, and response taxa in a wide range of scenarios.

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1 **Title:** A method for the objective selection of landscape-scale study regions and sites at the  
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27

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33

34

35 **Abstract**

- 36 1) Ecological processes operating on large spatio-temporal scales are difficult to  
37 disentangle with traditional empirical approaches. Alternatively, researchers can take  
38 advantage of “natural” experiments, where experimental control is exercised by  
39 careful site selection. Recent advances in developing protocols for designing these  
40 “pseudo-experiments” commonly do not consider the selection of the focal region and  
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42 selection protocol to study the impact of multiple landscape scale factors on pollinator  
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45 applied a novel hierarchical computation approach to select study sites that contrast as  
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47 comparability and national representativeness. There were three main steps to the  
48 protocol: i) selection of six 100 km x 100 km regions that collectively provided land  
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50 multivariate space with axes representing four key factors potentially influencing  
51 insect pollinator abundance, and iii) applying a selection algorithm which maximised  
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53 constraints.
- 54 3) Validation data for the site selection metrics were recorded alongside the collection of  
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57 sites that differed significantly in values for each of the four key variables. Between  
58 variable correlations were also reduced or eliminated, thus facilitating analysis of their  
59 separate effects.

60 4) This study has shown that national datasets can be used to objectively select  
61 randomised and replicated field sites within multiple regions and along multiple  
62 interacting gradients. Similar protocols could be used for studying a range of  
63 alternative research questions related to land use or other spatially explicit  
64 environmental variables, and to identify networks of field sites for other countries,  
65 regions, drivers, and response taxa in a wide range of scenarios.

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## 68 Introduction

69 A major challenge facing researchers of large-scale ecological processes is to find appropriate  
70 methods to characterise relationships between land use and biodiversity patterns (Diamond  
71 1983; Hargrove & Pickering 1992; Dilts, Yang & Weisberg 2010; Smart *et al.* 2012;  
72 HilleRisLambers *et al.* 2013). At the landscape scale, it is extremely difficult and expensive  
73 to apply a classical experimental approach involving establishing controls, manipulating  
74 “treatments”, assigning large-scale experimental units to treatments randomly or achieving  
75 true replication (Hargrove & Pickering 1992; Rundlof *et al.* 2015). In response to these  
76 issues, landscape ecology as a discipline has developed a number of tools to study large-scale  
77 natural phenomena (Diamond 1983; Hargrove & Pickering 1992; Sagarin & Pauchard 2010;  
78 HilleRisLambers *et al.* 2013). Many landscape-scale observational studies take place within  
79 “natural” or “accidental experiments”, making use of existing environmental variation  
80 occurring due to some sudden event or the gradual change brought about by humans or nature  
81 or both. When the goal of the study is to make statistical inferences about a broader  
82 population of landscapes, control of confounding factors can be applied through the careful,  
83 non-random selection of sites in so called “pseudo-experiments” (Diamond 1983; Fahrig *et*  
84 *al.* 2011). This kind of selection is important to avoid common statistical design flaws such as  
85 spatial dependence of sites, the use of a only a portion of the range of landscape variables and  
86 collinearity between variables (Eigenbrod *et al.* 2011; Pasher *et al.* 2013)

87 The recent development of this form of site selection methodology appears to perpetuate two  
88 common drawbacks (Table 1): a) the region(s) within which the study sites are selected are  
89 not explicitly considered, and b) the number of predictor variables is restricted to two  
90 (although see Watts *et al.* 2016). In this study, we argue that some research questions require  
91 that the broader study regions are representative of some larger area to enhance  
92 generalisability of results. Such regions should also be free from the potential biases and

93 problems of repeatability introduced by only studying well-known landscapes close to the  
94 study base or research institution (Dilts, Yang & Weisberg 2010). In addition, while there is a  
95 suitable method to select study sites that differ as much as possible in values of two variables  
96 (Fahrig *et al.* 2011), future studies seeking to disentangle multiple interacting drivers at large-  
97 scales will require a more advanced protocol. Watts *et al.* (2016) present the most promising  
98 of approaches to this need, developing a protocol that selects study sites that differ between  
99 three variables simultaneously. However, their protocol was not designed for hypothesis  
100 testing, is not applied to standardised sites and selects sites within subjectively chosen  
101 regions.

102 Our site selection protocol brings together the best aspects of its predecessors, enhances the  
103 objectivity and control of site selection, improves the description and testing of the protocol  
104 and allows application of the method to a broader array of situations. The method was  
105 originally developed to study the links between land use / management variables and insect  
106 pollinator populations and communities, but the approach is generic and could be used at a  
107 range of spatial scales and applied to almost any taxa or system. The objectives of the site  
108 selection methodology were to improve on previous landscape-scale pseudo-experimental  
109 designs by: i) enhancing objectivity of region selection (i.e., using a systematic approach with  
110 a transparent methodology which could be readily reproduced by other researchers), ii)  
111 enabling the study of several key factors simultaneously, and interactions between them, by  
112 selecting sites contrasting along multiple axes, and iii) enhancing the generality of results by  
113 selecting sites from areas that are representative of an entire country. To do this, national  
114 datasets were used to first select a set of focal regions that would be representative of Britain,  
115 and then to characterise each potential field site within those regions in terms of four key  
116 landscape-scale metrics that are thought to affect insect pollinator populations (habitat  
117 diversity, floral resource availability, insecticide loadings, managed honey bee density). Field

118 sites were chosen to contrast as much as possible in each of the four key metrics while  
119 attempting to maintain regional comparability and representativeness. Verification of the  
120 protocol was conducted by validating the values of the four metrics through *in situ* surveys.  
121 The data demonstrate that landscape scale variation can be estimated using available national  
122 datasets, and thus suggest that similar approaches may be effective in addressing other large-  
123 scale issues.

124

125

## 126 **Methods**

127 The site selection protocol consists of three parts: 1) focal region selection, 2) assigning  
128 values of key variables to potential sites within each region, and 3) a site selection algorithm.  
129 This is followed by validation of the variable estimates used in site selection. These aspects  
130 are outlined briefly below with full details given in the Supplementary material.

131

### 132 ***Focal Regions***

133 To simplify field logistics and costs by limiting the amount of travel between sites, it was  
134 decided to first select six representative “focal regions” of 100 x 100 km, and then choose  
135 study landscapes within them. The regions were selected to be as representative as possible  
136 of the British landscape across vegetation and environmental gradients and the number of  
137 regions was chosen as the minimum number to allow sufficient statistical power for paired  
138 contrasts. However, the protocol could easily be applied to a different number of regions.  
139 The selection of focal regions began with two 100 km resolution grids: the standard UK  
140 Ordnance Survey grid at 100 km resolution, and a second grid diagonally offset by 50 km to

141 the east and north. The second grid was used to double the pool of regions to choose from.  
142 All possible six-region combinations which did not include adjacent or overlapping cells  
143 were examined. For each six-region combination, the area of each broad habitat (from the  
144 2007 Land Cover Map (LCM2007); Morton *et al.* 2011) was summed and the proportional  
145 contribution to the overall area calculated. A national proportional contribution for each  
146 habitat type was also calculated. For each habitat type, the Euclidian distance between the  
147 six-region proportion and the national proportion was calculated, and then a mean distance  
148 for all habitat types was taken. This distance then corresponds to how well the six-region  
149 combination represents Britain in terms of land cover categories. This process was also  
150 completed for ITE Land Classes (Bunce *et al.* 1996) which represent topography, climate and  
151 human infrastructure. The combination of six regions that had the shortest mean distance for  
152 both classification schemes was considered to be most representative of Britain, and was  
153 chosen as the set of focal regions to be studied.

154

### 155 ***Survey sites***

156 The aim of the survey site selection protocol was to identify sites that contrasted as much as  
157 possible in four landscape-scale metrics: 1) habitat diversity, 2) floral resource availability, 3)  
158 insecticide loadings and 4) managed honey bee density. These four metrics were chosen  
159 because previous studies have demonstrated that they may be important drivers of local  
160 pollinator population decline in the UK. Strong links have been made between pollinator  
161 populations and the complexity of the landscape (Shackelford *et al.* 2013), the diversity and  
162 density of floral resources in agricultural settings (Potts *et al.* 2003; Gabriel & Tschamtker  
163 2007) and increased insecticide usage (Rortais *et al.* 2005; Brittain *et al.* 2010). There is also  
164 evidence that managed stocks of honey bees can affect the condition of wild pollinator stocks

165 either through spill-over of parasites (e.g., Evison *et al.* 2012) or through competitive  
166 interactions (Goulson & Sparrow 2009; Elbgami *et al.* 2014), although the landscape-scale  
167 population impact of honey bees on wild pollinators remains untested. In order to study the  
168 effects of these four factors individually and in combination, 16 sites in each study region  
169 were sought. We wanted these 16 sites to represent every possible combination of “high” and  
170 “low” values of each metric (i.e., site 1 = relatively “high” values for all four metrics, site 2 =  
171 “high” for three metrics and low for one metric, and so on) in a similar fashion to a full-  
172 factorial experiment. To this end, we used a computer algorithm technique to select sites  
173 with extreme values of each metric, as outlined below and in more detail in Supplementary  
174 material S1.1.

175

#### 176 *Data sources and manipulation*

177 Datasets were compiled using the UK Ordnance Survey National Grid reference system, the  
178 system of geographic grid references in the UK. The finest scale at which most agricultural  
179 and biodiversity datasets are available is the “tetrad” scale (2 x 2 km). Given the relatively  
180 high mobility of many pollinating insects (Westphal, Steffan-Dewenter & Tschardt 2006),  
181 we opted to define our sites at this scale. For each of the 2,500 potential sites or tetrads within  
182 a 100 x 100 km region, a value for each of the metrics was calculated from national datasets.  
183 Full details of the calculations are given in Supplementary material S1.1.1, but they are  
184 briefly outlined here:

- 185 1) **Habitat diversity** was calculated as a Shannon diversity index of broad habitats  
186 present, with each weighted by the area covered within each candidate tetrad. Habitat  
187 areas were derived from the LCM2007 (Morton *et al.* 2011).

- 188 2) **Floral resource availability** was calculated from nectar data only, as pollen data are  
189 less well recorded for British plants. This variable is expressed in terms of kilograms  
190 of sugar per hectare per year, and was derived by a) estimating flowering plant  
191 species cover per unit area of each habitat type in each site by combining finely-  
192 resolved regional vegetation quadrat data from Countryside Survey 2007 (CS2007;  
193 Carey *et al.* 2008) with the satellite-derived LCM 2007, b) modelling nectar sugar  
194 values for the 220 commonest insect-pollinated species based on published values for  
195 124 species at the time of the study (see Table S2 for details and references), c)  
196 accounting for additional floral resources in mass-flowering crops, agri-environment  
197 schemes and in organic arable fields.
- 198 3) **Insecticide loadings**, a score of the hazard to bees of different insecticide types and  
199 application rates, were calculated by multiplying the area under cultivation of each of  
200 36 crop groups within the sites estimated from national agricultural statistics, by a  
201 regional hazard score for agrichemicals used on that crop group, derived from  
202 Pesticide Usage Survey data for each crop combined with honey bee toxicity data for  
203 each insecticide applied.
- 204 4) **Managed honey bee population density** was estimated from data held by the  
205 national “Beebase” database ([www.nationalbeeunit.com](http://www.nationalbeeunit.com)). The number of adult bees  
206 present in mid-summer for an average colony was estimated and this was combined  
207 with the typical number of colonies present in each of three apiary classes. Honey bee  
208 density in surrounding landscapes was modelled by using published honey bee  
209 foraging data (Waddington *et al.* 1994; Beekman & Ratnieks 2000). The apiary  
210 location was used as a centroid and the estimated number of honey bee foragers  
211 grouped into concentric 200 m bins (see Supplementary material).
- 212

213 *Site selection algorithm*

214 Once assigned, the metric values were standardised by a Box-Cox transformation and  
215 converted to z scores (zero-centred), so that a score below 0 for a metric corresponded to a  
216 “low” value relative to regional norms, and a score above 0 represented a “high” value. The  
217 objective of the algorithm was to select a combination of 16 sites within a 100 x 100 km focal  
218 region to maximise the width of each of the four gradients sampled as well as the  
219 orthogonality between them. The number of ways of drawing unique sets of 16 sites from the  
220 2,500 options in a focal region is enormous ( $1.06055 \times 10^{41}$  combinations). It was therefore  
221 essential to reduce computing time by constraining the site combinations using a series of  
222 design criteria. These criteria included removing the sites closest to the mean value for any of  
223 the four variables, restricting the maximum distance between sites within a cluster to 50 km  
224 (for logistical reasons), restricting the amount of urban and water cover allowed per site, and  
225 ensuring topographic comparability between sites (e.g., to avoid comparing sites on mountain  
226 tops vs valley floors). See Supplementary material S1.1.2 for full details of the selection  
227 criteria. Once a feasible combination of field sites had been selected, landowners were  
228 identified and contacted for access permission. If access permission was refused to more than  
229 30% of the site, the next feasible combination of field sites was chosen.

230

231 *Site selection: validation*

232 As the four metrics were all assessed indirectly with varying degrees of reliability, their  
233 values were validated during a two-year field campaign. This aim of this fieldwork was both  
234 to validate the metrics and to sample the field sites for wild pollinators. The full details of the  
235 validation processes are given in Supplementary material S1.2 but are outlined briefly here:

- 236 **1) Habitat diversity** values were validated during field surveys by confirming or  
237 correcting the habitat types as mapped in the LCM2007. Corrected habitat areas were  
238 then used in new diversity index calculations.
- 239 **2) Floral resource availability.** Validation for this metric required several stages: a)  
240 actual floral reward production per flower per day was sampled for 175 species, and  
241 remodelled for a further 62 (2012) and 86 (2013) species (Baude *et al.* 2016), b)  
242 transect surveys were conducted to assess actual floral cover of each species for each  
243 broad habitat within each site, c) data from (a) and (b) were combined with corrected  
244 habitat areas to calculate the total floral resource per site.
- 245 **3) Insecticide loadings** were collated by conducting questionnaire surveys of all land  
246 managers for land within the field sites. The response rate to these questionnaires was  
247 approximately 50%, corresponding to an area of approximately 30% of the field sites.  
248 It was not possible therefore to validate the entire metric. Instead, direct comparison  
249 was made between the estimated and measured values for the fields covered by the  
250 questionnaire responses. Field values were summed for each tetrad.
- 251 **4) Managed honey bee density** was assessed by surveying each site using field  
252 observations along the predetermined transects used for floral resource validation, and  
253 using pan-trapping. Pan traps were set out on good weather days primarily to sample  
254 the wild pollinator community and any caught honey bees were added to the density  
255 count.

256

## 257 **Results**

### 258 *Region and site selection*



259 The six focal regions and 96 survey sites chosen by the protocol are shown in Fig. 1. From  
260 southeast to northwest, the focal regions covered parts of 1) Cambridgeshire, Suffolk and  
261 Norfolk, 2) Wiltshire and Gloucestershire, 3) Staffordshire, Cheshire, Shropshire and North  
262 East Wales, 4) North Yorkshire and Cumbria, 5) Ayrshire, Lanarkshire and East  
263 Renfrewshire, and 6) Inverness-shire.

264 Survey sites were generally well-selected in line with the criteria of the protocol, with some  
265 exceptions. Fig. 2 illustrates the contrasting values of the four estimated metrics for the  
266 Cambridgeshire/Suffolk region as an example. The goal of this part of the selection protocol  
267 was to effectively ensure that the bars were as high as possible for the “high” values (positive  
268 values in Fig. 2) and as low as possible for the “low” values (negative values in Fig. 2). In  
269 practice, we appreciated that the indirect assessment of focal variables (and regression  
270 towards the mean) would tend to narrow or erase the gap between high and low categories,  
271 such that each axis should be treated as continuous rather than categorical. Our protocol,  
272 however, helps ensure that as wide a range of variation as possible is sampled. Furthermore,  
273 although it was not a site selection criterion, the site selection protocol removed the inherent  
274 correlation between the estimated values of the four metrics both for all regions (Table 2),  
275 and within individual regions (Fig. S4 – S6).

276

### 277 *Validation*

278 In order to validate the site selection protocol, the observed values of each of the four metrics  
279 were tested against the predictions derived from national datasets using simple Spearman’s  
280 rank correlation tests (R base package; R Core Team 2014). These correlations are shown  
281 graphically in Fig. 3 and the coefficients are given in Table 3, together with results from  
282 linear mixed effects models using measured values as response variable, predicted values as

283 explanatory variable, and region as random effect. Mixed models were performed using the  
284 package *nlme* in R 3.1.1 (R Core Team 2014), and were considered valid following  
285 inspection of residuals for normal distribution, heteroscedasticity and influential values (Zuur  
286 et al. 2009). All four metrics showed significant positive relationships between the observed  
287 and predicted values. According to the correlation coefficients, the best predicted metric was  
288 habitat diversity, followed by insecticide loadings, floral resources, and honey bee density.  
289 However, it should be noted that the insecticide loading comparison omits tetrads for which  
290 questionnaire responses were not received, and tetrads for which measured insecticide could  
291 be assumed to be zero due to the absence of arable fields. If the latter are included, the  
292 Spearman's rank correlation coefficient is 0.57 ( $p < 0.001$ ) but the slope of the regression is  
293 only 0.25 ( $p < 0.01$ ).

294 In terms of the correlations between validated metrics, there were significant relationships  
295 between the metrics for three out of the six pair-wise comparisons overall (Table 4), although  
296 the correlation coefficients were all below the commonly used threshold of 0.7 for including  
297 variables in the same analysis. Measured floral resources was significantly correlated with  
298 measured honey bee density (Spearman's  $\rho = 0.31$ ,  $p = 0.002$ ) and with measured insecticide  
299 loadings (Spearman's  $\rho = -0.47$ ,  $p < 0.05$ ). In addition, measured honey bee density was  
300 strongly linked to measured insecticide loadings (Spearman's  $\rho = 0.54$ ,  $p < 0.05$ ). However,  
301 for the individual regions (Fig. S7 – S9) the only significant correlations were for measured  
302 habitat diversity vs measured honey bee density in Inverness (Spearman's  $\rho = 0.54$ ,  $p = 0.03$ ;  
303 Fig. S7), measured insecticide loadings vs measured habitat diversity in Wiltshire  
304 (Spearman's  $\rho = -0.92$ ,  $p < 0.01$ ; Fig S9) and for measured honey bee density vs measured  
305 insecticide loadings in Cambridgeshire (Spearman's  $\rho = -0.65$ ,  $p = 0.04$ ; Fig. S9).

306

## 307 **Discussion**

308 The methodology described here aimed to build on previous site selection protocols to select  
309 sites that varied in four main gradients, while at the same time ensuring comparability  
310 between sites and representation of Britain more widely. Although estimations of the four  
311 metrics were made with some uncertainty, the low level of correlation between verified  
312 metrics at the regional and national scales suggest that the site selection method provides a  
313 suitable sample of sites for investigating links between land management and pollinator  
314 biodiversity.

315

### 316 *Region selection*

317 One of the main differences between previous approaches and our protocol is in the objective  
318 selection of study regions, chosen here to represent Britain in terms of land class and land  
319 cover variables. Regions are often chosen in landscape studies because they are well known  
320 and have been used several times before in previous work. This manner of selecting focal  
321 regions is sufficient for studies that aim to understand basic or local mechanisms or  
322 processes. For example, Watts *et al.* (2016) chose two regions of the UK due to previous  
323 knowledge of the areas and of the variation in woodland habitats. Such a selection approach  
324 was expedient and suitable for the authors' study question which focused on landscape  
325 conservation and links between woodland biodiversity and gradients of woodland  
326 characteristics. Furthermore, the inferential scope of this study is likely restricted to British  
327 lowland woodlands within these two regions. By contrast, our research project sought to link  
328 the regional variation in land management drivers across a broad range of habitat types to the  
329 regional variation in pollinator diversity, thereby supporting inference about Britain as a  
330 whole. With this target of broader generality of results, the location of regions should ideally

331 be more objectively selected (Dilts, Yang & Weisberg 2010) and subject to the same levels of  
332 control as site selection. The addition of this regional selection protocol is therefore  
333 recommended for studies seeking broad statistical inference and a replicated pseudo-  
334 experimental design (Table 1).

335

### 336 *Site selection*

337 The second main difference in our approach was in the number of focal variables used  
338 simultaneously to select sites. Previous approaches have selected sites for different variables  
339 in a similarly hierarchical fashion, simultaneously selecting sites based on two variables  
340 (Holzschuh, Steffan-Dewenter & Tschardtke 2010; Hopfenmueller, Steffan-Dewenter &  
341 Holzschuh 2014; Steckel *et al.* 2014). Some such studies also detail selecting sites in the four  
342 quadrants of a 2-dimensional bivariate plot to remove the correlation between variables in the  
343 selected sites (Fahrig *et al.* 2011; Pasher *et al.*, 2013). Pasher *et al.* (2013) further suggested  
344 the extension of this selection system to  $n$  dimensions, and Watts *et al.* (2016) attempted it  
345 with three dimensions. However, each additional selection variable greatly increases the  
346 number of possible combinatorial possibilities, which can soon become unmanageable. Here,  
347 we have presented the first attempt to use four dimensions and provide detailed instructions  
348 for manageable repetition of the method.

349 While there was some uncertainty in estimating our four metrics, the set of sites selected was  
350 sufficiently dispersed in variable space to allow analysis using continuous variables with  
351 values across the full ranges of each (Pasher *et al.* 2013). Randomly selected focal sites tend  
352 to cluster around mean values, providing relatively low resolving power for discerning the  
353 effects of landscape-scale drivers. Our original choice of what were modelled to be extreme  
354 values might be criticised for missing out these typical parameter values, but in practice the

355 imprecise models combined with the inevitable regression towards the mean resulted in a  
356 wide exploration of parameter space of variables individually and in combination. An  
357 additional benefit of the protocol is that it greatly reduces the degree of correlation between  
358 focal variables, allowing valid inferences to be drawn about their separate and interacting  
359 impacts (Eigenbord *et al.* 2011; Pasher *et al.* 2013). Furthermore, studies of this kind do not  
360 normally assess correlations based on validated data, but we have demonstrated here that  
361 some caution is required if the calculation of focal variables is subject to high levels of  
362 uncertainty. Improvements to our metric estimates are likely to lead to further decoupling of  
363 metrics at the national scale.

364

#### 365 *Site validation*

366 The estimates of the four metrics varied in their accuracy quite widely. The most accurate  
367 was the habitat diversity metric which was based on the proportion of habitat covers  
368 calculated from remote sensing data. The high accuracy of this metric is not surprising as the  
369 estimates required the fewest steps in making the calculations, and verification was relatively  
370 straightforward. Even where the precise nature of land cover was misclassified on LCM2007,  
371 the spatial configuration of habitats as determined on the ground, and thus the Shannon index  
372 value, was generally quite close to our estimates from the LCM data. The level of accuracy is  
373 also similar to previous verification efforts (Morton *et al.* 2011).

374 The insecticide metric was also relatively well predicted when only considering those fields  
375 for which questionnaire responses were received. However, this result masks the large  
376 number of tetrads (especially in the North) for which large positive insecticide loadings were  
377 predicted when no arable fields were found on the ground. Although insecticides are applied  
378 on non-arable fields, the extent of application is unlikely to warrant a “high” insecticide

379 loading value. These inappropriate values were probably caused in part by the satellite  
380 classification of reseeded pastures as arable fields and partly by changes in the crop areas  
381 between the 2010 census and 2012/13 survey years due to normal crop rotation.

382 The floral resource metric proved to have relatively low accuracy for a number of reasons  
383 related to the data available for making estimates: 1) some habitat cover estimates were  
384 incorrect due to misclassification in LCM2007 as described above, 2) actual floral reward  
385 data were only available for relatively few species at the time of site selection, 3) estimates of  
386 species cover per habitat were based on regional averages per broad habitat and so were not  
387 sensitive to within-region variation, and 4) mean nectar availability reported in databases  
388 does not capture the high variability observed in the field due to site differences in climate,  
389 soil and nectar consumption. Validation of these factors inevitably led to some widely  
390 differing values of site-level floral resource availability.

391 The honey bee density metric was the least well verified of the four drivers partly because the  
392 methods used to count the number of honey bees visiting sites proved to be unsuitable. As  
393 honey bees are social foragers, using scouts to alert workers to rich floral resource patches,  
394 the use of pan trapping to sample them is extremely inefficient (Westphal *et al.* 2008).  
395 Further, attempts to observe honey bees on the wing or foraging along transects suffered from  
396 a lack of available survey time: only 3 full days per season per site were used, often in poor  
397 weather conditions. Where data are available, they show a good relationship with the  
398 estimated density. However, such is the noise in the data and the high presence of zeros that  
399 subsequent analysis will need to use the original estimated values as an explanatory variable.  
400 Better estimates of honey bee numbers would require either greater investment in survey time  
401 or an alternative method such as the use of baited traps or estimating the number of hives  
402 present through, for example, surveys of farmers and beekeepers. As a result of these

403 problems, we are not able to verify the accuracy of the honey bee population density  
404 estimation technique.

405

406 *Overall evaluation and implications*

407 The aims of this site selection methodology were to improve on previous landscape-scale  
408 natural experimental designs by i) increasing objectivity of region selection to enhance the  
409 ability to generalise results to the wider landscape, and ii) to improve the selection of sites  
410 based on the values of multiple focal variables. This has been achieved by developing a  
411 hierarchical region selection protocol and by explicitly testing previously conceived ideas of  
412 site selection using multiple variables simultaneously. The additional complexities we have  
413 introduced to landscape scale site selection will not be necessary for every research question,  
414 but provide a basis for increasing the inferential scope and complexity of landscape-scale  
415 pseudo-experiments.

416 We have also shown that it is possible to use national datasets to derive credible and objective  
417 sets of study sites that cover multiple environmental gradients, without bias from researcher's  
418 personal knowledge of landscapes in the site selection. The implications of this  
419 methodological development are important for landscape ecology and national scale  
420 monitoring programmes in any region or country with sufficient data, with a network of well-  
421 chosen sampling sites being a vital tenet of a well-designed national monitoring scheme.

422

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437

438 **Data Accessibility:** All primary collected datasets (datasets collected during the course of the  
439 project), are stored in the Centre for Ecology & Hydrology data repository and will be made  
440 available for download following publication of this manuscript. Other datasets used as cited  
441 in the article are available to download from the sources cited.

442

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548 **Tables**

549 Table 1: Comparison of previous and current site selection protocols of studies incorporating a landscape scale pseudo-experimental approach

Study	Number of simultaneous focal selection variables	Number of regions (size)	Number of study sites/ landscapes (size)	True population	Method useful for:	Limitations of method
Gabriel <i>et al.</i> (2010)	1	2 (not given)	16 (10x10km)	The two regions studied	Nested or multi-scale designs, paired landscapes, ensuring non-target environmental conditions remain similar	Regions selected subjectively, one categorical focal selection variable
Fischer, Thies and Tschardtke (2011)	2*	3 (not given)	100* (forests: 100 x 100m; grassland: 50 x 50m)	The three regions studied; Central European grassland and forest areas?	Selecting sites along variable gradients, multi-criteria selection, focus on particular habitat types	Regions selected subjectively, restricted to two selection variables, limited control of external factors
Pasher <i>et al.</i> (2013)	2	1 (~15,500k m <sup>2</sup> )	100 (100ha)	The study region	Avoiding correlations between landscape variables, maximizing variability in variables	Region chosen subjectively, restricted to two selection variables
Smart <i>et al.</i> (2014)	1	2 (~60,000k m <sup>2</sup> )	26 (5-100ha)	The study region; temperate lowland	Avoiding correlations between landscape variables, maximizing contrast between treatment of interest	Difficult to ensure equivalence of numerous other factors across treatment groups
Watts <i>et al.</i> (2016)	3	2 (~7335 km <sup>2</sup> & ~8570 km <sup>2</sup> )	106 (0.5-32ha)	The two regions studied; temperate lowland agricultural landscapes?	Selecting sites along variable gradients, multi-criteria selection, focus on particular habitat types, "natural experiments", analyzing relative effects of variables, landscape conservation studies	Regions chosen subjectively, focus on woodland only, variable site sizes, not designed for hypothesis testing
This study	4	6 (100 x	96 (2 x 2km)	The six regions,	Replicated pseudo-experimental	Time consuming, data

---

100km)

the British  
countryside

designs, broad generality of results, intensive  
hypothesis testing

---

550 \* corresponds to “experimental plots”

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551 Table 2: Spearman correlation coefficients for the four **estimated** metrics (i.e., before  
 552 ground-truthing; Box-Cox transformed Z-scores) for all six study regions. Coefficients are  
 553 calculated for all possible sites within all regions (n = 12,718 sites) and the sites selected for  
 554 study (n = 96). Asterisks denote significant correlations ( $p < 0.001$ ). Partial correlation  
 555 coefficients were calculated controlling for Region, but are not shown as they were not  
 556 different from the coefficients below.

	<b>Habitat diversity</b>		<b>Floral resources</b>		<b>Insecticide loadings</b>	
	All possible sites	Selected sites	All possible sites	Selected sites	All possible sites	Selected sites
Floral resources	0.14*	0.11	-	-	-	-
Insecticide loadings	-0.28*	-0.16	-0.20*	-0.16	-	-
Honey bee density	0.10*	0.10	-0.15*	-0.08	0.24*	0.11

557



558 Table 3: Spearman's rank correlation and partial correlation coefficients (controlling for  
 559 Region), and parameters of linear mixed models (Region as random effect) for the estimated  
 560 versus measured metrics in all regions. The data are Z-scores: box-cox transformed and zero  
 561 centred. "Mean floral resources" is the total amount of floral resources averaged over the two  
 562 years of field sampling. Asterisks indicate significant correlations: \*\*\* =  $p < 0.001$ , \*\* =  
 563  $p < 0.01$ , \* =  $p < 0.05$

	<b>Overall correlatio n</b>	<b>Partial correlatio n</b>	<b>Slope</b>	<b>Intercept</b>	<b>P</b>
Habitat diversity	0.77***	0.77***	0.56	-0.05	<0.001
Mean floral resources	0.28**	0.29**	0.20	-0.03	0.005
Insecticide loadings	0.67**	0.60**	0.67	-0.01	0.001
Honey bee density	0.22*	0.21*	0.16	0.03	0.002

564

565

566 Table 4: Spearman's rank correlation and partial correlation (controlling for region)  
 567 coefficients for the four **measured** metrics (i.e., corrected metrics after ground truthing; Box-  
 568 Cox transformed Z-scores) for all six study regions. Asterisks indicate significant correlations  
 569 (\* =  $p < 0.05$ , \*\* =  $p < 0.01$ ).

	<b>Habitat diversity</b>	<b>Floral resources</b>	<b>Insecticide loadings</b>
<i>All regions</i>			
Floral resources	0.18		
Insecticide loadings	-0.47*	0.10	
Honey bee density	-0.04	0.31**	-0.54*
<i>All regions (partial correlation)</i>			
Floral resources	0.16		
Insecticide loadings	NA	NA	
Honey bee density	-0.05	0.29**	NA

570

571

572

573 **Figure legends**

574

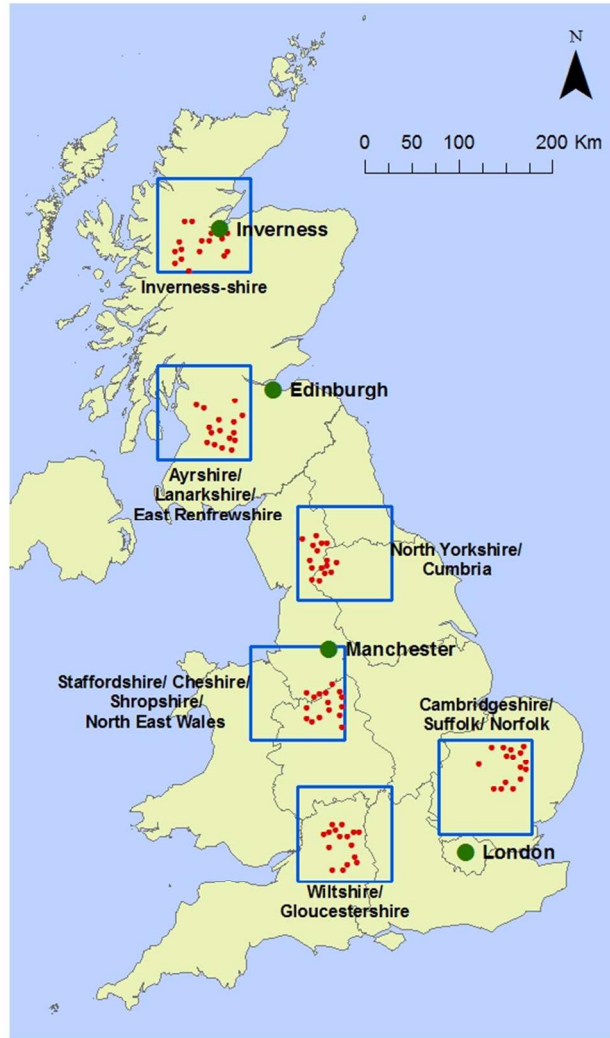
575 Fig. 1: The extent of the six 100 km<sup>2</sup> regions chosen by the region selection protocol (blue  
576 squares), and the 96 field sites (sixteen 2 x 2 km<sup>2</sup> sites per region) chosen by the site selection  
577 protocol (red circles). (Service Layer Credit: OS data; Crown copyright and database right  
578 2015)

579

580 Fig. 2: The estimated Z-scores (Box-Cox transformed and zero centred data) of the four  
581 metrics for the final 16 sites of the Cambridgeshire/Suffolk region, shown here as an  
582 example. The blue bars are Z-scores above 0, i.e., the site has a “high” score for that metric;  
583 the red bars are negative Z-scores, i.e., the site has a “low” score for that metric. The 16 sites  
584 represent every combination of high and low values of the four metrics, e.g., site 1 has high  
585 values of all four metrics, site 2 has a low value only for habitat diversity, and so on. The data  
586 for the remaining regions can be found in Fig. S3.

587

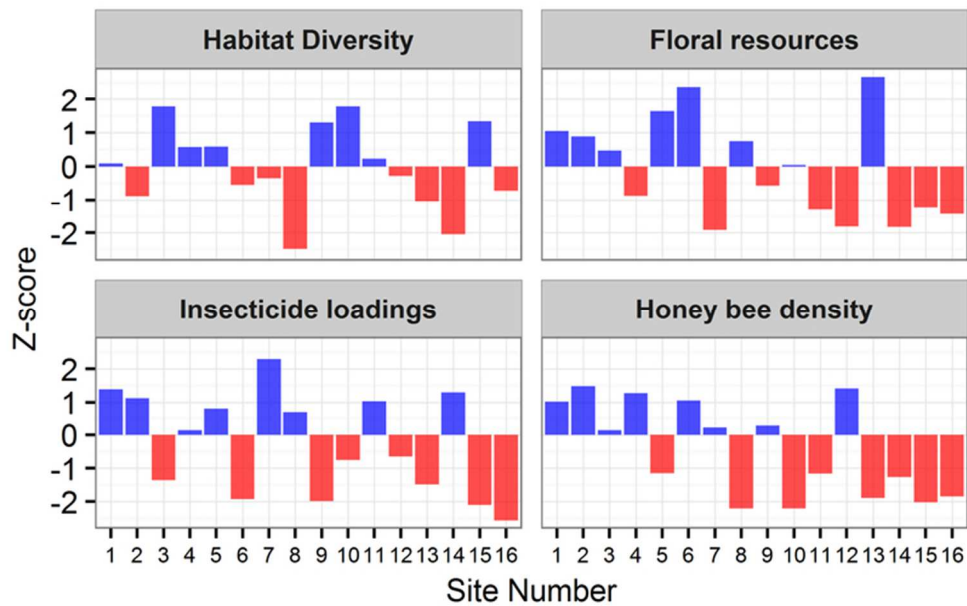
588 Fig. 3: “Ground-truthing” of the four key metrics. The data are Z-scores: box-cox  
589 transformed and 0 centred, and each point represents a single site. The straight bold line  
590 represents the linear regression line for all regions and the shaded area represents 95%  
591 confidence intervals. The blue lines are mixed effect regression lines for each of the six  
592 regions with “region” as a random effect, displayed here to demonstrate the variation in  
593 prediction accuracy between regions. “Mean floral resources” is the total amount of floral  
594 resources averaged over the two years of field sampling. Regional graphs are shown in Fig.  
595 S10.



The extent of the six 100 km<sup>2</sup> regions chosen by the region selection protocol (blue squares), and the 96 field sites (sixteen 2 x 2 km<sup>2</sup> sites per region) chosen by the site selection protocol (red circles). (Service Layer Credit: OS data; Crown copyright and database right 2015)

Fig. 1

210x296mm (96 x 96 DPI)



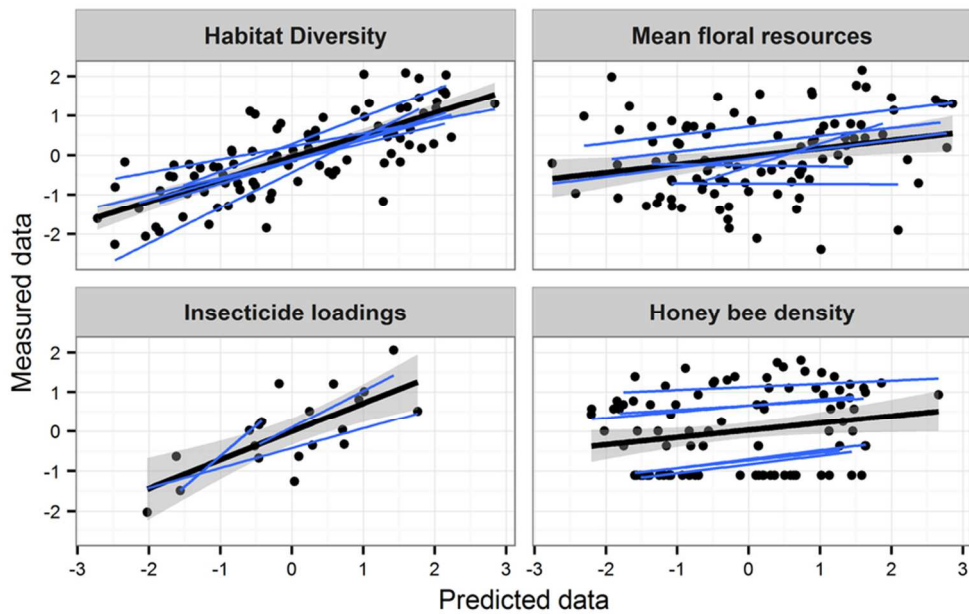
The estimated Z-scores (Box-Cox transformed and zero centred data) of the four metrics for the final 16 sites of the Cambridgeshire/Suffolk region, shown here as an example. The blue bars are Z-scores above 0, i.e., the site has a “high” score for that metric; the red bars are negative Z-scores, i.e., the site has a “low” score for that metric. The 16 sites represent every combination of high and low values of the four metrics, e.g., site 1 has high values of all four metrics, site 2 has a low value only for habitat diversity, and so on.

The data for the remaining regions can be found in Fig. S3.

Fig. 2

69x44mm (300 x 300 DPI)

Only



Validation of the four key metrics. The data are Z-scores: box-cox transformed and 0 centred, and each point represents a single site. The straight bold line represents the linear regression line for all regions and the shaded area represents 95% confidence intervals. The blue lines are mixed effect regression lines for each of the six regions with "region" as a random effect, displayed here to demonstrate the variation in prediction accuracy between regions. "Mean floral resources" is the total amount of floral resources averaged over the two years of field sampling. Regional graphs are shown in Fig. S10.

Fig. 3

80x51mm (300 x 300 DPI)

Only