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1 Running head: Biases in studies of insect herbivory

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3 **Biases in studies of spatial patterns in insect herbivory**

4

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8 *Abstract* The properties of the human mind are responsible for a number of biases that affect the quality
9 of scientific research. However, scientists working in the fields of ecology and environmental science
10 rarely take these biases into account. We conducted a meta-analysis of data extracted from 125
11 publications comparing woody plant damage by defoliating insects in different environments in order
12 to understand the extent to which our knowledge on spatial patterns in herbivory is affected by various
13 biases. We asked which research methods are most prone to biases and whether these biases lead to
14 overestimation of the effects under study. The effects sizes (ESs) decreased with increases in the
15 numbers of plant species involved in the study, with 61% lower ESs for herbivory estimated on all
16 plants growing in study plots compared to herbivory on selected species. ESs also depended on the leaf
17 sampling procedure: when all leaves from a tree or branch were sampled for measurements of
18 herbivory or when random or systematic selection protocols were applied, ESs were 74% smaller than
19 in cases of more subjective haphazard selection. In addition, ESs were 97 and 135% greater when the
20 person conducting sampling and measuring was aware of the research hypothesis or sample origin,
21 when compared with situations when the observer was blinded to these factors. The impacts of
22 cognitive biases on the study outcomes significantly decreased with the increase in publication year;
23 however, this pattern emerged mostly due to high-ranked journals and was non-significant for other
24 journals. Using the studies of spatial patterns in herbivory as an example, we showed that our
25 ecological and environmental knowledge is considerably biased due to an unconscious tendency of
26 researchers to find support for their hypotheses and expectations, which generally leads to
27 overestimation of the effects under study. Cognitive biases can be avoided by using different methods,
28 such as applying randomization procedures in sampling and blinding of research hypotheses and
29 sample origins. These measures should be seen as obligatory; otherwise, accumulation of the biased

30 results in primary studies may ultimately lead to false general conclusions in subsequent research
31 synthesis.

32

33 *Key words:* cognitive biases, meta-analysis, blind protocols, randomization, research methods, insect
34 herbivory, defoliators, haphazard selection, scientific methodology, spatial patterns in herbivory,
35 temporal trends in research, effect size.

INTRODUCTION

36

37 The human mind is undoubtedly the most important tool in scientific research, as only the human mind
38 can interpret the data in order to arrive at a logical conclusion (Leedy and Ormrod 2001). Nevertheless,
39 the properties of the human mind can affect the quality of the research through insertion of a number of
40 biases at the planning, data collection, analysis, and/or publication phases of scientific research
41 (Pannucci and Wilkins 2010). In science, bias is defined as systematic errors in results or inferences
42 that favor one outcome over others (Gluud 2006). Unfortunately, some biases are rarely considered by
43 scientists working in the fields of ecology and evolution.

44

45 Publication bias has a recognized influence on the understanding of ecological and evolutionary
46 processes and is widely appreciated (Fanelli 2010, 2012, Jennions et al. 2013). By contrast, the
47 occurrence and importance of biases introduced at pre-publication stages of ecological research have
48 received little attention (but see Kozlov and Zvereva 2009, 2015, Koricheva et al. 2013a, Kozlov et al.
49 2014). This is especially true for confirmation bias: a tendency to search for, interpret, and favor
50 information in a way that confirms one's pre-existing beliefs or hypotheses (Forstmeier et al. 2017).
51 Confirmation bias is a well-documented phenomenon in psychology and cognitive sciences; it results
52 primarily from automatic processes that occur unintentionally (Hergovich et al. 2010). Within
53 biological disciplines, confirmation bias has received sufficient attention only in studies of animal
54 behavior (Marsh and Hanlon 2007, van Wilgenburg and Elgar 2013, Traniello and Bakker 2015,
55 Tuytens et al. 2014, 2016).

56

57 Confirmation bias is usually demonstrated by comparing the results of studies conducted blindly (i.e.,
58 when the observer was not aware of the research hypothesis being tested) with the results of non-blind

59 studies (i.e., when the observer knew what results might be expected) (van Wilgenburg and Elgar 2013,
60 Holman et al. 2015). Blinding is a routine procedure in medical research, because non-blind protocols
61 were repeatedly found to overestimate the effects of a specific treatment (Noseworthy et al. 1994,
62 Saltaji et al. 2018). At the same time, blinding is only rarely reported in ecological and evolutionary
63 studies (Forstmeier et al. 2017), and the outcomes of blind and non-blind methods have rarely been
64 compared in ecological research. However, when this type of comparison has been conducted, the lack
65 of blinding with respect to the hypothesis being tested or the treatment condition of a sample usually
66 resulted in overestimation of the effects under study by the observers (Kozlov et al. 2014, Kozlov and
67 Zvereva 2015). Consequently, meta-analysis of experimental studies within the life sciences indicates
68 that non-blind studies tend to report higher effect sizes (ESs, hereafter) and more significant P values
69 when compared to blind studies (Holman et al. 2015). We expect that the impacts of various biases will
70 be stronger on research areas that rely on observational data than on experimental data, because
71 collection of observational data may be more prone to unconscious biases. Studies of spatial patterns in
72 herbivory and of environmental factors driving these patterns provide a representative example of this
73 type of research field.

74
75 The relationships between plants and herbivores are among the most intensively studied biotic
76 interactions (Tylianakis et al. 2008, Jamieson et al. 2012), and the amount of plant biomass consumed
77 by herbivores is the key characteristic of the intensity of these interactions. Explicitly or implicitly, the
78 numerical values reflecting the pressure imposed by herbivorous insects on plants are among the
79 cornerstones of numerous hypotheses/theories related to insect-plant relationships, such as the ‘green
80 world’ hypothesis (Hairston et al. 1960, Polis 1999), the exploitation ecosystem hypothesis (Oksanen et
81 al. 1981, Polis 1999), the Janzen–Connell hypothesis (Janzen 1970), the optimal defense theory

82 (Rhoades 1979), the growth–differentiation balance hypothesis (Herms and Mattson 1992), and many
83 others. These values also form the foundation of theories explaining the evolution of plant traits (Coley
84 and Aide 1991) and the formation of biogeographical patterns (Moles et al. 2011). However, the pool
85 of primary data regarding plant losses to insects that has served as the basis for numerous
86 generalizations has never been subjected to rigorous examination to confirm that these data provide
87 unbiased estimates of the amounts of plant biomass consumed by insects. The diversity in
88 methodologies of data collection was recently suggested as one possible reason underlying the lack of
89 consistency among studies exploring the levels of herbivory along environmental gradients (Andrew et
90 al. 2012). In particular, studies may differ in the extent to which various biases have impacted the
91 research process.

92
93 For example, the selection of plant species and study sites for measurements of foliar losses to
94 herbivores, as well as of the timing of the measurements, may be affected by the researcher’s
95 expectations or preconceptions, which in turn may depend on both the researcher’s personal experience
96 and on the hypothesis/theory that the researcher believes to be true. A striking example of this type of
97 effect was revealed in our previous study (Kozlov et al. 2014). For decades, insect herbivory was
98 commonly believed to be highest in tropical regions (Coley and Aide 1991, Coley and Barone 1996,
99 Schowalter and Lowman 1999), and this idea formed the foundation of a number of ecological
100 hypotheses and generalizations (Schemske et al. 2009, Moles et al. 2011). Nevertheless, we found that
101 records made in the tropics had overestimated the community-wide losses of plant foliage to insects
102 due to confirmation bias, i.e., collecting data in a way that confirms the influential theory. In line with
103 this result, many recent studies have found no support for the greatest levels of herbivory in the tropics
104 (Moles et al. 2011, Kozlov et al. 2015a). This example shows the importance of accounting for biases,

105 especially unconscious ones, in the development of scientific knowledge. However, the stages and the
106 specific parts of the research process where biases are most likely to occur are not clear, nor are the
107 consequences of biases for the generalizations based on primary studies. These consequences are
108 especially important due to the increasing use of meta-analysis as a tool for research synthesis in
109 ecological and environmental sciences (Koricheva et al. 2013a, Gurevitch et al. 2018).

110 The aim of the present study is to understand the extent of the influence of various biases on
111 publications addressing the impacts of different environmental factors that determine spatial patterns in
112 losses of woody plant foliage to insect herbivores, focusing on different stages of scientific research
113 from planning to publication. For this purpose, we conducted a meta-analysis of studies addressing
114 insect herbivory in different environments (habitats, geographical regions, or experimental treatments)
115 and compared the magnitude of the ESs between studies that were likely and unlikely to be influenced
116 by cognitive biases. We asked whether studies based on methods of data collection that are prone to
117 biases tend to overestimate the effects under study. To answer this question, we tested the following
118 specific predictions: (i) studies yield greater ESs when herbivory is measured in a single or a few plant
119 species than in multiple species (i.e., community wide); (ii) publications that formulate the research
120 hypothesis (or clearly state the authors' expectations) report greater ESs than publication that do not
121 formulate the hypothesis and do not state expectations; (iii) the method of selection of plants
122 individuals and individual leaves for the assessment of herbivory affects the magnitude of ESs, with
123 random or blinded selection resulting in the smallest ESs; (iv) awareness of the research hypothesis by
124 the sample collector and measurer of herbivory increases the ESs, whereas blinding of the sample
125 origin during measurements of herbivory reduces of the ESs. We also examined temporal trends in the
126 publication of case studies with potentially biased conclusions, and we asked whether these trends
127 differ between journals with high and low impact factors.

128

129

MATERIALS AND METHODS

130

Database

131 A huge number of publications is devoted to environmental factors driving spatial patterns of plant
132 losses to insects. We did not perform an exhaustive search of this literature, because we planned to
133 conduct a detailed examination of research methods through communication with the authors of the
134 studies selected for our meta-analysis. Therefore, to make the research feasible, we had to limit the
135 number of studies in our database to slightly over 100. A typical meta-analysis in plant ecology,
136 performed during the past decades, is usually based on several dozens of primary studies (mean 63,
137 median 41; Koricheva and Gurevitch 2014). Thus, the number of studies in our database (MetadataS1,
138 DataS1) is higher than the average number of publications in meta-analyses in the domain of ecological
139 and environmental studies, and we believe that this number is sufficient to uncover multiple sources of
140 variation in spatial patterns of herbivory, including differences in methodology among the primary
141 studies.

142

143 As the starting point for this study, we used a database that had been created for the analysis of global
144 patterns in the background losses of woody plant foliage to defoliating insects (selection criteria and
145 the earlier version of the database were published by Kozlov et al. 2015a,b). From this database, which
146 contained 490 publications as of 30 March 2018, we used all publications that explored three major
147 types of environmental factors that potentially affect insect herbivory: habitat disturbance (e.g.,
148 fragmentation, pollution, logging, burning, warming, and CO₂ enrichment), geographic variation (e.g.,
149 latitude, altitude, and biomes), and the types of natural habitats (e.g., wet vs. dry forest, nutrient-rich vs.
150 nutrient-poor habitats, and more diverse vs. less diverse plant community). The presence of quantitative

151 data on the background insect herbivory in natural ecosystems and the comparison between the two
152 environments were the only criteria for inclusion of the publication in our meta-analysis; therefore, we
153 presume that our choice of studies was not biased.

154 The 125 selected publications reported mostly the outcomes of observational studies, but eight of them
155 described large-scale field experimental studies that estimated natural herbivory (e.g., enhanced CO₂
156 level or soil warming).

157

158 *Collecting information about methods used in primary studies*

159 We inspected the Introduction section of each paper to understand the aim of the study, and we
160 recorded whether explicit research hypotheses or predictions were formulated concerning the
161 differences in herbivory between the compared environments, or whether the authors only searched for
162 differences between the environments. The studies aimed at hypothesis testing were further divided
163 into two groups, based on whether or not the direction of the effect was predicted.

164 To evaluate the quality of the research methodology and to decide whether the study was prone to
165 biases, we searched each paper for the following information:

- 166 1. In how many plots/sites per habitat/treatment (environment hereafter) and on how many plant
167 individuals was the herbivory measured?
- 168 2. In how many plant species was the herbivory measured? Was it measured in all plant species in
169 the plot/site (community-wide estimate), or in selected plant species?
- 170 3. If herbivory was measured on selected plant species: what was the reason for this selection?
- 171 4. For how many years was the herbivory measured?

- 172 5. How were plant individuals, branches within a plant (where appropriate), and individual leaves
173 selected for herbivory measurements? We distinguished between the following options: no
174 selection (all available plants or all leaves on the plant were sampled); random selection (some
175 randomization procedure was applied or trees/leaves were haphazardly selected before leaf
176 damage became apparent); systematic selection (e.g., every fourth leaf on the shoot was
177 selected); haphazard selection (selection was made non-blindly and did not involve any specific
178 algorithm aimed at a decrease in subjectivity). Haphazard selection clearly differs from other
179 ways of selection because it can be influenced by researcher's expectations, while random and
180 systematic selection cannot be affected by confirmation bias.
- 181 6. Was herbivory measured in situ (without sampling the leaves) or in the laboratory (from
182 collected leaves or their images)?
- 183 7. Was herbivory estimated from plant leaves or from their digital images?
- 184 8. Was herbivory estimated visually, or with a grid, or by image analysis?
- 185 9. Was the person who sampled leaves for measurements of herbivory (or conducted
186 measurements in situ) aware (author) or not aware (technician) of the aim of the study?
- 187 10. If herbivory was measured from sampled leaves or their images, were the measurements
188 conducted by a person who was aware or not aware of the aim of the study?
- 189 11. If herbivory was measured from sampled leaves or their images, was the information on sample
190 origin available (i.e., did a label include this information?) or not available (i.e., did a label
191 include only the code of the sample?) to the person who conducted the measurements of leaf
192 area lost to insects?

193 None of 125 publications selected for our meta-analysis, including 10 papers published by ourselves,
194 contained the complete information necessary to answer all these questions and to decide whether the

195 outcome of each individual study may have been affected by different biases. Therefore, we attempted
196 to contact the corresponding authors of all publications. In total, we sent 114 requests (some of which
197 contained questions on more than one paper published by the same author; some requests on the same
198 paper were sent to another co-author if the corresponding author did not respond to our e-mails). The
199 authors of four papers had retired long ago, so we were unable to contact them. Each request contained
200 one to nine questions (median value: five questions). Some authors never responded to our request, so
201 we ultimately obtained complete information on relevant aspects of the research methodology for 103
202 of the 125 publications. For remaining 22 publications, we used information reported unequivocally in
203 the papers; but when some details of research methodology were not described, we treated this
204 information as missing.

205 *Data extraction and meta-analysis*

206 From each publication, we calculated the Hedges' d measure of the ES; i.e., a normalized difference in
207 herbivory based on means, standard deviations (SD), and sample sizes (n) for the two compared
208 environments. As a rule, we calculated one ES from one paper; but six of 125 papers explored effects
209 of two different factors, so we calculated two ESs from each of these six papers. Therefore, we
210 performed 131 comparisons between the levels of damage of woody plant foliage by insects in
211 different environments (termed 'studies' hereafter).

212
213 The majority of the studies selected for our meta-analysis (105 studies) reported the data separately for
214 each of the compared environments. When the primary study used correlation analysis (26 studies), we
215 divided the data collected from the environmental gradients into two groups so that these two groups
216 could be attributed to contrasting environments (e.g., high- and low-altitude sites, high- and low-
217 diversity plots). These two groups of studies yielded effects of similar magnitudes (environmental

218 gradients: $d = 0.66$; contrasting environments: $d = 0.68$, respectively; $Q_B = 0.04$, $df = 1$, $P = 0.85$) and
219 were therefore combined in all further analyses.

220

221 The studies analyzed by us reported SD as variations between plant species, plant individuals, leaf
222 numbers, study sites, and sampling dates; consequently, sample sizes in these studies reflected different
223 aspects of spatio-temporal variation in insect herbivory. Presentation of data from 55 studies
224 (sometimes complimented by information received from the authors) allowed re-calculation of means,
225 SD, and n so that they reflected variation among plant individuals. For example, when the authors
226 calculated SD as among-year variation and presented the data as means and standard error (SE) or SD
227 reflecting variation among plant individuals for each study year, we calculated the weighted pooled SD
228 value from year-specific SE/SD values using a web-based calculator
229 (<https://home.ubalt.edu/ntsbarsh/Business-stat/otherapplets/Pooled.htm>). For these studies we
230 compared ESs based on both pooled and unpooled SD values and found that the differences in the
231 magnitude of ESs were not significant (pooled: $d = 0.69$, unpooled: $d = 0.64$; $Q_B = 0.25$, $df = 1$, $P =$
232 0.62) as well as for 86 studies based on plant individuals (pooled: $d = 0.72$, unpooled: $d = 0.66$, $Q_B =$
233 0.18 , $df = 1$, $P = 0.68$). Across all database, studies based on plant individuals (86 studies) and studies
234 based on study sites (36 studies) yielded ESs of similar magnitudes ($Q_B = 0.003$, $df = 1$, $P = 0.95$). Still,
235 whenever possible, we used sample sizes reflecting the numbers of plant individuals to minimize the
236 variation among studies caused by heterogeneity in SD and n .

237 In the majority of studies, such as those comparing herbivory in two habitats or correlating herbivory
238 with latitude, classification of the environments as either ‘control’ or ‘treatment’ was not possible.
239 Therefore, in most of our analyses, we considered only the absolute value of ES (i.e., the magnitude of
240 the differences in herbivory). However, when we tested the influence of the unconscious confirmation

241 bias on the outcome of the study, following Holman et al. (2015), we changed the sign of the ES to
242 negative if the reported result opposed the author's predictions on the direction of the effect (14
243 studies).

244 We performed our meta-analysis using the random effects categorical models in the MetaWin 2.0
245 program, assuming that studies differ by sampling error as well as by a random component in the ESs
246 (Rosenberg et al. 2000). The effects were considered statistically significant if the 95% confidence
247 interval of the mean ES (CI_{95}) did not overlap zero. The variation in the ES values within and among
248 the classes of explanatory variables was explored by calculating the heterogeneity indices (Q_T and Q_B
249 respectively) and testing them against χ^2 distribution (Gurevitch and Hedges 2001). Temporal trends in
250 ESs were studied by meta-regression of ES against publication year; temporal changes in sample size
251 were analyzed by calculating the Pearson correlation coefficient between the year of publication and
252 sample size. The proportions of studies that used different methods were compared by frequency
253 analysis (chi-square test; SAS Institute 2009).

254

255

RESULTS

256

Overview of the database

257 Our meta-analysis is based on 125 papers published from 1977–2018 (MetadataS1, DataS1). We
258 classified the environmental factors explored in the publications included in our meta-analysis into
259 three major groups: habitat type or habitat quality within the same geographical region (e.g., wet vs.
260 dry forests, nutrient-rich vs. nutrient-poor habitats, low diversity vs. high diversity forests, for a total of
261 56 ESs); habitat disturbances within the same geographical region (e.g., imposed by fragmentation,
262 pollution, logging, burning, warming, or CO₂ enrichment, for a total of 52 ESs); and geographic

263 variation (i.e., differences between habitats located in different latitudes, altitudes, or biomes, for a total
264 of 23 ESs).

265 *Overall variation in herbivory between the environments*

266 Overall differences in the losses of woody plant foliage to insects between the studied environments
267 did not overlap zero ($d = 0.66$, $CI_{95} = 0.56$ to 0.76), indicating that the papers selected for our meta-
268 analysis reported significant spatial variations in herbivory; this variation was highly heterogeneous (Q_t
269 $= 194.1$, $df = 130$, $P = 0.0002$).

270 The three major types of environmental factors listed above caused similar effects on herbivory ($d =$
271 0.70 , $d = 0.65$ and $d = 0.67$, respectively; $Q_B = 0.21$, $df = 2$, $P = 0.90$). Studies conducted in different
272 geographic zones (tropical, subtropical, temperate, or subpolar) yielded similar ESs ($Q_B = 0.90$, $df = 3$,
273 $P = 0.83$). Observational and experimental studies did not differ in the reported magnitude of the
274 detected effects on herbivory ($d = 0.66$ and $d = 0.66$, respectively; $Q_B = 0.0001$, $df = 1$, $P = 0.99$). Thus,
275 the discovered heterogeneity is associated with other differences among individual studies, most likely
276 with differences in the methods used by the authors.

277 *Factors affecting the outcome of the study*

278 *Numbers of plant species and study years.* - Only 17.6% of the studies explored the differences in the
279 community-wide levels of herbivory, as assessed either from abscised leaves or from leaves sampled
280 from all plant species on the plot. More than half of the studies (52.7%) measured herbivory in a single
281 plant species. The most frequent explanation for the selection of one or more particular plant species
282 was that the species was/were common in study area: this reason was mentioned in 94 of 108 (87%)
283 studies based on selected plant species.

284 Studies in which plant species (either single or several) were selected for measurements of insect
285 herbivory yielded greater ESs than studies where no selection was performed (i.e., where the authors
286 sampled all plant species in the plot and thus obtained community-wide estimates of herbivory) (Fig.
287 1). The magnitude of the reported differences between the environments was greatly influenced by the
288 number of plant species used to measure insect herbivory: a greater number of species assessed resulted
289 in smaller detected effects (Fig. 1). ESs also tended to be smaller in studies based on two or more years
290 of herbivory measurements than on single-year studies, and primarily in studies of geographic patterns
291 (Fig. 1).

292

293 *Selection of plants and leaves.* - Only about two-thirds (65.2%) of the information on the methods used
294 to select trees, branches, and leaves for measurements of herbivory were reported in the publications
295 used in our meta-analysis. Moreover, clarifications provided by the authors in response to our requests
296 revealed that 26.2% of this information was reported incorrectly. The most common error (44 identified
297 cases) was the use of the term ‘random selection’ in situations when the authors did not apply any
298 randomization procedure, so the selection should therefore have been classified as haphazard.

299 The review of research methodology associated with selection of plants, branches, and leaves and with
300 measurements of leaf area lost to insects suggested that a substantial fraction of the studies included in
301 our meta-analysis was prone to different kinds of cognitive biases. First, the selection of trees,
302 branches, and/or leaves was haphazard in 53.6% of studies. Second, 82.6% and 81.2% of the leaf
303 sampling and measurement of herbivory, respectively, were generally conducted by the authors, i.e., by
304 the persons who were aware of the hypotheses being tested and/or of the expected research results and

305 were interested in confirming their expectations. Third, only 10.1% of measurements of herbivory were
306 blinded with respect to leaf origin.

307 The method of selection of plant individuals for measurements of herbivory did not influence the
308 outcome of the study (Fig. 2). By contrast, leaf selection procedure affected the magnitude of the
309 reported differences in herbivory: the greatest differences between the environments were found when
310 leaves were selected haphazardly, and thus their selection may have been unconsciously influenced by
311 the attitudes of the researchers who made this selection. When leaves were not selected (i.e., all leaves
312 on the tree or branch were sampled), or a particular selection protocol (randomization procedure or
313 systematic sampling) was used to avoid the subjectivity in leaf choice, the reported differences were
314 considerably smaller than in the case of haphazard selection (Fig. 2).

315

316 *Measurement of herbivory.* - The differences between the environments in the percentage of leaf area
317 consumed by herbivores did not depend on whether the measurements were conducted in situ or in the
318 laboratory by processing collected leaves or their images ($d = 0.46$ and $d = 0.58$, respectively; $Q_B =$
319 1.06 , $df = 1$, $P = 0.30$). Similarly, visual estimations of leaf damage, the use of grids and image
320 processing yielded similar differences between the environments ($d = 0.47$, $d = 0.55$ and $d = 0.56$,
321 respectively; $Q_B = 0.51$, $df = 2$, $P = 0.77$). However, when sampling of leaves was conducted
322 exclusively by the authors, the reported differences were greater than in the cases, when technicians
323 were involved in sampling (Fig. 2). Moreover, when herbivory was measured in the laboratory, the
324 effect was greater when the measurer was aware of sample origin than when the measurer was blinded
325 to the sample origin (Fig. 2).

326

327 *Formulation of the predictions.* - The majority of publications (75.2%) used in our meta-analysis tested
328 at least one research hypothesis or formulated some prediction(s) about pattern(s) in herbivory; this
329 proportion was greater in journals with an impact factor (IF hereafter) exceeding 1.6 than in journals
330 with lower IF (72.9% and 61.5%, respectively; $\chi^2 = 5.67$, $df = 1$, $P = 0.017$). Of all stated predictions,
331 67% found at least partial support in the studies, and this proportion did not differ between high- and
332 low-impact journals ($\chi^2 = 2.11$, $df = 1$, $P = 0.15$). Of 74 studies that predicted the direction of the effect,
333 14 studies (18.9%) revealed patterns that opposed the research hypotheses or predictions.

334 The outcome of a study did not depend on prior formulation of the research hypothesis in the paper
335 (formulated: $d = 0.48$ and not formulated: $d = 0.62$; $Q_B = 1.13$, $df = 1$, $P = 0.29$), nor did it depend on
336 expectations about the direction of the effect under study (direction predicted: $d = 0.46$, direction not
337 predicted: $d = 0.54$; $Q_B = 0.24$, $df = 1$, $P = 0.62$).

338

339 *Journal impact factor and publication year.* – Across the entire database, the reported differences in
340 herbivory between the environments significantly decreased with the year of publication (meta-
341 regression: $Q = 6.09$, $P = 0.013$) and with the increases in a journal's IF (Fig. 3). In particular, 13 of 14
342 studies that revealed patterns opposing the research hypothesis or predictions were published in
343 journals with IFs exceeding 1.6, and 12 of them were published quite recently (from 2010–2018). The
344 decrease in the magnitude of the ES with time was mostly due to high-impact journals (with IFs
345 exceeding 2.5), while this trend was not significant in other journals (Fig. 3). Sample size did not
346 correlate with the year of publication ($r = -0.07$, $n = 131$, $P = 0.44$).

347 Methods of leaf selection that are prone to unconscious biases (e.g., haphazard selection) were used
348 twice as frequently in studies published in low-impact journals (IFs below 1.6) than in studies

349 published in journals with higher IF (31.4 and 15.9%, respectively; $\chi^2 = 3.72$, $df = 1$, $P = 0.05$). Also
350 the studies published in low-IF journals suffered more frequently from pseudoreplication in terms of
351 the number of study sites or experimental units (33.7% and 61.5%, respectively; $\chi^2 = 8.72$, $df = 1$, $P =$
352 0.003). Across all journals, the percentage of pseudoreplicated studies that used a single site in each
353 habitat decreased from 51.8% in 1977–2008 to 30.1% in 2010–2018 ($\chi^2 = 4.05$, $df = 1$, $P = 0.04$). The
354 percentage of these pseudoreplicated studies in high-impact journals dropped during the last years
355 (from 2010–2018) to 19%, compared to 47.5% in other journals ($\chi^2 = 6.48$, $df = 1$, $P = 0.01$).

356

DISCUSSION

357

Uncovering biases by means of meta-analysis

358 Psychologists have described a great number of cognitive biases that influence human perception,
359 reasoning, and memory (Forstmeier et al. 2017). Confirmation bias (Rosenthal 1976, Nickerson 1998)
360 is a ubiquitous phenomenon, particularly in science; however, within the biological sciences,
361 confirmation bias has received relatively little attention, and only very recently has the importance of
362 confirmation bias been recognized for ecological and environmental research (Kozlov et al. 2014,
363 Kozlov and Zvereva 2015, Holman et al. 2015, Kardish et al. 2015). Avoidance of confirmation bias
364 requires that experimenters or observers blind themselves by concealing any information about
365 research hypotheses or the treatment conditions of a specific sample (Noseworthy et al. 1994, van
366 Wilgenburg and Elgar 2013, Kardish et al. 2015). Therefore, confirmation bias is usually demonstrated
367 by comparing blind and non-blind studies (Kozlov et al. 2014, Tuytens et al. 2014, Kozlov and
368 Zvereva 2015), and meta-analyses that compare these two kinds of studies both across and within
369 disciplines have shown a tendency toward higher ESs in non-blind than in blind studies (van
370 Wilgenburg and Elgar 2013, Holman et al. 2015, Saltaji et al. 2018). Our meta-analysis of spatial

371 patterns in herbivory not only confirmed this tendency, but it also uncovered other sources of cognitive
372 biases in biological research (Table 1). In general, the use of research methods that are prone to
373 different biases results in overestimation of the effects under study and, consequently, leads to
374 overestimation of the average ESs in subsequent quantitative research syntheses (Table 1).

375 *Biases associated with selection of plant species and duration of the study*

376 The meta-analyses conducted in the field of ecology clearly demonstrated a disproportional amount of
377 information on certain ecosystems, high-rank taxa, and individual species (Gurevich and Hedges 1999).
378 This ‘biased sampling of the natural world’ (Jennions et al. 2013) may lead to wrong conclusions of
379 meta-analyses due to over-representation of some study systems. In some research areas, the studies
380 may be concentrated on just one or a few ‘model’ species, and this may lead to completely wrong
381 fundamental conclusions. One striking example concerns studies of gene expression that have drawn
382 conclusions about principal differences between plants and animals just because the model plant,
383 *Arabidopsis thaliana*, appeared exceptional among plants in the studied character (Lloyd and Davies
384 2013). In the area of the spatial patterns in herbivory almost no studies (and none of the studies used in
385 our meta-analysis) were performed on gymnosperms, despite the ecological and economic importance
386 of gymnosperms in many regions. This deficiency arose presumably due to practical difficulties
387 associated with measurements of herbivory in needle-bearing trees. Therefore, the detected patterns of
388 geographic variation in herbivory (e.g., by Kozlov et al. 2015a, b) may only be valid for woody
389 angiosperms and not for all woody plants.

390 One frequent example of non-random data collection is selection of species that occur at high densities
391 (Jennions et al. 2013). Indeed, in studies included in our meta-analysis, most plants were selected for
392 measurements of herbivory based on their high abundance, as declared in the publications. However,

393 our database shows no systematic bias toward a certain species because of the great variety of habitats
394 and geographic zones, each of which differ in the predominating plant species in the community.
395 Conversely, a possibility remains that foliar losses to insects systematically differ in terms of responses
396 to environmental factors when comparing abundant versus rare plant species. For example, more
397 abundant plants may suffer higher levels of herbivory due to the higher probability that these species
398 will be found by herbivores, as predicted by the resource availability hypothesis (Endara and Coley
399 2011).

400 In an earlier study (Kozlov et al. 2014), we found that the reported foliar losses to herbivory in Brazil
401 were two-fold higher in studies involving 1–3 species of woody plants than in studies involving more
402 than 10 species. In the current study, we also demonstrated that differences in herbivory between the
403 environments decreased with the number of plant species used for herbivory measurements, and were
404 lowest when the study involved more than 10 plant species. Among these studies, 80% measured
405 herbivory from all plant species growing in a study site. The community-wide estimates of herbivory
406 (or their approximations based on measurements of herbivory from multiple plant species) clearly
407 provide a more adequate reflection of the differences between the environments when compared to
408 results from studies based on a few selected plant species. Consequently, the outcomes of multi-species
409 studies are most important for understanding the impacts of environmental factors on herbivory, for
410 uncovering global patterns in herbivory, and for making predictions on the roles of herbivores in
411 ecosystem functioning. Therefore, we conclude that studies conducted on a single or few plant species
412 not only provide overestimated levels of herbivory (Kozlov et al. 2014), but they also generally
413 overestimate the magnitude of spatial variation in plant losses to insects.

414 Plant species differ considerably in the levels of herbivory they experience (Lowman 1995, Brenes-
415 Arguedas et al. 2008, Ruiz-Guerra et al. 2010). The differences in species-specific values of herbivory

416 between few-species and multi-species studies can be explained by unconscious avoidance by the
417 researchers of plant species that showed no or little foliar damage when using non-blind methods of
418 species selection (Kozlov et al. 2014). Only rarely (one study in our database) did the authors
419 acknowledge that a study species was selected because it showed greater damage from insects when
420 compared to other plant species (Peter et al. 2015).

421 The decrease in the magnitude of the responses to environmental factors with an increase in the number
422 of plant species demonstrated in our meta-analysis is more difficult to explain. Nevertheless, we
423 suggest that this pattern may arise for two reasons. First, plant species display diverse responses to
424 environmental factors. When a given species grows in two different environments (e.g., in dry and wet
425 forests or in disturbed and undisturbed sites), one of the habitats under study will always be less
426 favorable for that species than another habitat. This difference may affect plant losses to insects
427 because some insect species inflict higher damage on more stressed plants (White 1974), whereas other
428 insect species prefer more vigorous plants (Price 1991). In community-wide studies, the responses of
429 different plant species to the same environmental factors are likely to differ not only in their magnitude,
430 but also in their direction, resulting in variable consequences for plant-feeding insects. When
431 combined, these responses are likely to yield low or even zero overall effects on plant losses to insects.
432 This pattern was found in earlier meta-analysis of the effects of herbivory on the leaf life span of
433 woody plants, where stronger effects were observed in studies that included a single herbivore species
434 than in studies of multi-species insect assemblages (Zvereva and Kozlov 2014).

435 However, if the selection of species in single-species studies was random, the effect size in the meta-
436 analysis would not differ between single- and multi-species studies. Therefore, we suggest that greater
437 differences in herbivory between environments will be observed in studies in which species are
438 selected than in multi-species studies due to researcher bias. This bias is defined by Gurevitch and

439 Hedges (1999) as a tendency to collect data on organisms or under conditions in which one has
440 reasonable expectations of detecting statistically significant effects. This kind of bias is not necessarily
441 unconscious, because this selection could be made deliberately to increase probability of obtaining
442 statistically significant results. In this case, the authors might select the most responsive plant species,
443 based on their previous experience or on earlier studies of other researchers. The high occurrence of
444 this bias is indirectly confirmed by the fact that only one paper from our database (Nuckols and Connor
445 1995) mentioned that the plant species were selected without knowledge of herbivore attacks on them
446 in either of the compared habitats.

447 The differences observed between single-species and multi-species studies may also be due to selective
448 reporting. We have noticed that when herbivory on various studied plant species differs in response to
449 environmental factors, the authors present quantitative data (which can be used in meta-analysis) only
450 for the species that fit the research hypothesis, while ignoring or reporting incompletely data for species
451 that do not fit the research hypothesis (e.g., Knepp et al. 2005, Sobek et al. 2009). This selective
452 reporting is common in many scientific disciplines, including ecology and evolution (reviewed by
453 Parker et al. 2016). Moreover, the heterogeneity of the results is often not mentioned in either the
454 discussion or the abstract; for example, Knepp et al. (2005) found support for their hypothesis only in
455 one of three study years and for only a few of the 12 plant species selected; however, they concluded
456 that their study, in general, supported the hypothesis.

457 Similarly to the effects of the study species, the detected environmental effects tended to decrease when
458 observations or experiments extended beyond a single year. This difference may be explained by
459 among-year variation in the responses of herbivory to the environmental factors under study, which
460 was demonstrated by several studies from our database (Bogacheva 1986, Knepp et al. 2005, Kozlov
461 2015). We found especially large differences between single-year and multi-year studies addressing

462 geographic variation in herbivory. Changes in herbivory with latitude or altitude may differ among
463 years not only in magnitude, but even in their direction (Kozlov et al. 2013), indicating considerable
464 interaction between geographic pattern and current-year weather conditions. A striking example is
465 provided by the study of sap-feeding insects: their load on plants decreased with latitude in typical
466 summers but increased in an exceptionally hot summer and was independent of latitude during a warm
467 summer (Kozlov et al. 2015c).

468 Higher magnitudes of the effects detected for single-year studies compared to multi-year studies may
469 also be explained by selective reporting. Quite possibly, the results from the year(s) that did not fit the
470 research hypothesis were simply not included in some papers. However, this kind of selective
471 reporting, when some results go completely unreported, is hard to uncover (Parker et al. 2016).

472 In some situations, the selection of the study year may also be biased. For example, the meta-analysis
473 of the impact of point polluters on terrestrial insects showed that insect herbivore density was
474 preferentially measured in the years when plant damage in polluted sites was apparent (Zvereva and
475 Kozlov 2010), i.e. the pattern fitted the influential hypothesis (Führer 1985, Baltensweiler 1985) on the
476 increase of herbivory under pollution impact. This bias resulted in overestimation of the pollution
477 effects on insect herbivory. However, in the majority of the studies, the selection of study year was
478 determined by factors other than levels of herbivory and thus can be considered as random.

479 Nevertheless, the spatial pattern observed in a single-year study may be transient or accidental and may
480 not agree with the general trend. This calls for conducting field studies for at least two years before a
481 justified conclusion about a spatial pattern in herbivory can be made.

482 In summary, single-species studies conducted over a single year tend to overestimate the magnitudes of
483 the differences in plant losses to insects between the environments, and these results are less likely to
484 reflect the general patterns in herbivory than are multi-species and multi-year studies.

485

486 *Biases associated with leaf sampling and with measurements of herbivory*

487

488 Sampling procedure can considerably influence the conclusions of ecological studies (Albert et al.
489 2010, Mörsdorf et al. 2015). In our meta-analysis, the impact of a researcher's expectations on the
490 outcomes of the study is clearly seen from the greatest reported effects for leaves that were sampled by
491 authors only, while an involvement of technicians (who are usually not aware of the hypothesis or have
492 less interest in confirming it) decreased the magnitude of the differences in herbivory between the
493 environments. This bias was further detected in the next stage of sample processing in the laboratory:
494 the effect was higher when the measurer was aware of the sample origin. Thus, when the person who
495 selects leaves and measures herbivory is aware of the study predictions and is interested in confirming
496 these predictions (i.e., the research hypothesis is not blinded), and when that person is aware of the
497 sample origin (i.e., the treatment conditions are not blinded), the study is likely to overestimate the
498 impacts of environmental factors on levels of herbivory due to confirmation bias. This result is in line
499 other meta-analyses within medical (Saltaji et al. 2018) and biological (van Wilgenburg and Elgar
500 2013, Holman et al. 2015) sciences, in which non-blind studies generally yield higher effect sizes when
501 compared with studies using blinding protocols.

502 By contrast, we found similar ESs for studies where plant losses to insects were estimated visually or
503 by using relatively sophisticated (e.g., digital images) or less high-tech (e.g., grids) methods and

504 devices. We could expect that, in a meta-analysis, the visual estimates of leaf damage, which are
505 presumably less precise and accurate than other methods, would result in smaller ESs due to larger
506 SDs; but this expectation was not met. This result is in line with studies showing that visual estimates
507 of herbivory are as accurate as image processing (Kozlov and Zvereva 2018 and references therein),
508 and it indicates that when measurements are not blinded, the outcomes of these measurements are much
509 more influenced by ‘wishful thinking’ of the measurer than by the particular method used.

510 However, we discovered that factors other than lack of blinding may also lead to overestimation of the
511 effects. We found, on average, 72% greater effects for studies measuring herbivory from leaves
512 haphazardly selected by the researcher when compared to studies that measured herbivory from all
513 leaves of the plant or its branch (i.e., performing no selection of individual leaves), from leaves selected
514 before herbivory was apparent (blind selection), from leaves sampled systematically (e.g., every fourth
515 leaf on branch, starting from its tip, was sampled), or from leaves sampled randomly (in the strict sense
516 of this word, where some randomization procedure was applied) . This indicates that when a researcher
517 has the possibility of selecting leaves for estimations of herbivory, this selection introduces
518 considerable bias and leads to overestimation of the differences in herbivory between the environments.
519 This unconscious bias is likely caused by certain expectations or motivations to confirm the research
520 hypothesis, and can therefore be classified as confirmation bias.

521 Interestingly, we did not find any bias in the selection of plant individuals for sampling. In contrast to
522 leaf sampling, studies that sampled plant individuals haphazardly (i.e. were prone to confirmation bias)
523 yielded similar effect size to those obtained with studies that selected plants before herbivory was
524 apparent or that used some randomization procedure or that sampled all plants in a plot. This difference
525 hints that it is generally difficult to unconsciously estimate plant-wide level of herbivory when
526 selecting a tree for sampling, especially a large one (and large trees were used in the majority of the

527 studies included in our database), whereas during the leaf sampling, the collector can unconsciously
528 assess the level of damage of each individual leaf

529 Contrary to our expectations, the formulation of a research hypothesis appeared to be not necessary for
530 the emergence of an unconscious confirmation bias: our meta-analysis did not find any differences in
531 ESs between groups of studies that strictly stated or did not state any hypothesis and the aim of the
532 study was just to compare herbivory in two types of habitats. We expected to detect this difference not
533 only because formulation of the hypothesis creates a strong motivation to confirm it, but also because,
534 in many studies, the hypothesis is formulated after getting the result ('HARKing' – Hypothesizing
535 After Results are Known) (Kerr 1998). The latter should lead to higher ESs in studies that formulated a
536 hypothesis because if the study finds strong effect, the authors would tend to HARK more frequently
537 than if the study does not find an effect or finds a weak one. However, this did not appear to be the
538 case. Our result is in line with the opinion of Forstmeier et al. (2017) that a priori hypothesis testing or
539 HARKing does not substantially differ in terms of the likelihood of a positive finding. We suggest that
540 when a study compares habitats or treatments, researchers always search for differences, both
541 consciously and unconsciously, and this is sufficient to trigger confirmation bias. The formulation of a
542 hypothesis in the publication is more a matter of journal requirements (see below).

543 Our previous study (Kozlov et al. 2014), which compared blind and non-blind sampling, showed that
544 estimates of plant damage by insect herbivores in the tropics were considerably influenced by
545 unconscious selection of plants with higher-than-average levels of herbivory, and that this bias could
546 lead to overestimation of community-wide losses of plant foliage to insects. Our current meta-analysis
547 demonstrated that differences in herbivory between the environments may also be overestimated due to
548 unconscious biases. Ecological studies involving the assessment of herbivory may appear especially
549 prone to these biases because herbivore damage may be perceived visually during sampling, and these

550 biases may affect several stages of the study: selection of species, plant individuals, branches of the
551 plant (on woody plants), leaves for measurements, and the measurement process itself. Based on the
552 analysis of published papers and the responses of authors to our questions, we concluded that all 125
553 publications included in our database have potentially been affected by unconscious confirmation bias
554 at one or several stages of the research. This is much more than the 50.4% reported in other domains
555 within ecological science, as found by Kardish et al. (2015) who analyzed 248 publications in 11 high-
556 impact journals. We showed that these multiple possibilities of the occurrence of unconscious bias
557 during assessments of herbivory led not only to overestimation of herbivory levels, but also to
558 overestimation of the magnitude of the effects of different environmental factors on spatial patterns in
559 herbivory.

560 Unconscious biases in studies of insect herbivory may be avoided by different methods. First, selection
561 procedures should be avoided (e.g., by sampling all plants on the plot and all leaves on the plant, or by
562 selecting of plants and/or leaves before herbivore damage becomes apparent). Another way is to apply
563 a true randomization procedure or to sample plants/leaves systematically. However, even when the
564 sampling of plants and leaves is unbiased, confirmation bias may still affect the outcomes of the study
565 during the measurements of leaf losses to insects. This bias is unavoidable when herbivory is estimated
566 in situ, but when leaves (or their images) are collected and analyzed in the laboratory, confirmation bias
567 may be avoided by blinding the samples (i.e., labeling them in a way that does not provide a measurer
568 with any information about the sample origin). However, only three papers (2.4%) in our database
569 mentioned blinding of samples, while in the database of Kardish et al. (2015), this percentage was
570 13.3%. However, the authors of 11 additional studies reported blinding of sample origin when
571 responding to our direct question. This result indicates that the authors clearly underestimate the
572 importance of blinding and it justifies the necessity of requesting authors for details of their methods.

573 Furthermore, it suggests that the values provided by Kardish et al. (2015), which were based on the
574 published information only, underestimate the real frequency of the use of blind methods. In any case,
575 studies including an assessment of herbivory seem to use blind methods more rarely when compared to
576 studies from other research areas within the domain of ecological, evolutionary, and behavioral
577 research.

578

579 *Publication bias and temporal trends*

580

581 The number of hypotheses being tested per paper is increasing in ecology (Low-Décarie et al. 2014). In
582 our database, 76.3% of studies erected one or more research hypotheses or formulated one or more
583 predictions, and this proportion was greater in journals with higher IFs. The percentage of published
584 studies that support the hypothesis in our database (67%) was quite close to the percentage of studies
585 detected by Finelli (2010) for the research domain ‘Environment and Ecology’ (74%). In an earlier
586 paper (Csada et al. 1996), only 8.6% of studies were found to present non-significant tests of their main
587 hypothesis in a cross-section of biological journals. This difference may either reflect temporal trends
588 (see below), or it may indicate that our study domain (Environment and Ecology) is suffering less from
589 publication bias than are biological sciences in general, which is in line with the comparative data
590 presented by Fanelli (2010). Moreover, although the proportion of studies in our database that tested
591 research hypotheses is significantly greater in journals with high and medium impact factor than in
592 low-impact journals, the frequency of publications supporting the research hypothesis does not depend
593 on a journal's IF. Contrary to expectations on preferential publication of strong significant effects in
594 high-ranked journals (Leimu and Koricheva 2004), the mean magnitude of the effects detected by our

595 meta-analysis was smaller for papers published in these journals than in journals with lower IFs. This
596 result confirms the negligible impact of publication bias on the studies included in our database.
597 Significant changes in the magnitude of research findings over time have been reported in many meta-
598 analyses from different areas of ecology, and the majority of studies discovered a decrease in the ESs
599 with publication year (summarized by Koricheva et al. 2013b). This trend may have several
600 explanations. First, it can be due to the smaller sample sizes used in older studies (Ioannidis 2008).
601 However, we have not found any temporal trends in sample size in our study, in line with the
602 conclusions by Koricheva et al. (2013b) based on 54 meta-analytical studies in ecology and evolution.
603 Jennions and Møller (2002) suggested that the decrease in ESs with time results from delayed
604 publication of studies reporting small or non-significant effects; however, evidence for time-lag bias
605 was not found in ecological studies (Jennions et al. 2013). Another explanation lies in the development
606 of the evidence for any ecological hypothesis. This process starts from strong supportive evidence of a
607 newly formulated hypothesis, but later on accumulation of disconfirming evidence leads to a decrease
608 in the ESs with time (Leimu and Koricheva 2004). However, this explanation is not applicable to our
609 database, because different papers test different hypotheses that have different ‘dates of birth’ and
610 different histories of their development. Temporal changes may also be caused by changed in the
611 preference toward particular study organisms and systems (e.g. Nykänen and Koricheva 2004,
612 Saikkonen et al. 2006). In our case, this trend could be due to temporal shifts in the proportion of multi-
613 species studies, which yield smaller effect sizes than do single species studies; however, in our study,
614 the proportion of multi-species studies did not change with time.

615 Lastly, temporal trends in ESs may result from changes in research methodology. A number of studies
616 demonstrated that improvement in methods, including statistical analysis, resulted in a decrease in the
617 magnitude of the effect (reviewed by Koricheva et al. 2013b). In our opinion, this is most plausible

618 explanation for the temporal trends observed in our study, because a significant trend was detected only
619 in high-impact journals, while the decrease was weak and non-significant in other journals. Stronger
620 demands with respect to research methodology in high-impact journals are confirmed in our meta-
621 analysis by a two-fold less frequent use of methods that are prone to unconscious confirmation bias
622 (haphazard selection of plants or leaves in the first line) when compared to other methods that are not
623 prone to this bias. High-impact journals also publish more multi-species studies, which reflect habitat-
624 specific levels of herbivory more adequately when compared with studies exploring herbivory on a
625 single or few plant species. Moreover, the frequency of pseudoreplication (expressed primarily in the
626 use of a single site in each of the compared environments) is significantly lower in high-impact journals
627 than in other journals, and this difference has especially increased during last decade. In high-impact
628 journals, the ES for environmental effects reported in the publications from 2010-2018 were as low as
629 0.3, which indicates a high proportion of papers that either did not detect significant effects or that
630 detected effects contradicting the research hypothesis.

631 The detected temporal trends in the improvement of methodology may at least partly result from
632 Transparency and Openness Promotion (TOP)—a framework that emerged in 2015 and is currently
633 supported by many high-impact journals, with many TOP guidelines that request or require more
634 thorough reporting of methods and results (Parker et al. 2016). Our meta-analysis demonstrated that
635 progress in the quality, presentation, and transparency of methodology has already led to a decrease in
636 the frequencies of methodological flaws and, as a result, to a decrease in the magnitude of
637 environmental effects on herbivory measured during last decade. This decrease in the magnitude of the
638 effect with time, and even an increase in the number of publications that oppose the existing
639 hypotheses (12 of 14 these papers were published after 2010), indicates not only an improvement of
640 methodology, but also a considerable decrease in publication bias against non-confirming results in

641 high-impact journals. A similar shift in publication bias has been demonstrated by Low-Décarie et al.
642 (2014) for three ecological journals. From these patterns, we conclude that studies published in the
643 1960s–1990s considerably overestimated the magnitude of environmental effects on insect herbivory.
644 Our results also oppose the opinion that publication bias is especially strong in high-ranked journals,
645 which prefer to publish studies reporting large, highly significant effects (Murtaugh 2002) and/or
646 support a prevailing hypothesis over those that reject it (Leimu and Koricheva 2004). We found that
647 this kind of publication bias is weaker in high-impact journals than in journals with lower ranking,
648 especially in recent years. A similar trend has been demonstrated in the meta-analyses of the impact of
649 pollution on arthropods, plant growth, and plant diversity, where ESs were lower in high-impact
650 publications (Zvereva et al. 2008, 2010, Zvereva and Kozlov 2010). This means that, in our research
651 domain (spatial patterns in herbivory), the pressure on researchers to publish only strong and
652 supportive results is relatively low and steadily decreasing. We conclude that the current trends in
653 herbivory studies overcome the overestimation of the effects due to different biases and thereby
654 improve our knowledge, mostly due to the changing editorial policy of high-impact journals.

655

656

CONCLUSIONS

657

658 Using the studies of spatial patterns in herbivory as an example, we showed that the properties of
659 human mind may lead to overestimation of the magnitudes of the effects recorded in the course of
660 ecological and environmental studies (Table 1). The magnitude of this overestimation is of high
661 ecological significance: methods that are not prone to biases and thus reflect the real situation more
662 adequately yield small to medium ESs (0.2 and 0.5 according to Cohen 1988), while methods that are

663 prone to biases generally yield large ESs (0.8). Importantly, factors related to methodology appeared
664 the only significant sources of variation in magnitudes of spatial patterns in insect herbivory, and most
665 of this variation likely emerged due to various biases. Whether a hypothesis is formulated or not,
666 researchers always have some expectations concerning the studied patterns and processes, and these
667 expectations trigger unconscious psychological processes, unavoidably biasing the outcome of the
668 study. The biases are especially frequent in studies based on sampling of data from natural ecosystems,
669 and a number of measures should be taken to avoid these biases (Table 1). Selection of study species
670 may be prone to bias and should therefore be avoided when comparing different environments—for
671 example, by sampling all species on the plot. At lower hierarchical levels, when the selection of study
672 units (such as individual leaves within a large tree) is unavoidable, sampling should be randomized in
673 the strict sense (i.e., by applying randomization procedures). Finally, the studies should be blinded with
674 respect to the research hypothesis and sample origin. The information about all these measures taken to
675 avoid biases should be explicitly provided in the publications. Researchers must remain aware of the
676 existence of unconscious biases that occur at different stages of the research and that can considerably
677 influence the magnitudes of the effects under study. Accumulation of biased results in primary studies
678 may ultimately lead to false general conclusions when the outcomes of these studies are summarized in
679 research syntheses.

680

681

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682

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Table 1. Overview of the potential and actual effects of research methodology on the outcomes of studies of spatial patterns in insect herbivory.

| Stage of research | Procedure | Expected | Compared groups of studies | Conclusions of meta-analysis and relative differences in effect sizes ¹ | Recommendations |
|-------------------|-----------------------------|--|--|--|---|
| Planning | Selection of plant species | Conscious and unconscious biases: selection of species that are likely to produce the expected results | Selected species > entire plant community | Confirmed: 61% | Study multiple plant species; whenever possible, obtain community-wide estimates of herbivory |
| | Selection of study duration | Conducting the study in a year when the | Single-year studies > multi-year studies (for studies of | Confirmed ² : 87% | Repeat measurements of herbivory during at least |

¹ The absolute difference in effect sizes relative to smaller effect size; reported only for significant ($P < 0.05$) and marginally significant ($0.05 < P < 0.08$) differences.

² The difference is marginally significant ($0.05 < P < 0.07$).

| | expected effect is apparent | geographic patterns) | | two years |
|--------------|--|----------------------|--|--|
| | Formulation of HARKing predictions (Hypothesizing After Results are Known) | Confirmation bias | Studies that formulated predictions > studies that did not formulate predictions | Not confirmed No |
| Sampling | Selection of individual plants | Confirmation bias | Haphazard selection > random/systematic/no selection | Not confirmed To be on the safe side, random or systematic selection is recommended |
| | Selection of individual leaves | Confirmation bias | Haphazard selection > random/systematic/no selection | Confirmed: 74% Sample all available leaves or apply strict randomization protocol |
| | Selection of individual leaves | Confirmation bias | Leaves collected by authors > leaves collected by technicians | Confirmed ² : 97% Blind the hypothesis to collectors |
| Measurements | Assessment of leaf damage | Confirmation bias | In situ > in the laboratory | Not confirmed No |
| | Assessment of | Confirmation bias | Visual > grids > image analysis | Not confirmed No |

| | | | | | |
|-------------|--|--|---|----------------------------|---|
| | leaf damage | | | | |
| | Assessment of leaf damage | Confirmation bias | Sample origin known to measurer > sample origin unknown to measurer | Confirmed: 135% | Blind sample origin to measurers |
| Publication | Selection of results for publication | Publication bias | High-impact (impact factor > 2.5) journals > low-impact (impact factor < 1.6) | Opposed ² : 58% | Increase demands for the quality and presentation of research methodology |
| | Peer reviewing in low-impact journals | Decrease in publication bias with time | Old publications (before 2010) > new publications (2010–2018) | Not confirmed | Increase demands for the quality and presentation of research methodology |
| | Peer reviewing in high-impact journals | Decrease in publication bias with time | Old publications (before 2010) > new publications (2010–2018) | Confirmed: 137% | Not needed |

Figure legends

Fig. 1. Effects of the number of plant species involved in the study and the study duration on the magnitude of the detected differences in herbivory between the environments. Horizontal lines denote 95% confidence intervals; sample sizes are shown in parentheses.

Fig. 2. Effects of unconscious selection bias on differences in insect herbivory between environments. No selection: all trees per plot or all leaves per tree/branch were sampled; random or systematic selection involved pre-defined selection protocols; haphazard selection was made intuitively by a researcher without the use of any pre-defined protocol. Effects of sample collection and information about sample origin are calculated for measurements of herbivory conducted in the laboratory. In original studies, the effect size was considered negative when the outcome of the study opposed the predictions (i.e., a significant effect was observed in the direction opposite to the predicted direction). Horizontal lines denote 95% confidence intervals; sample sizes are shown in parentheses.

Fig. 3. Effects of the impact factor (IF) of the journal and of the publication year on differences in herbivory between the environments. Because we were interested in the willingness of journals to publish negative results (i.e., results opposing the predictions), the effect size in those studies was considered negative (i.e., a significant effect in the direction opposite to the predicted direction). Horizontal lines denote 95% confidence intervals; sample sizes are shown in parentheses.

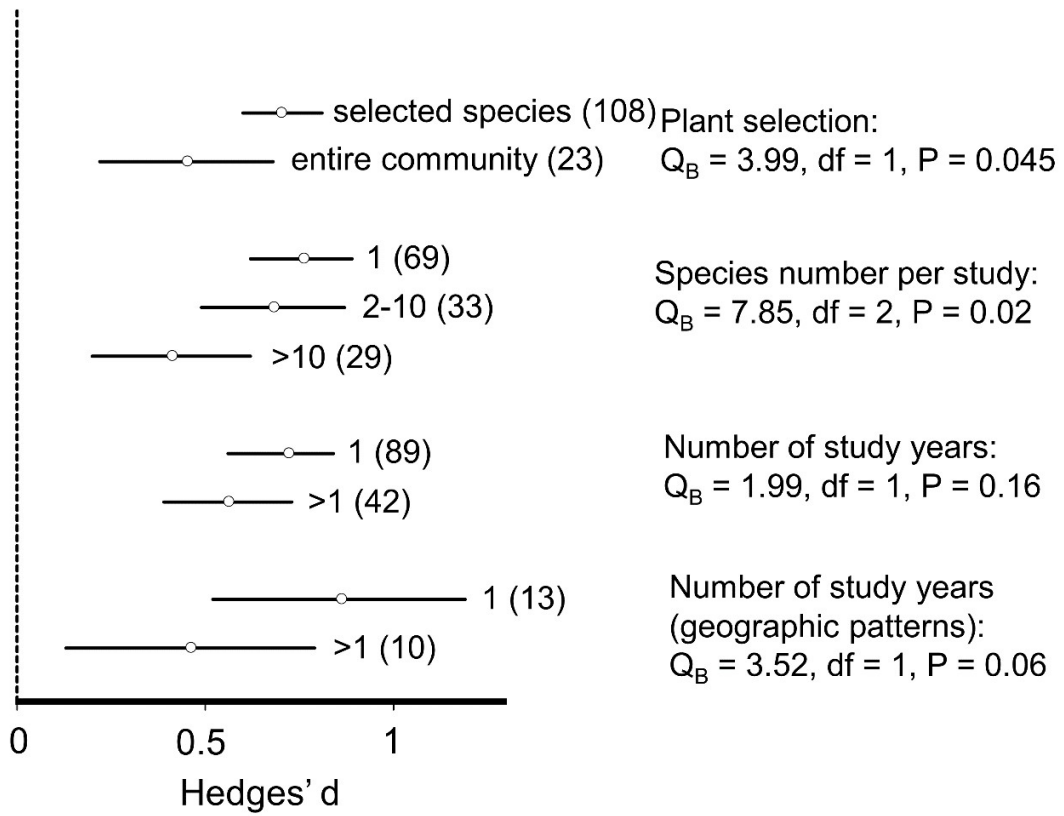


Fig. 1

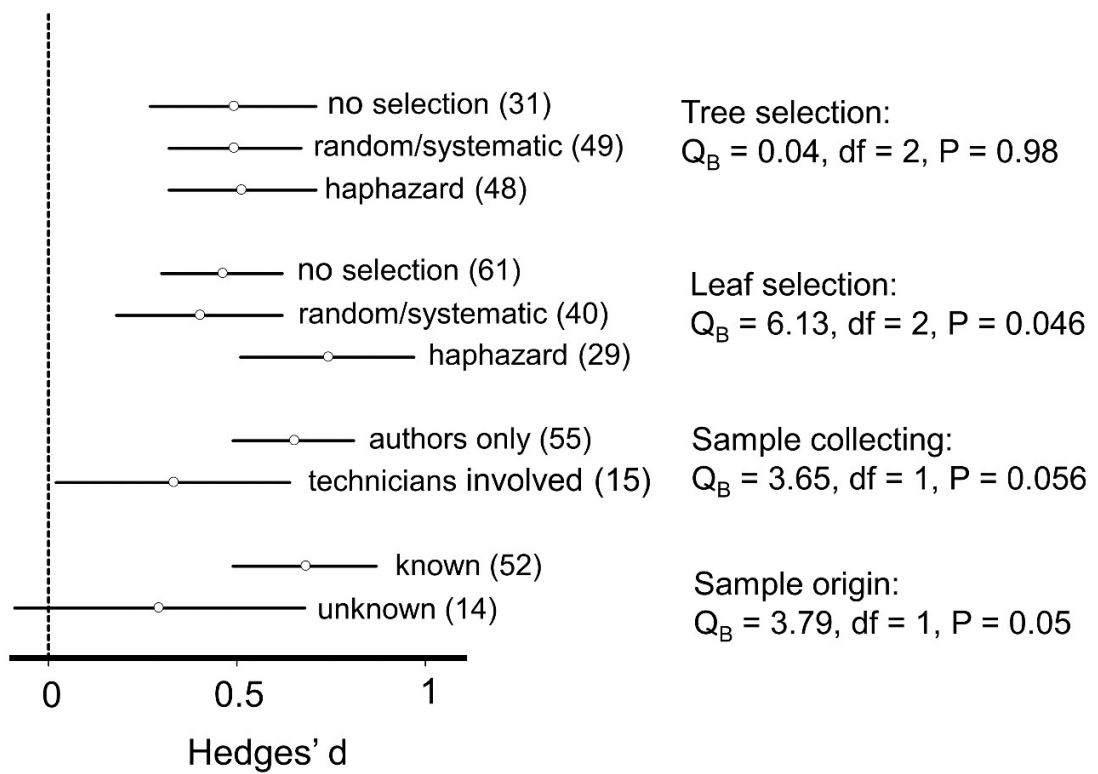


Fig. 2

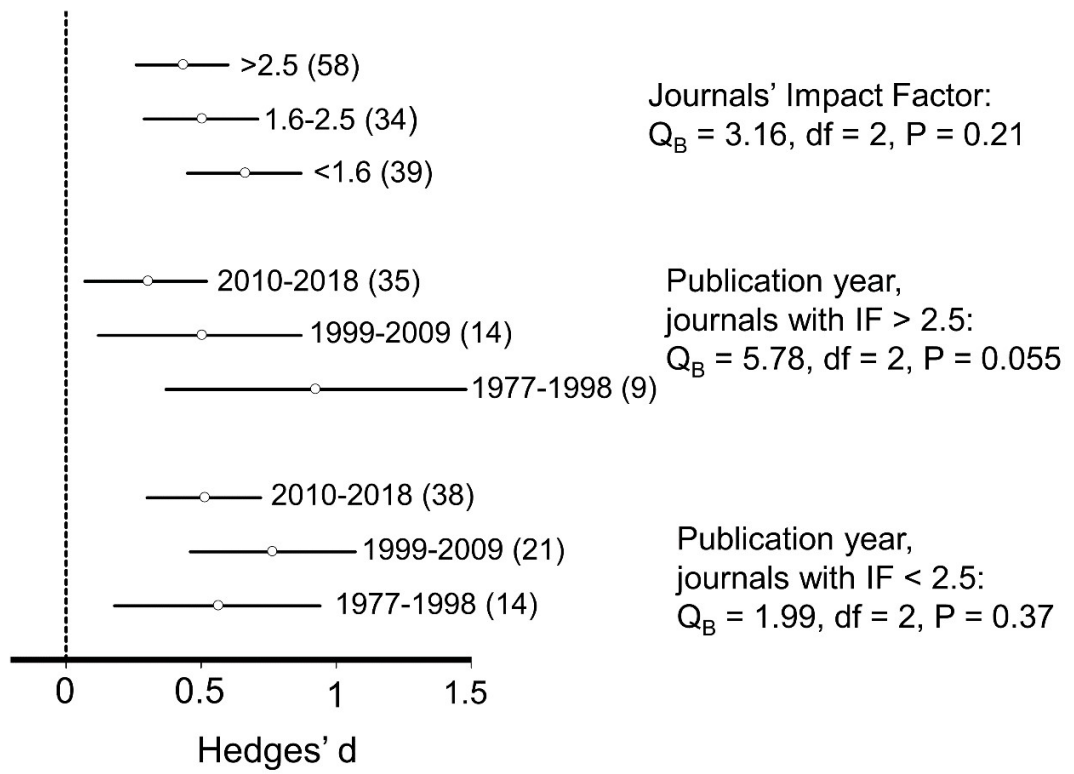


Fig. 3