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

3  
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7  
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13

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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 Tscharrntke 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 Tscharrntke 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.

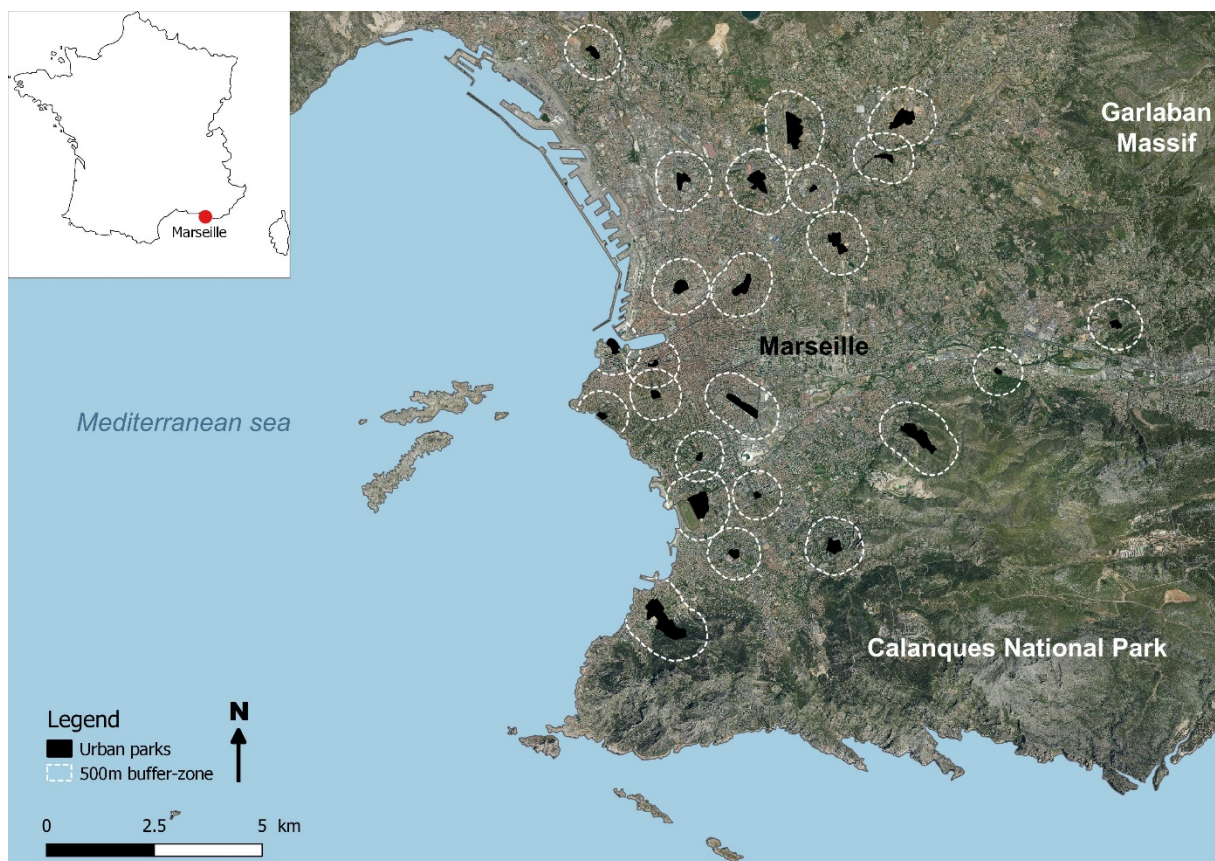
131

## 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 km<sup>2</sup> 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**

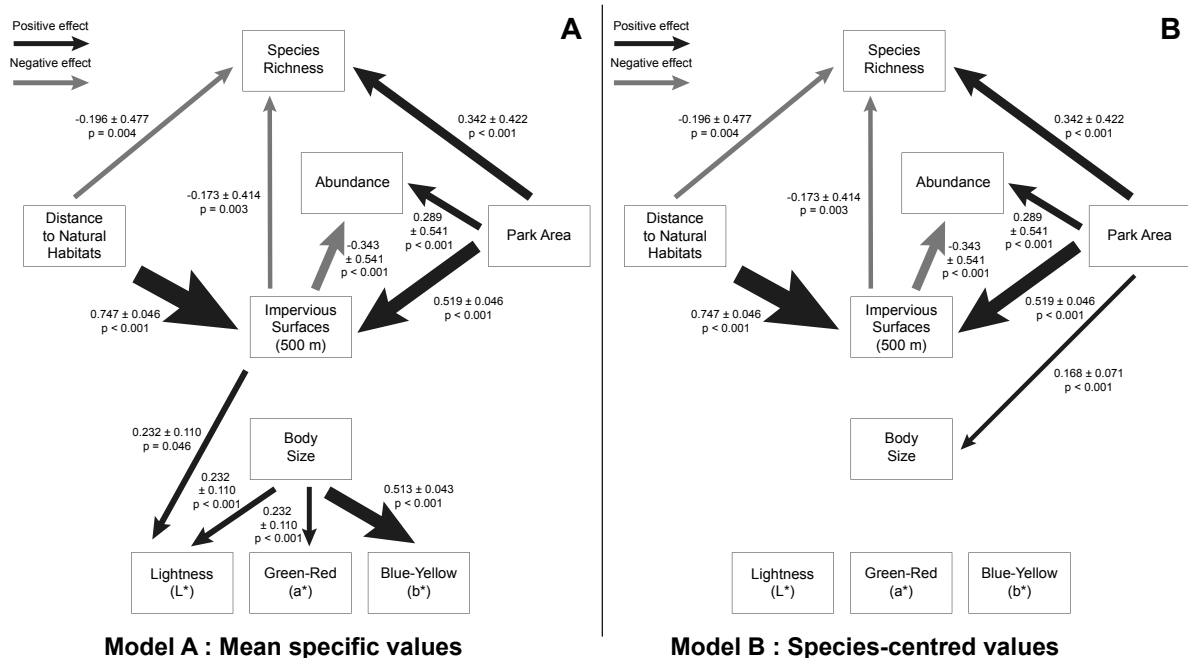
280 From April to July 2016, we sampled a total of 435 wild bees belonging to 121 species,  
281 30 genera, and 5 families, and we recorded the presence of 994 honeybees (*Apis mellifera*). We  
282 were able to successfully capture only 373 of the 435 wild bees observed. According to the  
283 Chao1 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  $\pm 0.477$ ,  $p < 0.001$ ), 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  $0.343 \pm 0.541$ ,  $p < 0.001$ ).

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  $\pm 0.048$   $p < 0.001$ ), 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 ( $\beta$   
 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

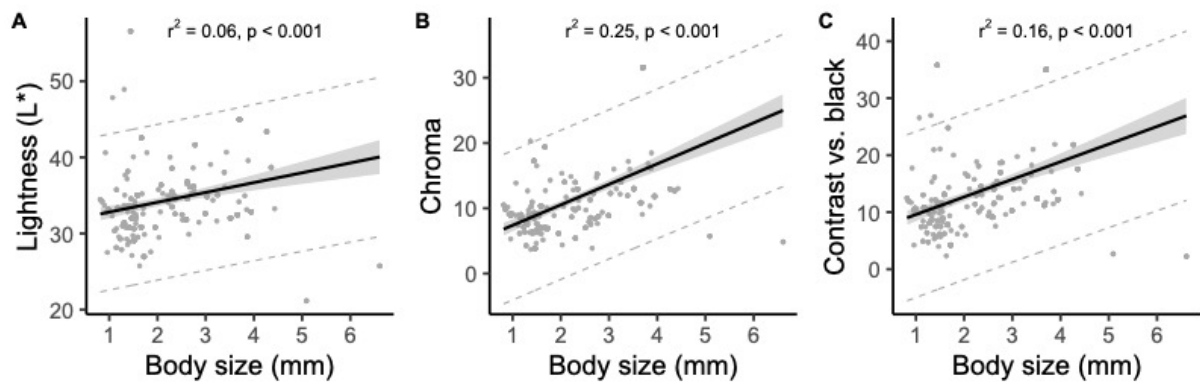
371 between park size and the amount of vegetation within parks in our data,  $r^2 = 0.78$ ), thus  
372 allowing individuals to grow larger than in smaller parks, where resources may be scarcer.  
373 This further strengthens the idea that larger parks are beneficial to bees, not only in terms of  
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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457 *Conflict of interests*

458 The authors have no conflict of interest to declare that are relevant to the content of this

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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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