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Effect of urbanization and its environmental stressors on the intraspecific variation of flight functional traits in two bumblebee species — [Source link](#)

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24 **ABSTRACT**

25 Landscape anthropization replaces natural areas with agricultural or urban covers,
26 leading to land-use intensification. This worldwide phenomenon affects biodiversity,
27 but little is known about the effects on the intraspecific variation of functional traits
28 related to ecosystem services. By combining field data collection, remote sensing and
29 land cover analysis we investigated how land use intensification affects functional
30 traits in two bumblebee species. Namely, the impact of different land use cover (i.e.,
31 semi-natural, urban, and agricultural) and of multiple biotic and abiotic stressors (i.e.,
32 temperature, resource availability, and air pollutants) was investigated. Along a
33 gradient of landscape anthropization, we sampled populations of two European
34 bumblebee species (*Bombus terrestris* and *B. pascuorum*) at 37 sites in Northern Italy.
35 Through geometric morphometrics we investigated the variation of morphological
36 traits related to flight performance (i.e., wing centroid size and shape and size
37 fluctuating asymmetry FA), previously used as indicators of stress during insect
38 development. Our results point out an idiosyncratic response of the two species to
39 landscape anthropization. Smaller individuals of *B. pascuorum* were observed in
40 response to increased impervious cover and temperature. No similar patterns were
41 noticed in *B. terrestris*, which was characterized by larger individuals in response to
42 floral resource availability. Wing size FA was positively associated with warmer
43 temperatures and increased levels of NO₂ only in *B. terrestris*. Overall, this study
44 found taxon-specific functional trait variation at the intraspecific level in syntopic
45 organisms, expanding our understanding about the effects of land-use intensification
46 on the ecological activity of pollinator model species.

47

48 **Keywords:** Functional diversity, heat islands, pollination, urban ecosystems, wing
49 asymmetry

50

51 **INTRODUCTION**

52

53 Widespread phenomena, such as the progressive expansion of agricultural and
54 urban lands are driving deep changes on ecosystems, among which the replacement of
55 natural areas and the creation of novel ecosystem conditions. Plants and animals
56 respond to these marked environmental variations by shifting their distribution (Colla
57 et al., 2012) and phenology (Huchler et al., 2020), and/or shaping some morphological
58 traits considered “functional”, i.e., relevant for their ecology, fitness and behaviour
59 (Alberti et al., 2017; Eggenberger et al., 2019; Nooten & Rehan, 2020). Trait variation
60 due to environmental alteration is particularly relevant in those organisms responsible
61 for ecosystem services, such as pollinators, since changes in their traits could
62 jeopardize the service provided (Buchholz & Egerer, 2020). For instance, land-use
63 intensification and degradation (i.e., the growing proportion of anthropized or
64 productive surfaces across landscapes) could lead to limited floral resource
65 availability to pollinators (Steffan-Dewenter et al., 2001). This scenario, in turn, could
66 impose changes in pollinator functional traits, such as body size decline due to less
67 food supplied to larvae. Furthermore, anthropization of landscapes changes the local
68 microclimate, thus determining an alteration of pollinator insects ecology,
69 development and activities (Radmacher & Strohm, 2010). Specifically, higher degrees

70 of impervious land cover are often associated with increasing temperatures (Chun &
71 Guldmann, 2018), a phenomenon known as “heat island effect”, that characterizes
72 both urban and suburban areas. Observations from previous studies have strengthened
73 the hypothesis that pollinator insects could face a shift towards smaller body size as an
74 adaptation to reduce risk of overheating in presence of warmer conditions (Peters et
75 al., 2016; Gérard et al., 2018a). Considering that the steady growth of the human
76 population is driving a dramatic increase in the area allocated to intensive agriculture
77 and urban expansion worldwide, new insights in the study of pollinator response are
78 necessary to predict which scenarios would likely occur for urban biodiversity and its
79 mediated services.

80 Previous studies investigated the morphological responses of pollinators to
81 anthropogenic pressures, mainly focusing on key functional traits, such as body size
82 (e.g., Chown & Gaston, 2010; Eggenberger et al., 2019; Theodorou et al., 2020). In
83 bees, this character responds rapidly to environmental changes (Chown & Gaston,
84 2010), it shows little heritability and its variation mainly depends on the amount of
85 food received during the larval development (Couvillon et al., 2010). Bee size directly
86 influences their mobility and so their foraging range (Greenleaf et al., 2007), but also
87 determines the metabolism rate and resource needs of adult imagos, with larger bees
88 having higher metabolism rate (Kelemen et al., 2019) and thus potentially being more
89 susceptible to variation in floral resource availability leading to starvation (Couvillon
90 & Dornhaus, 2010). However, to date, the investigation of pollinator body size
91 variation in anthropogenic habitats yielded heterogeneous results. A recent study on
92 bumblebees found bigger workers in cities (Theodorou et al., 2020). This pattern has

93 been interpreted as an adaptation to longer flights for collecting resources, due to
94 severe habitat fragmentation and dispersion caused by land use intensification
95 (Greenleaf et al., 2007). Conversely, a study by Eggenberger et al. (2019) found
96 smaller bumblebee foragers in cities, interpreted as an effect of both limited local
97 resource availability and warmer temperature in urban areas.

98 A promising research field in the study of morphological traits is to identify
99 possible stress biomarkers of habitat quality and population health status (Adams et
100 al., 2001). The advantage of using trait variation to measure stress resides in the fact
101 that changes of phenotypes are detectable before a decrease in population viability
102 occurs (Hoffmann et al., 2005). Therefore, quantifying traits variation could become
103 an essential practice when evaluating local- and landscape-level stressors. A metric
104 that has grown in popularity is the fluctuating asymmetry (FA) (Klingenberg, 2001;
105 Beasley et al., 2013; Alves-Silva et al., 2018), defined as the presence of small,
106 randomly placed deviations from perfect bilateral symmetry due to the occurrence of
107 developmental instability, driven by exogenous environmental conditions
108 (Klingenberg, 2015). FA differs from another type of bilateral asymmetry, the
109 directional asymmetry (DA), that occurs when one specific side tends to be steadily
110 larger than the other. While DA have a genetic basis and therefore could be less
111 impacted by the environment (Palmer & Strobeck, 2003), the FA is considered a valid
112 proxy of stress exposure to conditions that typically occur in anthropized
113 environments (e.g., temperature, air pollutants, and pesticides) (Beasley et al., 2013).
114 For instance, laboratory-based studies have demonstrated that CO₂ or low temperature
115 leads to wing FA, supporting the possible role of traffic pollutant in determining

116 developmental instability (Klingenberg et al., 2001; Hoffmann et al., 2002).
117 Interesting, however, these studies also indicated how wing shape and wing size
118 asymmetries had different responses to the same stressor type. This aspect is
119 ecologically very relevant. Both wing size and shape are important functional traits in
120 pollinators. This is because wing size is believed to be related to flight length and it
121 influences metabolic costs (Fernandez et al., 2017; Soule et al., 2020), while shape is
122 considered as important for flight maneuverability (Kolliker et al., 2003, Grilli et al.,
123 2017). Thus, their asymmetry could reduce flight performance ability and thus could
124 reduce fitness and even impact on species interactions and pollination efficiency.

125 In order to characterize the effects of landscape anthropization and related
126 pressures on pollinator insects, we quantified the morphological variation in two
127 species of bumblebee (i.e., *Bombus pascuorum* and *B. terrestris*), by investigating
128 different populations across a gradient of growing land-use alteration, from semi-
129 natural areas to rural sites and urban places. Specifically, we chose the region
130 surrounding Milan (Northern Italy). This is one of the major European cities and lies
131 on a territory that experienced a strong anthropogenic footprint and ongoing efforts
132 are trying to mitigate it. We expected to find quantitative variation in bumblebee
133 functional traits (i.e. body size and wing FA) in response to increased land-use
134 intensification. Our hypothesis was that in anthropized areas, pressures eliciting a
135 decrease in body size (e.g., high temperature and low floral resource availability)
136 would be prevailing and would lead to smaller foragers in urbanized and agricultural
137 habitats. We also expected to find increased wing size and shape FA in response to
138 higher levels of biotic and abiotic stressors (e.g., floral resources abundance,

139 temperature, and air pollutants). Hence, our ultimate goal was to determine which type
140 of response prevails and the putative pressures that are determining it to better
141 understand the impact of landscape anthropization on the ecology of pollinator insects.

142

143 **MATERIALS AND METHODS**

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145 **Study species**

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147 This study was focused on two co-occurring species of bumblebee: *Bombus*
148 *terrestris* (Linnaeus 1758) and *B. pascuorum* (Scopoli 1763). Both species are
149 efficient pollinators, common in Europe, and can be found in different habitats from
150 the natural to the agricultural and urban ones (Polce et al., 2018). Given these
151 characteristics, these species are reliable models to investigate responses by
152 pollinating insects to landscape anthropization (Eggenberger et al., 2019; Theodorou
153 et al., 2020). The two selected species have slightly different foraging ranges, with
154 estimated maximum dispersal range of 449 and 758 m for *B. pascuorum* and *B.*
155 *terrestris*, respectively (Knight et al., 2005). Nesting habits are also dissimilar as *B.*
156 *terrestris* builds its nest in subterranean holes, while *B. pascuorum* on top of or
157 slightly beneath the soil surface (Goulson, 2010). Another important difference is
158 represented by their dietary regimes with the *B. pascuorum* usually having a narrower
159 trophic niche and a preference for deep-corolla flowers (Harder, 1985).

160

161 **Study design and sampling**

162

163 Samplings were conducted at 37 sites (Fig. 1), mainly lying within the
164 metropolitan area of Milan in July 2019, only on days with sunny and not windy
165 weather conditions.

166 The study sites were distributed in an area of about 1800 km² covering four
167 administrative provinces, Milano, Monza e della Brianza, Lecco and Como. A
168 minimum distance between sites of 1 km was imposed to avoid the non-independence
169 of sites (Phillips et al., 2019) since it is above the mean foraging range observed for
170 the two species (Knight et al., 2005). A Moran test was also applied to confirm the
171 absence of spatial auto-correlation within sites ($P>0.05$).

172 Study sites spread along a gradient of landscape anthropization, ranging from areas
173 dominated by semi-natural hay meadows close to forest, to agriculture dominated
174 landscapes (i.e., sites at the edges of crop fields of mainly large monoculture) and sites
175 characterized by a high degree of impervious surface (i.e., concrete, building, asphalt).
176 For each species, five to six specimens, excluding queens and males, were captured in
177 a plot of about 50 m x 50 m at each site using an entomological net. After collection,
178 the insects were stored at -80 °C until further analysis.

179

180 **Landscape and environmental variables**

181

182 In order to assess landscape anthropization, land-use data were obtained from
183 the latest version of land cover database provided by Lombardy region (2018-DUSAF
184 6.0; <https://www.dati.lombardia.it/Territorio/DUSAF-6-0-Uso-del-suolo->

185 [2018/7rae198](#) fng6). Using QGIS 3.10.11, a 1 km radius buffer area was created
186 around each site where landscape composition was evaluated arranging DUSAF
187 original level and sub-level of land-use classification into three macro categories:
188 impervious (i.e., buildings, infrastructures, roads, and cemented surface), agricultural
189 (i.e., arable land devoted to crop production) and semi-natural land (i.e., meadows,
190 forest and urban green spaces) (Online resource 1 for a list of DUSAF codes assigned
191 to each grouping). For each site, the ratio between impervious and semi-natural land
192 (Impervious / Natural) as well as the ratio between agricultural and semi-natural land
193 (Agricultural / Natural) was computed to describe the urbanization and agricultural
194 intensification processes, respectively. Habitat fragmentation was quantified by
195 computing the edge density (ED), as the ratio of edge length of green and semi-natural
196 patches over their total area (Wang et al., 2014).

197 Other environmental biotic and abiotic features were considered to test for their
198 potential effects on altering body size and wing size/shape FA. Specifically, land
199 surface temperature was calculated as the mean value in the period June-July using
200 data retrieved through remote sensing imaging spectroradiometer (MODIS)
201 MOD11A2 from the NASA database
202 (<https://modis.gsfc.nasa.gov/data/dataproduct/mod11.php>) with a resolution of 1 km. The
203 choice of considering temperature for these two months was dictated from the
204 supposition that our sampled specimens developed during that period, according to the
205 biology of two selected species (Goulson, 2010). Air pollution was estimated as the
206 mean of daily concentrations of NO₂ over two months (June and July) registered by
207 Regional agency for environmental protection (ARPA) through monitoring stations

208 located nearby our sampling sites ([https://www.arpalombardia.it/Pages/Aria/qualita-](https://www.arpalombardia.it/Pages/Aria/qualita-aria.aspx)
209 [aria.aspx](https://www.arpalombardia.it/Pages/Aria/qualita-aria.aspx)).

210 Floral resources at each site (i.e., the total number of flowers) was estimated in
211 sampling plots by using six randomly placed quadrats 1 m x 1 m (covering a
212 proportion of sampling area similar to that reported in Fisher et al., 2017) and
213 summing the number of flowers found within each quadrat.

214

215 **Specimens imaging and wings measurement**

216

217 The forewings of all individuals were detached at the base and scanned at high
218 resolution. The obtained images were converted into TPS files using tps-UTIL 1.74
219 and digitised using the tps-Dig 2.31 software (Rohlf, 2015), with two-dimensional
220 cartesian coordinates of 15 landmarks positioned at wing vein junction (Fig. 2) (as in
221 Aytekin et al., 2007; Klingenberg et al., 2001). Bumblebees with damaged or badly
222 worn wings were excluded from further analyses.

223 The analysis of landmark configuration was conducted in MorphoJ 1.07 software
224 (Klingenberg, 2011). To remove all differences unrelated to shape (i.e., rotation,
225 translation, and scale) a generalized least square Procrustes superimposition was
226 applied (Klingenberg, 2011). The output of this procedure is a new set of
227 superimposed landmark coordinates, called ‘Procrustes shape coordinates’, that
228 contains all the shape information. Wing size was estimated as the centroid size (i.e.,
229 the square root of the sum of squared distances from the centroid of each landmark
230 configuration) and used as a proxy of body size (hereafter “body size”, as in

231 Outomuro & Johansson, 2011 and Dellicour et al., 2017). Wing size asymmetry was
232 computed by dividing the absolute difference between left and right centroid sizes by
233 the mean centroid size and multiplying by 100 (Leonard et al., 2018). To estimate
234 wing shape variation, Procrustes distances were computed for each individual
235 (Klingenberg, 2015). These represent the measure of an individual's overall
236 asymmetry (i.e., sum of DA and FA components), obtained by taking the square root
237 of the sum of squared distances between corresponding right and left Procrustes'
238 coordinates.

239

240 **Statistical analysis**

241

242 Since the levels of asymmetry in bilateral traits are subtle, we estimated the
243 measurement error, which could possibly cause considerable variation in the
244 assessment of asymmetry levels, by double-scanning wings and digitizing their
245 landmarks for a subset of 50 specimens, in MorphoJ with the Procrustes ANOVA
246 (Klingenberg, 2001), in order to evaluate if measurement error was negligible. The
247 presence of directional asymmetry (DA) was also tested with the same approach, and
248 if DA occurred, the total asymmetry measures were corrected subtracting the mean
249 DA from the overall individual asymmetry, thus isolating the FA component as in
250 Costa et al. (2015).

251 To investigate the relationship between morphological traits and covariates (i.e., ratio
252 between impervious and natural cover, ratio between agricultural and natural cover,
253 edge density, temperature, NO₂, and floral resources) a series of linear mixed models

254 were used. In order to improve the fit between the predictors and the response
255 variable, mathematical transformations were applied on the covariates as reported in
256 Table 1. The goodness of these transformations was assessed through the AIC
257 criterion. ($\Delta AIC > 2$).

258 The response of the two species was assessed separately, furthermore different models
259 were used to evaluate the effects of highly correlated variables ($r > 0.7$). Specifically,
260 the response of centroid size was tested in two different models to account for
261 covariates collinearity. The first one (Table 1, model id A) tested the effect of i) edge
262 density of green areas, ii) ratio between impervious and semi-natural land cover, iii)
263 ratio between agricultural and semi-natural land-cover, and iv) floral resources.
264 Conversely, the second model (Table 1, model id B) evaluated separately the impact
265 of temperature because of its correlation with the ratio between impervious and semi-
266 natural land cover. Regarding FA, the effects of covariates known to influence the
267 developmental stability as temperature (Table 1, model id C), and NO_2 (Table 1,
268 model id D) were tested individually both for wing size FA and wing shape FA
269 because of their correlation. In all models, the sampling site was included as the
270 random effect. For all models we applied a stepwise model selection based on AIC
271 values removing variables that did not improve the model fit (Zuur et al., 2009).
272 Statistical analyses were conducted using MorphoJ 1.07 and JMP 14.2.0 (© SAS
273 Institute Inc. Cary, North Carolina, US).

274

275 **RESULTS**

276 Overall, the sampling dataset was similar in the two bumblebee species,
277 encompassing a total of 179 *B. pascuorum* (mean per site = 4.8 ± 0.4) and 169 *B.*
278 *terrestris* (mean per site = 4.5 ± 0.3) that were considered for morphometric analyses.

279 The measurement error was negligible because not significant for wing size (df = 79,
280 $F = 2.67$ $p = 0.4578$) and shape (df = 2054, $F = 0.51$, $p = 0.9976$). Different patterns of
281 size variation were found in the two bumblebee species. *B. pascuorum* decreased its
282 body size in response to both the increasing temperature (Fig. 3a; Table 1: model id B)
283 and the higher ratio of impervious on semi-natural land cover (Online resource 2 - Fig.
284 S1 ; Table 1: model id A). Conversely, no significant effect of these two predictor
285 variables was found on *B. terrestris*, that increased body size in response to the
286 availability of floral resources (Fig. 3b; Table 1: model id A).

287 Concerning wing asymmetry, both species showed a significant level of shape DA (*B.*
288 *pascuorum* df = 26, $F = 4.66$; $p < 0.0001$; *B. terrestris* df = 26, $F = 5.60$; $p < 0.0001$),
289 while size DA was statistically significant only in *B. pascuorum* (df = 1, $F = 29.77$; p
290 < 0.0001 ; in *B. terrestris* df = 1, $F = 0.51$; $p = 0.4779$). Measures of FA were then
291 obtained correcting for directional components.

292 *B. terrestris* size FA was positively correlated to temperature (Fig. 4; Table 1: model
293 id C) and concentration of NO₂ (Online resource2 - Fig. S2; Table 1: model id D), but
294 no similar response was observed in *B. pascuorum*. None of the predictor variables
295 (i.e., temperature, NO₂, resource abundance) showed any significant effect on wing
296 shape asymmetry in both bumblebee species.

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305 Table 1: Results of the regression analysis of centroid size and fluctuating size asymmetry as a function of
 306 landscape and biotic and abiotic covariates. Only final models selected through AIC criterion with significant
 307 covariates are reported. β_i : regression coefficient; F: F-value; P: p-values. Models and results of size FA for *B.*
 308 *pascuorum* and of shape FA for both species are not reported in the table as they were inconsistent.

Response variable	Model ID	Covariates	Bi	Df; F	P > F
<i>B. pascuorum</i> Centroid size	A	Impervious/Natural (Log)	-0.00869	1; 4.2661	0.0462*
	B	Temperature	-0.00341	1; 7.7271	0.0088*
<i>B. terrestris</i> Centroid size	A	Floral resources (Log)	0.0725	1; 11.0222	0.0026*
<i>B. terrestris</i> Fluctuating size asymmetry	C	Temperature	0.05259	1; 11.5565	0.0019*
	D	NO ₂	0.01433	1; 7.2304	0.0118*

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320 **DISCUSSION**

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322 In this study, we quantified the spatial intraspecific variation of functional traits
323 that can shape the ecology of two common bumblebee species, possibly altering their
324 dispersion ability, and pollination efficiency. We focused on their variation along the
325 main trajectories of land use alteration, namely urbanization and agriculture
326 intensification of landscapes, by relating variation of body size and wing asymmetry
327 with landscape features and environmental stressors.

328 Landscape anthropization was found to be associated with intraspecific
329 variation of body size (i.e., estimated as wing centroid size) across bumblebees
330 populations. Specifically, foragers of one of the two investigated species, *B.*
331 *pascuorum*, showed a shift toward smaller body size in response to increasing
332 proportion of impervious surfaces and temperature, two conditions that characterize
333 urban landscapes and emerged as highly correlated from our analysis. A similar
334 pattern of body size reduction in urban bumblebees was reported by Eggenberger et al.
335 (2019) and it agrees with observations showing a general decrease of body size in
336 urban insect communities (Merckx et al., 2018). These works suggest that a potential
337 driver of size reduction in bumblebees is represented by the diminished, and spatially
338 dispersed, availability of floral resources that is often associated with higher

339 impervious cover (Eggenberger et al., 2019). However, in our study, neither the
340 estimation of floral resources, nor the fragmentation of green areas (i.e., the edge
341 density), correlated with size variation in *B. pascuorum*, the species that responded to
342 urbanization. Hence, a more reliable driver of urban-induced size shrinkage could be
343 warmer temperatures linked to the urban “heat island effect” (Merckx et al., 2018),
344 and this is also supported by the results of our study. Indeed, temperature has a strong
345 effect on insect body size, with multiple historical series collections-based and
346 experimental studies that revealed how higher environmental temperatures represent a
347 driver of body size reduction in different species of bees (e.g., Nooten & Rehan, 2020;
348 Theodorou et al., 2020). Higher temperature accelerates larval development, which
349 likely result in smaller adults (Sibly & Atkinson, 1994). Furthermore, smaller sizes in
350 warmer areas could also be a strategy for reducing overheating risks while foraging,
351 due to an increased convective heat loss in smaller bees (de Farias-Silva & Freitas,
352 2020). Functionally, smaller foragers could travel shorter foraging distances
353 (Greenleaf et al., 2007) and could also load less pollen and nectar (Goulson et al.,
354 2002). As a consequence, the shift towards smaller body size in *B. pascuorum* in more
355 urbanized environments could imply that it will pollinate less plants or handle flowers
356 less efficiently (Földesi et al., 2020), a concerning aspect in view of colony provision
357 and pollination.

358 In our study *B. terrestris* body size was found to respond only to the abundance of
359 flower resources, with larger individuals observed where potentially more food is
360 available. This is in accordance with the observation that adult size is strictly
361 correlated with the amount of food received during larval development (Couvillon &

362 Dornhaus 2009). However, this trend seems to be not clearly confirmed by *B.*
363 *pascuorum* probably due to the higher specialization of this species (Harder, 1985)
364 that may prevent it from benefiting from higher availability of flowers, since this not
365 necessarily results in higher possibility to exploit adequate resources for their diet.

366 Despite *B. pascuorum* and *B. terrestris* belong to the same genus, the responses
367 of these two species were not equal. Similarly, idiosyncratic responses were also
368 observed in other bumblebee species, where if the body size of some decreased over
369 warming decades, others responded in the opposite way (Gérard et al., 2020). In our
370 study the invariant size of *B. terrestris* in warmer conditions could be explained by the
371 high heat tolerance of this species (Martinet et al., 2020). In addition, *B. terrestris*
372 nests further underground compared to the other bumblebee, and might be less
373 exposed to warm, urban air temperatures during larval stages. These aspects
374 strengthen the hypothesis that temperature is a major determinant of pollinator size
375 reduction in cities because they affected the body size of the more temperature-
376 sensitive species, but not the heat-tolerant one. These species-level, idiosyncratic
377 responses are very relevant for our understanding of the potential mechanism of
378 intraspecific trait variation associated with landscape anthropization. This supports the
379 need to consider a wider panel of species when investigating the impact of landscape
380 anthropization on functional biodiversity.

381 Flight performance does not only depend on body size, but it is also affected by
382 asymmetries in shape and size between wings (Grilli et al., 2017; Soule et al., 2020).
383 Here, we found that wing size FA was positively correlated with environmental
384 stressors such as increased temperatures and NO₂ concentration in *B. terrestris*. The

385 absence of variations in shape asymmetry registered for both the bumblebee species
386 could confirm the results from other studies that have indicated shape variation as less
387 susceptible to stressors than size asymmetry (e.g., Gérard et al., 2018b). Variation in
388 wing asymmetry was observed in other insect taxa; the effect of temperature was
389 previously investigated under controlled laboratory conditions (Mpho et al., 2002)
390 while the role of pollutants was discussed interpreting the results of a field
391 experiment evaluating the impact of road traffic exposure (Leonard et al., 2018).
392 Studies associated the increased wing FA to environmental stressors, indicating that
393 impairment of developmental processes might take place (e.g., Klingenberg et al.,
394 2001; Kerr et al., 2013). Concerning pollutants, Klingenberg et al. (2001)
395 hypothesized that they could alter development through mechanisms related to gas
396 exchange. Trait variation does not only show developmental instability, but it also has
397 ecological implications. Wing size FA impacts the management of lengthy flights
398 (Fernandez et al., 2017, Soule et al., 2020), compared to wing shape which is often
399 associated with flight maneuverability. Thus, size asymmetry could specifically
400 impact flight performance, flight length in time and space, and even affect bumblebee
401 interactions with plants, the provisioning of larvae with food and their pollination rate.

402

403 **CONCLUSIONS**

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405 Overall, the experimental results obtained in this study suggest that landscape
406 anthropization could affect different functional traits, and it occurs heterogeneously in
407 different pollinator species. Eventually, these responses could bring to the alarming

408 outcome of decreased pollination efficiency, because the studied traits are often
409 involved in flying abilities. Temperature emerged as one of the main drivers of these
410 phenotypic variations, affecting the two species in a different manner. The multiple
411 responses to the same stressor, observed for the two species, underline the necessity
412 for future studies to consider a wider panel of taxa instead of single model species.

413 From a conservation perspective, the comprehension of how pollinators cope with the
414 challenging conditions occurring in novel anthropogenic habitats, plays a key role in
415 informing suitable policy efforts to conserve their biodiversity and the ecosystem
416 service they provide. In the future, cities are predicted to expand constantly and thus
417 designing of urban landscapes will become a fundamental step for achieving
418 sustainability outcomes. The creation and the wise management of urban green spaces
419 will allow not only to create conditions that could support pollinators species
420 abundance and diversity but also sustain the biological interactions that are the basis
421 of a correct ecosystem functioning. At the same time, urban forestry and greenery
422 practices (e.g., plantation of street and residential trees and the creation of urban
423 greenbelts or greenways) could represent a valid solution to mitigate stressful
424 conditions related to the urban environment, such as the “heat island effect” (Chun &
425 Guldmann, 2018) and the high level of air pollution (Ottosen & Kumar, 2020) that
426 here were found to influence functional diversity issues.

427

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432

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438 **Conflicts of interest/Competing interests:** The authors declare that no competing
439 interests exist.

440 **Ethics approval:** Sampling permits were obtained when needed from local authorities

441 **Availability of data and material:** All relevant data are within the paper or stored in
442 a public repository (<https://doi.org/10.6084/m9.figshare.13637594>).

443 **Authors' contributions:** NT, EP and AG conceived the ideas and designed
444 methodology; NT, EP and PB collected the data; NT and EP analysed the data, PB
445 contributed to analyses checking/development and checked the final model outcomes;
446 EP, NT and PB led the writing of the manuscript. All authors contributed critically to
447 the drafts and gave final approval for publication.

448

449 **REFERENCES**

450

- 451 Adams, S. M., Giesy, J. P., Tremblay, L. A., & Eason, C. T. (2001). The use of biomarkers in
452 ecological risk assessment: Recommendations from the Christchurch conference on
453 Biomarkers in Ecotoxicology. *Biomarkers*, 6(1), 1–6.
454 <https://doi.org/10.1080/135475001452724>
- 455 Alberti, M., Correa, C., Marzluff, J. M., Hendry, A. P., Palkovacs, E. P., Gotanda, K. M.,
456 Hunt, V. M., Apgar, T. M., & Zhou, Y. (2017). Global urban signatures of phenotypic
457 change in animal and plant populations. *Proceedings of the National Academy of*
458 *Sciences*, 114(34), 8951–8956. <https://doi.org/10.1073/pnas.1606034114>
- 459 Alves-Silva, E., Santos, J. C., & Cornelissen, T. G. (2018). How many leaves are enough?
460 The influence of sample size on estimates of plant developmental instability and leaf
461 asymmetry. *Ecological Indicators*, 89, 912–924.
462 <https://doi.org/10.1016/j.ecolind.2017.12.060>
- 463 Aytekin, M. A., Terzo, M., Rasmont, P., & Çağatay, N. (2007). Landmark based geometric
464 morphometric analysis of wing shape in *Sibiricobombus* Vogt (Hymenoptera: Apidae:
465 *Bombus* Latreille). *Annales de la Société entomologique de France (N.S.)*, 43(1), 95–
466 102. <https://doi.org/10.1080/00379271.2007.10697499>
- 467 Beasley, D. A. E., Bonisoli-Alquati, A., & Mousseau, T. A. (2013). The use of fluctuating
468 asymmetry as a measure of environmentally induced developmental instability: A
469 meta-analysis. *Ecological Indicators*, 30, 218–226.
470 <https://doi.org/10.1016/j.ecolind.2013.02.024>
- 471
- 472 Chown, S. L., & Gaston, K. J. (2010). Body size variation in insects: a macroecological
473 perspective. *Biological Reviews*, 85(1), 139–169. [https://doi.org/10.1111/j.1469-](https://doi.org/10.1111/j.1469-185X.2009.00097.x)
474 [185X.2009.00097.x](https://doi.org/10.1111/j.1469-185X.2009.00097.x)

- 475 Buchholz, S., & Egerer, M. H. (2020). Functional ecology of wild bees in cities: Towards a
476 better understanding of trait-urbanization relationships. *Biodiversity and*
477 *Conservation*, 29(9), 2779–2801. <https://doi.org/10.1007/s10531-020-02003-8>
- 478 Chun, B., & Guldman, J.-M. (2018). Impact of greening on the urban heat island: Seasonal
479 variations and mitigation strategies. *Computers, Environment and Urban Systems*, 71,
480 165–176. <https://doi.org/10.1016/j.compenvurbsys.2018.05.006>
- 481 Colla, S. R., Gadallah, F., Richardson, L., Wagner, D., & Gall, L. (2012). Assessing declines
482 of North American bumble bees (*Bombus* spp.) using museum specimens.
483 *Biodiversity and Conservation*, 21(14), 3585–3595. [https://doi.org/10.1007/s10531-](https://doi.org/10.1007/s10531-012-0383-2)
484 [012-0383-2](https://doi.org/10.1007/s10531-012-0383-2)
- 485 Costa, M., Mateus, R. P., & Moura, M. O. (2015). Constant fluctuating asymmetry but not
486 directional asymmetry along the geographic distribution of *Drosophila antonietae*
487 (Diptera, Drosophilidae). *Revista Brasileira de Entomologia*, 59(4), 337–342.
488 <https://doi.org/10.1016/j.rbe.2015.09.004>
- 489 Couvillon, M. J., & Dornhaus, A. (2010). Small worker bumble bees (*Bombus impatiens*) are
490 hardier against starvation than their larger sisters. *Insectes Sociaux*, 57(2), 193–197.
491 <https://doi.org/10.1007/s00040-010-0064-7>
- 492 Couvillon, Margaret J., Jandt, J. M., Duong, N., & Dornhaus, A. (2010). Ontogeny of worker
493 body size distribution in bumble bee (*Bombus impatiens*) colonies. *Ecological*
494 *Entomology*, 35(4), 424–435. <https://doi.org/10.1111/j.1365-2311.2010.01198.x>
- 495 Couvillon, Margaret J. & Dornhaus, A. (2009) Location, location, location: larvae position
496 inside the nest is correlated with adult body size in worker bumble bees (*Bombus*
497 *impatiens*). *Proceedings of the Royal Society B*, 276, 2411–2418.

- 498 de Farias-Silva, F. J., & Freitas, B. M. (2020). Thermoregulation in the large carpenter bee
499 *Xylocopa frontalis* in the face of climate change in the Neotropics. *Apidologie*.
500 <https://doi.org/10.1007/s13592-020-00824-8>
- 501 Dellicour, S., Gerard, M., Prunier, J. G., Dewulf, A., Kuhlmann, M., & Michez, D. (2017).
502 Distribution and predictors of wing shape and size variability in three sister species of
503 solitary bees. *PLOS ONE*, *12*(3), e0173109.
504 <https://doi.org/10.1371/journal.pone.0173109>
- 505 Eggenberger, H., Frey, D., Pellissier, L., Ghazoul, J., Fontana, S., & Moretti, M. (2019).
506 Urban bumblebees are smaller and more phenotypically diverse than their rural
507 counterparts. *Journal of Animal Ecology*, *88*(10), 1522–1533.
508 <https://doi.org/10.1111/1365-2656.13051>
- 509 Fernández, M. J., Driver, M. E., & Hedrick, T. L. (2017). Asymmetry costs: Effects of wing
510 damage on hovering flight performance in the hawkmoth *Manduca sexta*. *The Journal*
511 *of Experimental Biology*, *220*(20), 3649–3656. <https://doi.org/10.1242/jeb.153494>
- 512 Fisher, K., Gonthier, D. J., Ennis, K. K., & Perfecto, I. (2017). Floral resource availability
513 from groundcover promotes bee abundance in coffee agroecosystems. *Ecological*
514 *Applications*, *27*(6), 1815-1826.
- 515 Földesi, R., Howlett, B. G., Grass, I., & Batáry, P. (2020). Larger pollinators deposit more
516 pollen on stigmas across multiple plant species—A meta-analysis. *Journal of*
517 *Applied Ecology*. <https://doi.org/10.1111/1365-2664.13798>
- 518 Gérard, M., Martinet, B., Maebe, K., Marshall, L., Smagghe, G., Vereecken, N. J., Vray, S.,
519 Rasmont, P., & Michez, D. (2020). Shift in size of bumblebee queens over the last
520 century. *Global Change Biology*, *26*(3), 1185–1195.
521 <https://doi.org/10.1111/gcb.14890>

- 522 Gérard, M., Vanderplanck, M., Franzen, M., Kuhlmann, M., Potts, S. G., Rasmont, P.,
523 Schweiger, O., & Michez, D. (2018a). Patterns of size variation in bees at a
524 continental scale: Does Bergmann's rule apply? *Oikos*, *127*(8), 1095–1103.
525 <https://doi.org/10.1111/oik.05260>
- 526 Gérard, M., Michez, D., Debat, V., Fullgrabe, L., Meeus, I., Piot, N., Sculfort, O., Vastrade,
527 M., Smaghe, G., & Vanderplanck, M. (2018b). Stressful conditions reveal decrease
528 in size, modification of shape but relatively stable asymmetry in bumblebee wings.
529 *Scientific Reports*, *8*(1), 15169. <https://doi.org/10.1038/s41598-018-33429-4>
- 530 Combined effects of global change pressures on animal-mediated pollination. *Trends in*
531 *Ecology & Evolution*, *28*(9), 524–530. <https://doi.org/10.1016/j.tree.2013.05.008>
- 532 Goulson, D. (2010). *Bumblebees: Behaviour, Ecology, and Conservation*. OUP Oxford.
- 533 Goulson, D., Peat, J., Stout, J. C., Tucker, J., Darvill, B., Derwent, L. C., & Hughes, W. O. H.
534 (2002). Can alloethism in workers of the bumblebee, *Bombus terrestris*, be explained
535 in terms of foraging efficiency? *Animal Chown, S. L., & Gaston, K. J. (2010). Body*
536 *size variation in insects: a macroecological perspective. Biological Reviews*, *85*(1),
537 *139-169. Behaviour*, *64*(1), 123–130. <https://doi.org/10.1006/anbe.2002.3041>
- 538 Greenleaf, S. S., Williams, N. M., Winfree, R., & Kremen, C. (2007). Bee foraging ranges
539 and their relationship to body size. *Oecologia*, *153*(3), 589–596.
540 <https://doi.org/10.1007/s00442-007-0752-9>
- 541 Grilli, M. G., Lambertucci, S. A., Therrien, J.-F., & Bildstein, K. L. (2017). Wing size but not
542 wing shape is related to migratory behavior in a soaring bird. *Journal of Avian*
543 *Biology*, *48*(5), 669–678. <https://doi.org/10.1111/jav.01220>
- 544 Harder, L. D. (1985). Morphology as a Predictor of Flower Choice by Bumble Bees. *Ecology*,
545 *66*(1), 198–210. <https://doi.org/10.2307/1941320>

- 546 Hoffmann, A. A., Collins, E., & Woods, R. (2002). Wing Shape and Wing Size Changes as
547 Indicators of Environmental Stress in *Helicoverpa punctigera* (Lepidoptera:
548 Noctuidae) Moths: Comparing Shifts in Means, Variances, and Asymmetries.
549 *Environmental Entomology*, *31*(6), 965–971. [https://doi.org/10.1603/0046-225X-](https://doi.org/10.1603/0046-225X-31.6.965)
550 [31.6.965](https://doi.org/10.1603/0046-225X-31.6.965)
- 551 Hoffmann, A. A., Woods, R. E., Collins, E., Wallin, K., White, A., & McKenzie, J. A.
552 (2005). Wing shape versus asymmetry as an indicator of changing environmental
553 conditions in insects. *Australian Journal of Entomology*, *44*(3), 233–243.
554 <https://doi.org/10.1111/j.1440-6055.2005.00469.x>
- 555 Huchler, K., Schulze, C. H., Gamauf, A., & Sumasgutner, P. (2020). Shifting Breeding
556 Phenology in Eurasian Kestrels *Falco tinnunculus*: Effects of Weather and
557 Urbanization. *Frontiers in Ecology and Evolution*, *8*.
558 <https://doi.org/10.3389/fevo.2020.00247>
- 559 Karra, S., Malki-Epshtein, L., & Neophytou, M. (2011). The Dispersion of Traffic Related
560 Pollutants Across a Non-Homogeneous Street Canyon. *Procedia Environmental*
561 *Sciences*, *4*, 25–34. <https://doi.org/10.1016/j.proenv.2011.03.004>
- 562 Kelemen, E. P., Cao, N., Cao, T., Davidowitz, G., & Dornhaus, A. (2019). Metabolic rate
563 predicts the lifespan of workers in the bumble bee *Bombus impatiens*. *Apidologie*,
564 *50*(2), 195–203. <https://doi.org/10.1007/s13592-018-0630-y>
- 565 Kerr, E. D., Phelan, C., & Woods, H. A. (2013). Subtle direct effects of rising atmospheric
566 CO₂ on insect eggs. *Physiological Entomology*, *38*(4), 302–305.
567 <https://doi.org/10.1111/phen.12034>
- 568 Klingenberg, C. P. (2011). MorphoJ: An integrated software package for geometric
569 morphometrics. *Molecular Ecology Resources*, *11*(2), 353–357.
570 <https://doi.org/10.1111/j.1755-0998.2010.02924.x>

- 571 Klingenberg, C. P. (2015). Analyzing Fluctuating Asymmetry with Geometric
572 Morphometrics: Concepts, Methods, and Applications. *Symmetry*, 7(2), 843–934.
573 <https://doi.org/10.3390/sym7020843>
- 574 Klingenberg, C. P., Badyaev, A. V., Sowry, S. M., & Beckwith, N. J. (2001). Inferring
575 Developmental Modularity from Morphological Integration: Analysis of Individual
576 Variation and Asymmetry in Bumblebee Wings. *The American Naturalist*, 157(1),
577 11–23. <https://doi.org/10.1086/317002>
- 578 Knight, M. E., Martin, A. P., Bishop, S., Osborne, J. L., Hale, R. J., Sanderson, R. A., &
579 Goulson, D. (2005). An interspecific comparison of foraging range and nest density of
580 four bumblebee (*Bombus*) species. *Molecular Ecology*, 14(6), 1811–1820.
581 <https://doi.org/10.1111/j.1365-294X.2005.02540.x>
- 582 Kölliker & Ott, U. M., Blows, M. W., & Hoffmann, A. A. (2003). Are wing size, wing shape
583 and asymmetry related to field fitness of *Trichogramma* egg parasitoids? *Oikos*,
584 100(3), 563–573. <https://doi.org/10.1034/j.1600-0706.2003.12063.x>
- 585 Leonard, R. J., Wat, K. K. Y., McArthur, C., & Hochuli, D. F. (2018). Urbanisation and wing
586 asymmetry in the western honey bee (*Apis mellifera*, Linnaeus 1758) at multiple
587 scales. *PeerJ*, 6, e5940. <https://doi.org/10.7717/peerj.5940>
- 588 Martinet, B., Dellicour, S., Ghisbain, G., Przybyla, K., Zambra, E., Lecocq, T., Boustani, M.,
589 Baghirov, R., Michez, D., & Rasmont, P. (2020). Global effects of extreme
590 temperatures on wild bumblebees. *Conservation Biology*.
591 <https://doi.org/10.1111/cobi.13685>
- 592 Merckx, T., Souffreau, C., Kaiser, A., Baardsen, L. F., Backeljau, T., Bonte, D., Brans, K. I.,
593 Cours, M., Dahirel, M., Debortoli, N., De Wolf, K., Engelen, J. M. T., Fontaneto, D.,
594 Gianuca, A. T., Govaert, L., Hendrickx, F., Higuti, J., Lens, L., Martens, K., ... Van

- 595 Dyck, H. (2018). Body-size shifts in aquatic and terrestrial urban communities.
596 *Nature*, 558(7708), 113–116. <https://doi.org/10.1038/s41586-018-0140-0>
- 597 Mpho, M., Callaghan, A., & Holloway, G. J. (2002). Temperature and genotypic effects on
598 life history and fluctuating asymmetry in a field strain of *Culex pipiens*. *Heredity*,
599 88(4), 307–312. <https://doi.org/10.1038/sj.hdy.6800045>
- 600 Nooten, S. S., & Rehan, S. M. (2020). Historical changes in bumble bee body size and range
601 shift of declining species. *Biodiversity and Conservation*, 29(2), 451–467.
602 <https://doi.org/10.1007/s10531-019-01893-7>
- 603 Ottosen, T.-B., & Kumar, P. (2020). The influence of the vegetation cycle on the mitigation
604 of air pollution by a deciduous roadside hedge. *Sustainable Cities and Society*, 53,
605 101919. <https://doi.org/10.1016/j.scs.2019.101919>
- 606 Outomuro, D., & Johansson, F. (2011). The effects of latitude, body size, and sexual selection
607 on wing shape in a damselfly. *Biological Journal of the Linnean Society*, 102(2), 263-
608 274. <https://doi.org/10.1111/j.1095-8312.2010.01591.x>
- 609 Palmer, A. R., & Strobeck, C. (2003). CH 17. Fluctuating asymmetry analyses
610 revisited. *Developmental Instability: Causes and Consequences*, Oxford University
611 Press, Oxford, 279-319.
- 612 Peters, M. K., Peisker, J., Steffan-Dewenter, I., & Hoiss, B. (2016). Morphological traits are
613 linked to the cold performance and distribution of bees along elevational gradients.
614 *Journal of Biogeography*, 43(10), 2040–2049. <https://doi.org/10.1111/jbi.12768>
- 615 Phillips, B. B., Gaston, K. J., Bullock, J. M., & Osborne, J. L. (2019). Road verges support
616 pollinators in agricultural landscapes, but are diminished by heavy traffic and summer
617 cutting. *Journal of Applied Ecology*, 56(10), 2316–2327.
618 <https://doi.org/10.1111/1365-2664.13470>

- 619 Polce, C., Maes, J., Rotllan-Puig, X., Michez, D., Castro, L., Cederberg, B., Dvorak, L.,
620 Fitzpatrick, Ú., Francis, F., Neumayer, J., Manino, A., Paukkunen, J., Pawlikowski,
621 T., Roberts, S., Straka, J., & Rasmont, P. (2018). Distribution of bumblebees across
622 Europe. *One Ecosystem*, 1. <http://dx.doi.org/10.3897/oneeco.3.e28143>
- 623 Radmacher, S., & Strohm, E. (2010b). Factors affecting offspring body size in the solitary
624 bee *Osmia bicornis* (Hymenoptera, Megachilidae). *Apidologie*, 41(2), 169–177.
625 <https://doi.org/10.1051/apido/2009064>
- 626 Rohlf, F. (2015). The tps series of software. *Hystrix, the Italian Journal of Mammalogy*,
627 26(1). <https://doi.org/10.4404/hystrix-26.1-11264>
- 628 Sascha, B., & Egerer, M. H. (2020). Functional ecology of wild bees in cities: Towards a
629 better understanding of trait-urbanization relationships. *Biodiversity & Conservation*,
630 29(9–10), 2779–2801. <http://dx.doi.org/10.1007/s10531-020-02003-8>
- 631 Schneider, S. S., Leamy, L. J., Lewis, L. A., & DeGrandi-Hoffman, G. (2003). The
632 Influence of Hybridization Between African and European Honeybees, *Apis*
633 *Mellifera*, on Asymmetries in Wing Size and Shape. *Evolution*, 57(10), 2350–2364.
634 <https://doi.org/10.1111/j.0014-3820.2003.tb00247.x>
- 635 Sibly, R. M., & Atkinson, D. (1994). How Rearing Temperature Affects Optimal Adult Size
636 in Ectotherms. *Functional Ecology*, 8(4), 486–493. <https://doi.org/10.2307/2390073>
- 637 Soule, A. J., Decker, L. E., & Hunter, M. D. (2020). Effects of diet and temperature on
638 monarch butterfly wing morphology and flight ability. *Journal of Insect*
639 *Conservation*, 24(6), 961–975. <https://doi.org/10.1007/s10841-020-00267-7>
- 640 Steffan-Dewenter, I., Münzenberg, U., & Tschardtke, T. (2001). Pollination, seed set and
641 seed predation on a landscape scale. *Proceedings of the Royal Society of London.*
642 *Series B: Biological Sciences*, 268(1477), 1685–1690.
643 <https://doi.org/10.1098/rspb.2001.1737>

- 644 Theodorou, P., Baltz, L. M., Paxton, R. J., & Soro, A. (2020). Urbanization is associated with
645 shifts in bumblebee body size, with cascading effects on pollination. *Evolutionary*
646 *Applications*, 14(1), 53–68. <https://doi.org/10.1111/eva.13087>
- 647 Wang, X., Blanchet, F. G., & Koper, N. (2014). Measuring habitat fragmentation: an
648 evaluation of landscape pattern metrics. *Methods in Ecology and Evolution*, 5(7),
649 634-646. <https://doi.org/10.1111/2041-210X.12198>
- 650 Zuur, A., Ieno, E. N., Walker, N., Saveliev, A. A., & Smith, G. M. (2009). *Mixed Effects*
651 *Models and Extensions in Ecology with R*. Springer Science & Business Media.

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658 FIGURE LEGENDS

659 Fig.1: Map showing the distribution of the sampling sites and the border of the different provinces included in
660 the study area.

661 Fig.2: Right forewing of *B. terrestris* with landmark locations

662 Fig. 3: Regression analysis of centroid size as a function of (a) temperature, and (b) floral resources . *B.*
663 *pascuorum* is represented by black dots and lines, *B. terrestris* is represented by grey triangles and lines.
664 Continuous lines indicate the significant relationships while dashed lines represent the non-significant ones.
665 Dots and triangles represent observed values. The two species were tested in separate models but represented
666 together to facilitate the comparison.

667 Fig. 4: Regression analysis of fluctuating size asymmetry as a function temperature . *B. pascuorum* is
668 represented by black dots and lines, *B. terrestris* is represented by grey triangles and lines. Continuous lines
669 indicate the significant relationships, while dashed lines represent the non-significant ones.

Fig.1

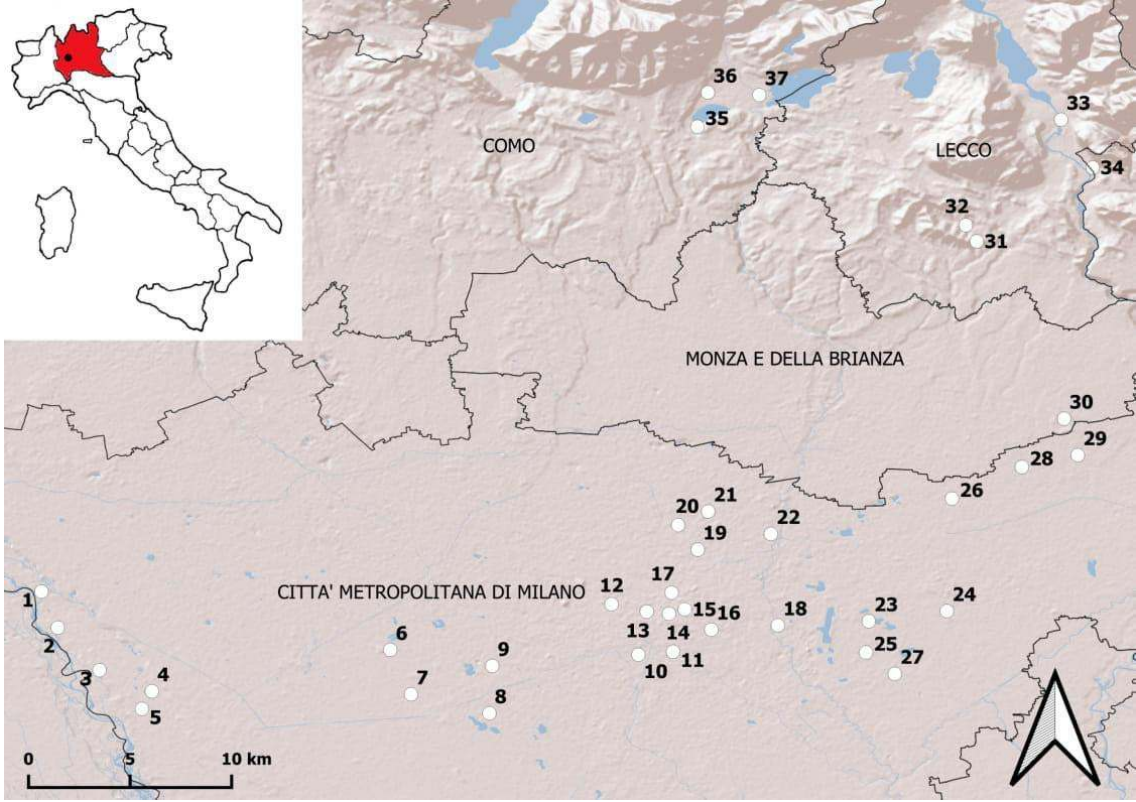


Fig.2

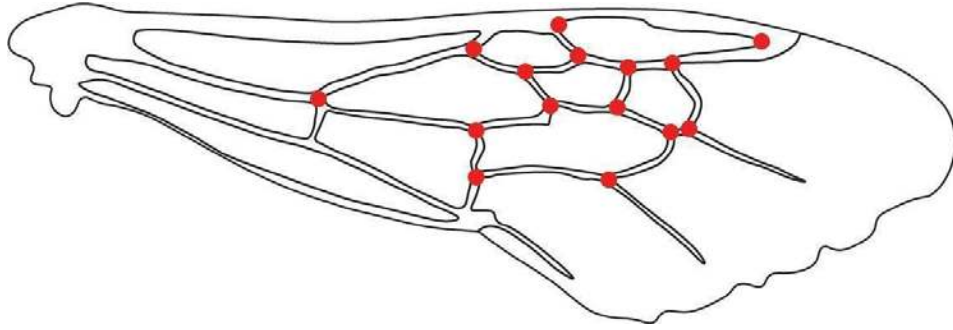


Fig.3

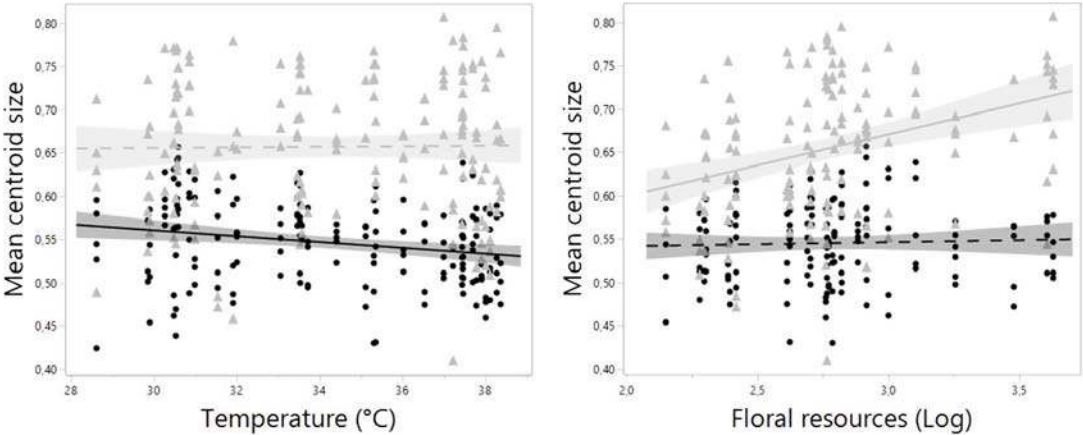


Fig.4

