

1 **Urbanisation impacts the diversity, coloration, and body size of**
2 **wild bees in a Mediterranean city**

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19 fieldwork.

20 **Abstract**

21 Urbanisation is a growing phenomenon causing the decline of wild bees globally. Yet, bees
22 manage to persist in the urban matrix thanks to islands of vegetation in public parks and
23 private gardens. While we begin to comprehend the impact of urbanisation on bees' diversity
24 and abundance, our understanding of its impact on the functional diversity of wild bees is
25 limited. Here, we use an integrative approach to investigate the response of wild bees to
26 urbanisation at the community, species, and individual levels. To do so, we sampled wild bees
27 in 24 public parks along an urbanisation gradient in the Mediterranean city of Marseille. We
28 found that species richness and abundance decreased in more urbanised areas, but increased in
29 larger city parks. Moreover, larger individuals within species, but not larger species, were
30 found in larger city parks, suggesting that park size is crucial for the persistence of bees in
31 cities. Interestingly, we show that brighter species were found in parks surrounded by a large
32 amount of impervious surface, highlighting the importance of colour traits in the response to
33 environmental changes. Finally, our results revealed that larger species, but not larger
34 individuals, were also more colourful. In summary, our study not only confirmed that
35 urbanisation negatively impacts community-level traits, but that it also affects species'
36 coloration and individuals' body size, thus improving our understanding of the functional
37 response of wild bees to urbanisation. We suggest that increasing park size may compensate
38 for the negative effects of urbanisation on wild bees.

39

40 **Key words**

41 Urban ecology; Conservation; Coloration; Mediterranean; City; Parks

42 **Introduction**

43 Bees constitute one of the major groups of pollinators of wild plants and crops worldwide
44 (Potts et al. 2016; Hung et al. 2018; Zattara and Aizen 2021). Over the past sixty years, bees
45 experienced a sharp decline globally (Zattara and Aizen 2021). Several anthropogenic factors
46 are responsible for this decline, including urbanisation and agricultural intensification causing
47 habitat and floral resource loss, the use of pesticides, parasites, the introduction of invasive
48 species and climate change (Potts et al. 2010; Goulson et al. 2015; Sánchez-Bayo and
49 Wyckhuys 2019). Among these causes, urbanisation is especially preoccupying because urban
50 areas are growing at an unprecedented rate (United Nations, 2018), transforming semi-natural
51 and agricultural habitats into impervious surfaces (McKinney 2002) detrimental to bees
52 (Cardoso and Gonçalves 2018; Baldock 2020). Yet, islands of vegetation subsist in urban
53 landscapes, such as private gardens, allotments, and public parks, allowing bees to persist in
54 these environments (Baldock et al. 2015, 2019; Geslin et al. 2015; Theodorou et al. 2020).
55 The urban matrix therefore acts as an environmental filter and its permeability, which can be
56 highly variable among cities, depends on the amount, extent, quality, and degree of isolation
57 of these islands of vegetation (Mcintyre and Hostetler 2001; Braaker et al. 2014; Fattorini
58 2016; Banaszak-Cibicka et al. 2018). When city parks are managed so as to offer favourable
59 conditions for bee assemblages, urban environments can harbour a bee species diversity and
60 abundance comparable to what is found in natural habitats, but not necessarily in terms of
61 functional diversity (Hall et al. 2017; Banaszak-Cibicka et al. 2018). An increasing amount of
62 work focuses on the functional aspects of urban impacts on bees, examining the
63 morphological and life-history traits allowing or preventing bees to cope with urban
64 environments (e.g. Geslin et al. 2013, 2016; Zaninotto et al. 2021) and references therein).

65 Several functional traits have been found to promote the presence of bees in large
66 cities. Indeed, social behaviour, broad dietary niche (i.e., polylectism), cavity-nesting habits,

67 and early spring phenology seem to be favoured in urban landscapes whereas solitary and
68 parasitic behaviours, narrow dietary niche (i.e., oligolectism), ground-nesting habits, and late
69 spring phenology appear to be unsuccessful traits in cities (Zanette et al. 2005; Hernandez et
70 al. 2009; Banaszak-Cibicka et al. 2018; Buchholz et al. 2020; Ayers and Rehan 2021;
71 Zaninotto et al. 2021). Regarding body size, however, evidence is more contrasted. On one
72 hand, some studies found that large-sized species decreased in abundance and diversity in
73 urban environments (Banaszak-Cibicka and Żmihorski 2012; Geslin et al. 2016; Banaszak-
74 Cibicka et al. 2018) possibly because large body size correlates with extinction risk in insects
75 (e.g., Nolte et al. 2019). On the other hand, other studies found that small species were less
76 common in urban centres because of their reduced dispersal abilities whereas large-sized
77 species were less affected by urbanisation as they have good flight abilities allowing them to
78 penetrate the urban matrix and hop from a suitable patch to another (Gathmann and
79 Tscharncke 2002; Ahrné et al. 2009). Reduced flight abilities generally make smaller bees less
80 mobile and more sensitive to habitat fragmentation in general than larger bee species (e.g.,
81 Steffan-Dewenter and Tscharncke 1999; Greenleaf et al. 2007; Warzecha et al. 2016; Gérard
82 et al. 2021). Interestingly, body size correlates with several phenotypic traits fulfilling
83 important ecological functions. For instance, small-sized bees usually have small mouthparts,
84 which associates with a narrower dietary niche because they cannot exploit some types of
85 flowers (e.g., tubular - Stang et al. 2006) whereas the reverse is true for larger species. It is
86 therefore pertinent to use body size when assessing the effect of urbanisation on the functional
87 diversity of bees (Theodorou et al. 2021).

88 Urbanisation may also affect other traits that play important functions in bees, such as
89 coloration. Bees indeed display a great variety of colours. Some species are entirely black or
90 darkly coloured, while others display bright colours including yellow, orange, red, green,
91 blue, violet, and white (Michez et al. 2019). These colour traits play various functions. Bright

92 colours often act as Mullerian and Batesian aposematic signals in bees (Badejo et al. 2020),
93 especially when black coloration associates with bright stripes (Mappes et al. 2005; Caro and
94 Ruxton 2019). Melanin pigments responsible for the dark coloration can also contribute to
95 defence functions by encapsulating pathogens (Siva-Jothy et al. 2005) and protecting against
96 UV radiations (Badejo et al. 2020). Body coloration can also serve as camouflage (Williams
97 2007) and play a role in thermoregulation processes, for example via the thermal melanism
98 hypothesis (Clusella Trullas et al. 2007) stating that darker colours should be favoured in
99 colder environments. Hence, given the functional importance of body coloration in bees,
100 urbanisation can affect bee coloration via its impact on the multiple processes involving
101 colour traits. For instance, urbanisation reduces predation pressures (Lagucki et al. 2017;
102 Eötvös et al. 2018, 2020), which in turn may affect aposematic signals (Valkonen et al. 2012).
103 Moreover, the urban heat-island effect in cities (Memon et al. 2008) impacts water balance
104 and thermoregulation processes of bees (Hamblin et al. 2017) such that bee species are close
105 to their critical thermal limit and/or their critical water content (Burdine and McCluney
106 2019a). Thus, we could hypothesise that darker species reach their critical thermal limit faster
107 than brighter ones, which would make them less successful in cities, especially in cities
108 located in warm regions. Finally, urban landscapes have a different background colour than
109 surrounding natural habitats due to buildings and impervious surfaces, and this may alter
110 camouflage and colour signal efficacy (Delhey and Peters 2017). Because the selective forces
111 affecting coloration detailed above have conflicting effects, it is challenging to predict how
112 urbanisation will affect bee coloration. Thus, exploring whether urban environments promote
113 or hinder colourful traits in bees will bring new insights into the ecological impacts of
114 urbanisation processes.

115 In this study, we aimed to assess the impact of urbanisation on wild bee assemblages
116 in the Mediterranean city of Marseille, France. While the Mediterranean region is a hotspot

117 for bee diversity (Nielsen et al. 2011; Ropars et al. 2020a), it also suffers from anthropogenic
118 pressures including increasing urbanisation (García-Nieto et al. 2018), enhancing the need to
119 improve our understanding of the response of bees to urbanisation. Our study focuses on wild
120 bees only and excludes the honey bee (*Apis mellifera*) because the latter is a non-native,
121 managed species with possible negative impacts on wild bee communities (Ropars et al. 2019,
122 2020b). We sampled urban parks along an urbanisation gradient in Marseille in order to
123 investigate the extent to which landscape variables related to urbanisation affect wild bees at
124 the community level (i.e. species diversity and abundance), at the species level (i.e. mean
125 specific body size and coloration), and at the individual level (i.e. within species variation in
126 body size and coloration). This study design allows us to assess the impact of urbanisation at
127 three biological scales so that we can improve our understanding of the response of wild bees,
128 and other species, to urbanisation. In addition, we also explored the relationship between
129 body size and coloration in wild bees, both at the inter- and intra-specific level, as it has never
130 been empirically studied in the past.

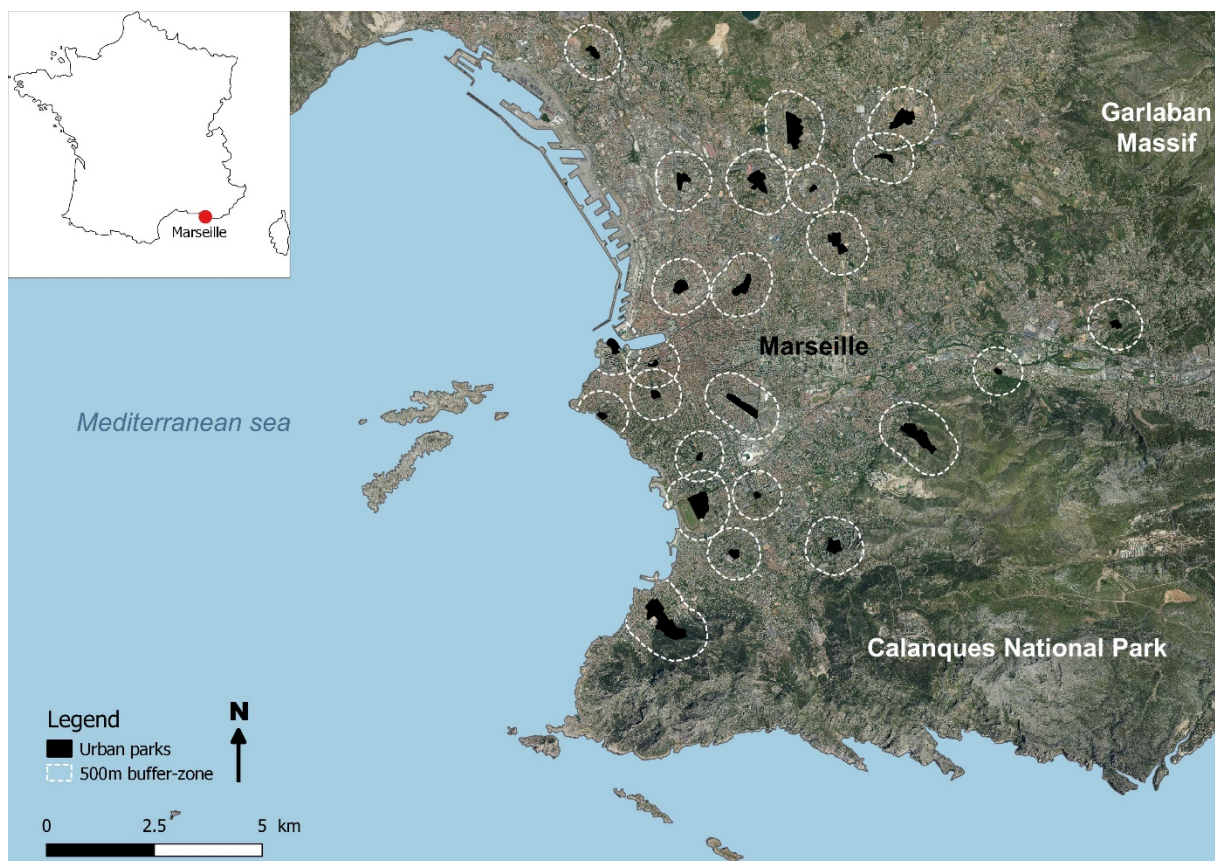
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132 **Material and Methods**

133 *Study sites*

134 The study was conducted in the Mediterranean city of Marseille (France) during the spring
135 and summer of 2016 (April to July) for fieldwork and during the spring of 2020 for laboratory
136 analyses. With 240 km² and 871,103 inhabitants (INSEE, 2020), Marseille is the second-
137 largest and one of the oldest cities of France. The region is characterised by a Mediterranean
138 climate with cool winters and hot summers accompanied by irregular precipitations in spring
139 and autumn and pronounced summer drought. In contrast to most European cities, Marseille
140 is not surrounded by agricultural crops but by calcareous massifs dominated by biodiversity-
141 rich areas such as shrublands. This configuration thus offers interesting gradients from natural

142 habitats to highly urbanised areas (Lizée et al. 2012; Lizée et al. 2016), which is ideal to study
143 how animals cope with urbanisation.
144 We selected 22 city parks and 2 university campus (similarly managed) covering an
145 urbanisation gradient within the city of Marseille, from the highly urbanised city centre to less
146 urbanised areas on the periphery (Figure 1). These parks vary in size (range 1-31 ha, mean = 9
147 ha), and offer various land-use contexts, with various amounts of surrounding vegetation and
148 impervious surface, and various degrees of isolation from natural areas, as the distance from a
149 park to the closest natural areas ranges from 0.5 km to 7.5 km. One urban park was excluded
150 from the following analyses because no native bees were found foraging in the park (only
151 *Apis mellifera*).



152

153 **Figure 1**

154 *Location of the 24 urban parks (in black) sampled within the city of Marseille with their 500m*
155 *buffer-zone (white dotted lines).*

156

157 *Landscape variables*

158 To characterize landscape variables, we used the land cover map from Lizée et al. (2012) built
159 with SPOT and IGN data (SPOT5 - 2004; BD1000-2006; BD Carto® IGN - 2004). We
160 combined these data using QGIS software on a 10 m-resolution raster map and contains 5
161 classes: impervious surface, rocky habitat, sparsely vegetated area, herbaceous stratum, tree
162 stratum.

163 First, we calculated the distance from each park to the closest natural area by drawing a
164 straight line between the park and the closest natural area, which in Marseille corresponds
165 mostly to the closest mountain range. Then, we created a polygon around each of the 24 parks
166 and calculated their area. We also drew a buffer-zone of 500 m around each of the 24 urban
167 parks in order to calculate the area of each land cover class, and made sure to exclude the area
168 within the parks. We chose a 500-m buffer because it encompasses the mean foraging range
169 of most wild bee species we observed as mentioned in Wright et al. (2015). We counted the
170 number of pixels of each class in the 500-m band around each urban park. Then, we combined
171 rocky habitats and impervious surfaces as one class and three vegetation classes (i.e., grasses,
172 scarce vegetations, and trees) all together to only have two classes: impervious surfaces and
173 vegetation surfaces.

174

175 *Bee sampling and pollination network description*

176 In each of the 24 parks, we surveyed 16 transects of 10 m during five minutes at a pace of one
177 meter every 30 seconds. To maximize the bee species richness, we placed eight transects along
178 a linear of shrub or bush, and eight transects within a lawn totalizing 384 transects. We
179 prospected each transect three times during the period April to July 2016 for a total of 1152
180 transect visits.

181 We captured with a net all wild bees observed foraging within 2 meters on both sides of each
182 transect. We identified each plant species on which bees were foraging. Bee specimens
183 collected were pinned and dried prior to identification by professional taxonomists (E. Dufrêne
184 for cuckoo bee species, D. Genoud for Andrenidae, Anthophorini, *Colletes* sp. and Halictidae
185 and M. Aubert for Megachilidae, Ceratini and *Hylaeus* sp.).

186 To evaluate the completeness of our samplings and estimate the potential maximum bee
187 species richness in the city of Marseille, we used the Chao and jackknife indexes including
188 captures and observations on plant species (Gotelli and Colwell 2011). We calculated these
189 indexes using the function *ChaoSpecies* within the *Spade-R* package in R version 3.6 software
190 (Chao et al. 2016).

191

192 *Body size and colour variables*

193 We took calibrated photographs of the dorsal part of each captured bee using a DSLR Nikon
194 D500 mounted with a Tokina 100-mm macro lens. For each photograph, we placed a
195 millimetric scale and a colour chart with a grey scale (i.e., SpyderCheckr, Datacolor Inc.).
196 Then, we imported the pictures in raw format in the software ImageJ (Schneider et al. 2012)
197 and used the ‘line’ tool to measure the intertegular span of each individual, which is a reliable
198 proxy of body size in bees (Cane 1987).

199 To objectively assess bee coloration, we used the Quantitative Colour Pattern Analysis
200 (QCPA) framework (van den Berg et al. 2020) implemented in the Multispectral Image
201 Analysis and Calibration (MICA) Toolbox (Troscianko and Stevens 2015), an ImageJ plugin.
202 First of all, we created a cone-catch model for our camera setup using a colour chart (X-Rite
203 colorCheckr passport) of known reflectance. This step allows us to convert the RGB values
204 recorded by our photography setup into the standardised colorimetric values of the CIELAB
205 colour space. We used the CIELAB, a colour space based on human vision, because we did

206 not have access to the UV range, and since bees are capable of UV vision, we could not use
207 the bee visual system (Menzel and Blakers 1976; Peitsch et al. 1992). CIELAB is a three-
208 dimensional colour space in which each colour is defined by three chromatic variables or
209 coordinates: L^* , a^* and b^* . Lightness (L^*) is the percentage of light reflected from a surface
210 and goes from black (0) to white (100). The coordinate a^* corresponds to a green-to-red
211 colour variation and coordinate b^* corresponds to a blue-to-yellow colour variation. Then, we
212 generated a multispectral image from RAW photographs using the MICA Toolbox and
213 adjusted the white balance with the 96% white standard from the colour chart. We then
214 selected two body regions of interest to be measured, namely the thorax and the abdomen, by
215 surrounding these body parts, excluding wings and artefacts such as the entomological pin.
216 After having converted our multispectral image into the CIELAB cone-catch model, we
217 obtained the mean $L^*a^*b^*$ values for the whole thorax and the whole abdomen of each
218 individual. Finally, we calculated the $L^*a^*b^*$ values of the entire body by taking the averaged
219 values between the thorax and the abdomen, therefore characterising the body coloration of
220 each individual.

221

222 *Statistical analyses*

223 To better understand the impact of urbanisation on wild bee communities, we explored the
224 relationships between urbanisation variables, community-level variables (i.e., species
225 diversity, abundance) and individual-level variables (i.e., body size, coloration) using a
226 piecewise structural equation modelling (SEM) in combination with (generalised) linear
227 (mixed-effects) models. To do so, we used R v.3.6.2 (R Core Team 2019) with the R
228 packages *piecewiseSEM* v2.1 package (Lefcheck 2016) and *nlme* (Pinheiro et al. 2019). SEM
229 is a suitable tool to evaluate direct and indirect effects in descriptive analyses of ecological
230 systems (Grace et al. 2010). In addition, piecewise SEM tests for missing paths between

231 variables using Shipley's test of d-separation (Shipley 2013), allowing us to adjust our initial
232 model to improve its fit and biological significance. Adequate model goodness-of-fit is first
233 indicated by a non-significant p-value based on the Chi-squared test (Shipley 2009). Then,
234 goodness-of-fit can be improved using a combination of indices, including Akaike's
235 Information Criterion corrected for small sample size (AICc) obtained from Fisher's C
236 statistic, and the Bayes-Schwarz Information Criterion (BIC), the latter being the most
237 reliable for model selection using piecewise SEM (Hertzog 2018).

238 We built two similar models that differ in the way individual-level variables are
239 accounted for. Indeed, when assessing the impact of urbanisation on body size and colour,
240 two variables that are measured on each individual, we are actually mixing two different
241 questions. The first one (i.e. Model A) tests the effect of urbanisation on the bee traits at the
242 species level (how do larger or smaller species respond to urbanisation?) whereas the second
243 one (i.e. Model B) deals with within-species trait variation (how do larger or smaller
244 individuals within each species respond to urbanisation?). In order to disentangle these two
245 questions, we transformed the individual-level variables so as to obtain two different sets to
246 include in two different versions of the same model. First, we took the average specific values
247 of body size and the three colour variables ($L^*a^*b^*$) and assigned it to each individual from a
248 given species. Thus, all individuals from the same species had the same value for these four
249 individual-levels variables, and we could account only for inter-specific differences in our
250 model. In the second version of these variables, we subtracted the mean specific value of each
251 individual variable such that the mean specific value of each species is equal to 0. This allows
252 us to control for inter-specific variation and to account only for within-species variation in
253 these variables.

254 Here, we built an initial model (model list detailed in Supp. Info. S1) with the distance
255 to the closest natural habitat and park area having direct effects on the four individual-level

256 variables (i.e., three colour variables and body size), on both community-level variables (i.e.,
257 species richness and abundance), and on the amount of impervious surface in a 500-m buffer.
258 In addition, we added a direct effect of the amount of impervious surface in a 500-m buffer on
259 all community- and individual-level variables. We also added body size as a direct predictor
260 of the three colour components. Moreover, we specified correlated errors between our three
261 colour variables, and between species richness and abundance. This step allows the residual
262 errors of two variables to be correlated for a reason not explained by our model when a direct
263 causal effect is not ecologically relevant, for example when two variables correlate with a
264 third unknown variable. We used a linear model (LM) for the amount of impervious surface
265 in a 500-m buffer, a generalised linear model for species richness and abundance since these
266 variables follow a Poisson distribution, and a linear mixed-effects model (LMM) for the four
267 individual-level variables with park ID as random intercept factor. We did not include the
268 amount of vegetation in a 500-m buffer in our model because this variable induced a high
269 level of collinearity in the model (VIF = 14.57). As explained above, this model was built in
270 two versions: Model A included the mean specific values of body size and the three colour
271 variables while Model B included the species-centred values of these same variables. This
272 initial model therefore allows us to test the direct and indirect effects of urbanisation features
273 on both community- and individual-level traits as well as the relationship between coloration
274 and body size in wild bees. We then discarded the non-significant terms until we obtained the
275 lowest values of BIC. We checked model performance using the R package *Performance*
276 (Lüdecke et al. 2020), and we calculated marginal (fixed effect) and conditional (fixed and
277 random effects) R^2 for each model (Nakagawa and Schielzeth 2013).

278

279 **Results**

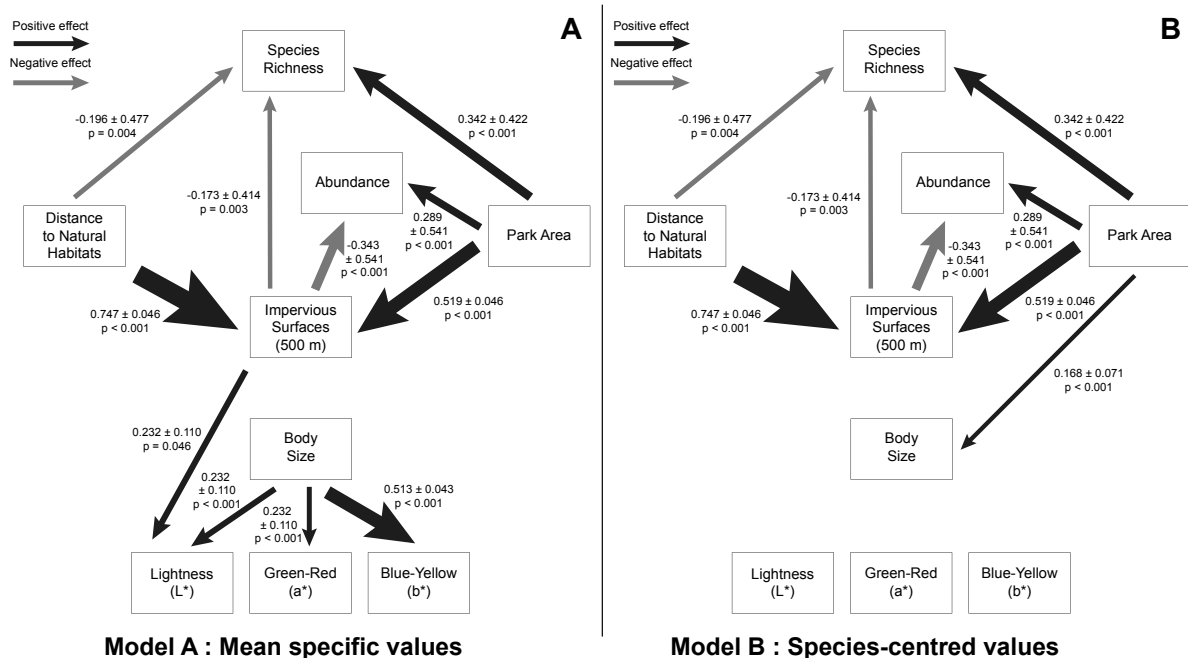
280 From April to July 2016, we sampled a total of 435 wild bees belonging to 121 species,
281 30 genera, and 5 families, and we recorded the presence of 994 honeybees (*Apis mellifera*). We
282 were able to successfully capture only 373 of the 435 wild bees observed. According to the
283 Chao1 method to estimate the total species richness, observed wild bee species richness
284 represented 52.8% of the potential maximum richness. Using Jackknife 1 and 2 indexes, the
285 observed richness represented from 55.8% (Jackknife 2) to 68.7% (Jackknife 1) of the potential
286 maximum richness. Finally, 55 species (45.4%) were represented by only one individual
287 (singleton). We provide a more detailed description of bees' ecological traits in Supp. Info. S2,
288 and the structure of their interaction network with flowering plants in Supp. Info. S3.

289 Our two final models resulting from the piecewise SEM approach are presented in Figure 2
290 and statistics are fully summarised in Supp. Info S4. Model A, including mean specific values
291 of individual-level variables, has a Fisher's C statistics of 26.81 with a p-value of 0.867 and
292 36 degrees of freedom. Similarly, Model B, including species-centred values of individual-
293 level variables, has a Fisher's C statistics of 22.75 with a p-value of 0.958 and 36 degrees of
294 freedom, implying that both our models provide a good fit to our data.

295 We found in both our models that species richness increased in larger parks ($\beta = 0.342 \pm$
296 0.477 , $p = 0.004$), but decreased in parks located further away from natural habitat ($\beta = -0.196$
297 ± 0.477 , $p < 0.001$), and in parks surrounded by more impervious surfaces ($\beta = -0.173 \pm$
298 0.414 , $p < 0.001$). Abundance also increased in larger parks ($\beta = 0.289 \pm 0.541$, $p < 0.001$)
299 and decreased when the amount of impervious surface surrounding the park increased ($\beta = -$
300 0.343 ± 0.541 , $p < 0.001$).

301 In addition, Model A indicated no significant relationship between average specific body size
302 and the distance to the closest natural habitat ($p = 0.075$). We found that average specific
303 lightness (L^*) increased with the amount of impervious surface ($\beta = 0.232 \pm 0.110$, $p =$
304 0.046). Moreover, larger bee species (average specific body size) were brighter (L^* ; $\beta = 0.228$

305 ± 0.048 $p < 0.001$), redder (a^* ; $\beta = 0.191 \pm 0.051$ $p = 0.001$) and yellower (b^* ; $\beta = 0.513 \pm$
 306 0.043 $p < 0.001$) than smaller bee species.
 307 In contrast, Model B showed that individuals were larger (species-centred value of body size)
 308 in larger parks ($\beta = 0.168 \pm 0.071$ $p = 0.028$). However, we found that none of the three
 309 colour variables (species-centred values) were correlated to species-centred values of body
 310 size.
 311 Finally, we found positively correlated errors between all three colour components for mean
 312 specific values ($L^* \sim a^*$: $\beta = 0.313$, $p < 0.001$; $L^* \sim b^*$: $\beta = 0.774$, $p < 0.001$; $a^* \sim b^*$: $\beta =$
 313 0.708 , $p < 0.001$), but only between L^* and b^* ($\beta = 0.422$, $p < 0.001$), and a^* and b^* ($\beta =$
 314 0.341 , $p < 0.001$) for species-centred values as L^* and a^* were negatively correlated ($\beta = -$
 315 0.133 , $p = 0.005$). We also found correlated errors between species richness and abundance (β
 316 $= 0.937$, $p < 0.001$).
 317



318

319 **Figure 2**

320 *Best selected path diagrams representing the direct effects of urbanisation-related variables*

321 *on the species richness, the abundance, the body size and the coloration of wild bees in the*
322 *city of Marseille. Each arrow represents a statistically significant effect, which can be either*
323 *negative (grey arrows) or positive (black arrows), and arrows thickness is proportional to*
324 *their effect size. We provide effect size \pm standard error along with the p-value. Model A (A)*
325 *represents the model in which body size and the three colour variables were included as mean*
326 *specific values. Model B (B) represents the same model but the values of body size and the*
327 *three colour variables correspond to species-centred values (between-species variation has*
328 *been eliminated by subtracting the mean specific values each time).*

329

330 **Discussion**

331 We investigated the extent to which urbanisation impacts wild bees in the Mediterranean city
332 of Marseille, and found that wild bees responded to urbanisation variables at the community,
333 species, or individual level. As we detailed below, our study across biological scales provides
334 invaluable insights into the multifaceted impacts that urbanisation has on wildlife. This
335 integrative approach allows us to capture subtle effect variations, mainly between the inter-
336 and intra-specific level, that would be otherwise undetectable when comparing separate
337 studies, because of confounding factors specific to each study. We therefore encourage the
338 use of holistic approaches across biological scales to precisely assess the impact of
339 environmental change on animals.

340 The negative impact of urbanisation on bee diversity has been documented in the past
341 (Schochet et al. 2016; Cardoso and Gonçalves 2018). Yet, some studies found no or little
342 reduction in terms of species richness and abundance in cities (Buchholz et al. 2020;
343 Theodorou et al. 2020). Several factors may explain these differences. For example, habitat
344 connectivity can be highly variable among cities (Beninde et al. 2015) and may explain why
345 some cities are more or less permeable to wildlife and bees in particular (Steffan-Dewenter

346 and Tschardtke 1999; Buchholz et al. 2020). Furthermore, the nature and quality of the less
347 urbanised end of the gradient can also vary. Many cities are surrounded by a more or less
348 extended suburb and further by agricultural fields, which can have a negative impact on bee
349 diversity depending on how crops are managed (e.g., Le Féon et al. 2010). The city of
350 Marseille is ideal to study urbanisation gradient because it is directly surrounded by natural
351 massifs and diversified scrubland, one of which being highly protected by the Calanques
352 National Park, a protected area with little anthropogenic impact for bees (Ropars et al. 2020a).
353 Therefore, we could estimate directly the extent to which wild bee assemblages penetrate into
354 and respond to the urban matrix. More specifically, we found that the amount of impervious
355 surface in a 500-m buffer around a park had a negative impact on both species' richness and
356 abundance. As impervious surfaces reduce the availability of resources and nesting sites for
357 bees, measuring the amount of impervious surface around a park reflects its degree of
358 isolation and its lack of connectivity with vegetation patches. This result corroborates
359 previous findings showing that the amount of impervious surface in a 500-m buffer correlated
360 with reduced species richness and abundance of bees (Geslin et al. 2016; Burdine and
361 McCluney 2019b). Furthermore, our results revealed that higher species richness and
362 abundance were found in larger city parks. This is consistent with previous studies identifying
363 large patches of habitat as the most important factor to maintain high levels of biodiversity
364 within cities (Beninde et al. 2015; Baldock et al. 2015; Quistberg et al. 2016). Our study thus
365 highlights the need to create larger city parks and denser corridor networks between these
366 parks so as to make the city of Marseille more permeable to wild bees.

367 Urbanisation variables did not affect the body size of bees in our study, except park
368 size, as larger parks harboured larger individuals within species but not larger species. This
369 effect, albeit weak based on the marginal R^2 of the model, may reflect a higher resource
370 availability both in terms of quality and quantity in larger parks (strong, positive correlation

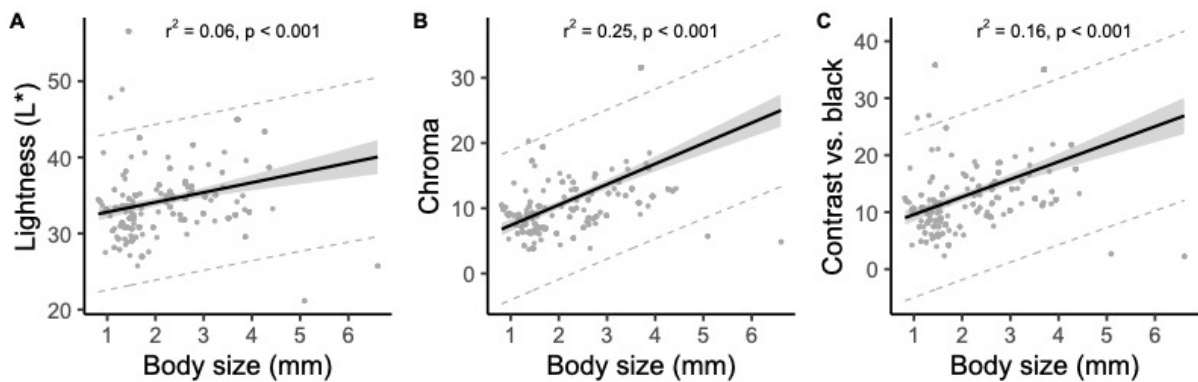
371 between park size and the amount of vegetation within parks in our data, $r^2 = 0.78$), thus
372 allowing individuals to grow larger than in smaller parks, where resources may be scarcer.
373 This further strengthens the idea that larger parks are beneficial to bees, not only in terms of
374 species richness and abundance, but also in terms of individual quality (Quistberg et al. 2016).
375 With this finding, we also emphasise the need to use individual-level variables such as body
376 size (Buchholz and Egerer 2020) to precisely assess the health of a given community of
377 species because one can disentangle the observed effects occurring at the species level from
378 those occurring at the individual level within species. Assessing the amount and quality of
379 resources within parks could also improve how parks should be managed to reduce the impact
380 of urbanisation.

381 In our study, we characterised the coloration of each individual we captured to assess
382 how colour traits respond to urbanisation on one hand, and to explore the relationships
383 between coloration and body size in bees on the other. The effect of urbanisation on animal
384 coloration has been relatively overlooked, and although most studies focussed on birds,
385 current evidence suggest that diurnal animals in urban areas are darker due to thermal
386 melanism, protection against pollution, or camouflage, and display duller colour signals than
387 their rural counterparts (e.g., Chatelain et al. 2014; Biard et al. 2017; Leveau 2021). Our
388 results indicate that brighter (i.e., high lightness values) species are more successful than
389 darker ones in parks surrounded by a greater amount of impervious surface. In other words,
390 darker species are under-represented in highly urbanised areas. This is consistent with the
391 thermal melanism hypothesis (Clusella Trullas et al. 2007) stating that darker ectotherms
392 should be favoured in colder habitats because they heat their body up faster than bright
393 individuals, since dark colours are more efficient at absorbing external heat. Urban
394 environments, especially in a hot Mediterranean city such as Marseille, are particularly warm
395 and bees are forced to live near their critical thermal maximum (Burdine and McCluney

396 2019a). Therefore, a possible interpretation of our results is that in the most urbanised areas,
397 which are presumably warmer, thermoregulation is more challenging for dark species than for
398 bright ones because they reach their critical limit too fast (Pereboom and Biesmeijer 2003). In
399 addition, even though we cannot completely rule them out, alternative hypotheses relative to
400 camouflage or aposematism are unlikely. First, urban-induced colour change related to
401 camouflage usually has an opposite effect, driving urban animals towards darker coloration
402 (Bishop and Cook 1980; Leveau 2019). Second, in the context of aposematism, having more
403 brightly coloured species in more urbanised areas would mean that darker species are more
404 predated in these parks. This hypothesis either implies that predation pressures in urbanised
405 city parks is higher for darker species or lower for brighter ones compared with less urbanised
406 city parks. Although this is plausible, this explanation is far from parsimonious and would
407 involve too many layers of presumptions. In any case, we advocate future studies to further
408 investigate the relationship between urbanisation and coloration in bees taking into account all
409 ecological determinants of body coloration.

410 Interestingly, we found positive correlations between species body size and species
411 coloration. More specifically, larger species are brighter, redder, and yellower while smaller
412 species are darker, greener, and bluer. In simple terms, large species often have conspicuous
413 colours while smaller species are much darker (Figure 3). Surprisingly perhaps, bee coloration
414 has received relatively little attention compared with other traits but their bright coloration
415 seems to have an aposematic function (Badejo et al. 2020). If so, our results suggest that
416 aposematic colours are much more present in large than in small species. Two non-exclusive
417 hypotheses could explain why larger species are more brightly coloured than smaller ones.
418 First, aposematism signals are more efficient in large preys because predators can detect them
419 and identify them more easily than small preys (Gamberale and Tullberg 1996 but see
420 Rimmel and Tammaru 2009). Second, larger species of bees may suffer from a higher

421 predation pressure from birds than smaller species which are only a few millimetres in body
422 length since birds prefer larger insect preys (Rommel and Tammaru 2009). Thus, the cost-
423 benefit balance of producing conspicuous colours may be more advantageous for larger
424 species than for smaller ones. Caution should be given with these possible interpretations
425 since dark coloration can also have an aposematic function, especially when iridescent
426 colours are involved, as demonstrated in carpenter bees from the genus *Xylocopa* (Blaimer et
427 al. 2018).
428



429

430 **Figure 3**

431 *Linear regressions between colour components, i.e. lightness (A), chroma (B), and contrast*
432 *against a black colour (C), and body size at the species level (mean specific values). Chroma*
433 *was calculated as $C = (a^{*2} + b^{*2})^{1/2}$ and corresponds to colour saturation. Contrasts vs.*
434 *black corresponds to the distance between each colour point and a black point within the*
435 *CIELAB colour space, with higher values corresponding to more colourful species. Contrast*
436 *vs. black was calculated as $\Delta_{black} = ((L^* - L^*_{black})^2 + (a^* - a^*_{black})^2 + (b^* - b^*_{black})^2)^{1/2}$.*
437 *Shaded area represents the 95% confidence interval and dashed lines the 95% prediction*
438 *interval. We also provide r^2 and p -value associated with each linear regression.*

439

440 To conclude, our study shows that urbanisation has a negative impact on wild bees
441 across biological scales, with distinct responses at the community, species, and individual

442 levels. Species richness and abundance of wild bees decrease along an urbanization in the
443 Mediterranean city of Marseille, mainly because of the amount of impervious surface around
444 the city parks. We also identified the size of city parks as a key factor positively affecting the
445 wild bee community, in terms of species richness and abundance on one hand, and in the
446 body size of individuals within species on the other. This strongly advocates for the inclusion
447 of larger parks in city centres to maintain acceptable levels of biodiversity. Brighter species
448 are also more successful in urbanised areas, perhaps due to the thermal advantage that their
449 bright colours confer them, suggesting that coloration is an important trait to consider when
450 assessing the impact of environmental change of functional diversity. Finally, we uncovered a
451 positive correlation between species size and colour in wild bees and urge future studies to
452 explore the details and ecological function of these relationships.

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460

461 *Authors' contributions*

462 A.B., L.R., L.S., F.F. and B.G. conceived the study. L.S., M.D.C., C.R., M.Z. and B.G.

463 participated in fieldwork or data collection. A.B. and L.R. performed the statistical analyses

464 and L.R. extracted landscape variables. A.B. wrote the manuscript with L.R., F.F. and B.G.

465 and all authors reviewed it and provided feedback.

466

467 *Consent for publication*

468 All the authors consent for the publication of this manuscript

469

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