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1 Urban colonies are more resistant to a trace metal than their forest counterparts in the ant

2 *Temnothorax nylanderi*

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8  
9 **Keywords:** Urbanization, common garden, cadmium resistance, social insects

10 **Abstract:** Cities can be used as open-sky replicates to assess the responses of organisms to  
11 environmental changes, such as increased heat or pollution. In contrast with the numerous community-  
12 level studies, population-level studies are rare, hugely biased towards birds, and rarely based on multiple  
13 city replicates. They are also mostly based on solitary species, despite some social insects being  
14 ecologically dominant and economically important. Our study aimed at assessing whether colonies of  
15 the ant *Temnothorax nylanderi* from forest and urban populations exhibit distinct responses to pollution.  
16 We collected colonies from four pairs of forest and urban habitats and reared them in the laboratory  
17 under normal or cadmium-supplemented diets. We then measured the emergence rate and size of newly-  
18 produced workers as well as the mortality rate of adult workers. We also assessed the effect of some  
19 social factors such as colony size or size of field workers. In all four forest/city replicates, urban colonies  
20 were less negatively affected by cadmium than forest colonies. We did not find any effect of social  
21 factors on cadmium resistance, in contrast with what was found in other eusocial insects. We discuss  
22 the potential adaptive nature of this differential response.

## 23 **Introduction**

24 United Nations predict that by 2030, more than 60% of the worldwide human population will live in  
25 cities, resulting in a threefold increase of total urban areas (1). Urbanization is associated with many

26 environmental changes, both abiotic and biotic (e.g. modified land use, habitat fragmentation, decreased  
27 soil permeability, increased temperatures known as Urban Heat Island effect, noise, light, and chemical  
28 pollutions including trace metals, exotic species (2–4)). Because these changes are quite fast and recent  
29 on the evolutionary timescale, cities can be seen as open sky laboratory replicates for understanding the  
30 responses of species facing rapid environmental changes (5,6). Most studies investigating the impacts  
31 of urbanization on wildlife have been conducted at the community level, with a taxonomic skew toward  
32 birds, although fishes, lizards, amphibians, arthropods (7–14) and plants (15,16) have been occasionally  
33 studied. Urbanization alters the composition of communities (higher or lower species abundance),  
34 functional traits (e.g. reduced body size), and interaction networks (10–14,16–19). In contrast, species-  
35 level studies are less common (see (6) for a good summary) Relative to non-urban populations, animals  
36 from urban populations express altered behavior (e.g. songs with distinct pitches, loss of migratory  
37 behavior (17,18)), changes in physiology (e.g. lower glucocorticoid secretion in response to stress,  
38 higher oxidative stress (19)), phenology (earlier reproduction (20,21)), circadian rhythm (faster  
39 circadian oscillations (22)), health (higher parasitism rate (23)) and/or morphology (7,24). These shifts  
40 in life-history traits are often seen as a response, adaptive or not, to urban stressors. However, because  
41 cities are extremely diverse and because most studies are based on a single city and not multiple city  
42 replicates (but see (21,25)), it is difficult to make general conclusions and identify a robust syndrome of  
43 urbanization for population-level traits. In addition, population studies are generally correlative  
44 (17,24,26–34), and do not quantify the respective contributions of genetics and phenotypic plasticity.  
45 So far, common garden experiments between urban and non-urban populations highlighted a genetic  
46 basis for the lower dispersal ability of urban plants (15), earlier reproduction of urban birds and ants  
47 (20,21) and for specific behaviors of urban birds (lower migratory behavior, increased boldness and  
48 stress). Additional common garden experiments are thus needed to complement correlative studies and  
49 assess causality and determination of various other life-history traits associated with urban life.

50 Pollution is one of the main chronic urban stressors. Urban habitats are usually more polluted because  
51 of traffic and industrial activities (35). For example, traffic-generated air pollution is responsible of  
52 increased oxidative stress leading to genetic damages, respiratory diseases and brain inflammation

53 (29,36,37) as well as higher susceptibility to parasites (23) in animals. Cities are also contaminated by  
54 trace metals. For example, cadmium has a concentration of  $0.30 \text{ mg.kg}^{-1}$  in soil of rural woods and  $2.45$   
55  $\text{mg.kg}^{-1}$  in urban woods (35). Cadmium alters plant physiology by interfering with calcium uptake (38).  
56 It also impacts various life history traits, causing longer development in fishes and arthropods, decreased  
57 hatching rate in fishes, sea stars and arthropods (39–41), decreased body size in fishes (40) and  
58 immunosuppression in rats (42).

59 Ants represent a large portion of the total earth animal biomass (43). They are found in a large diversity  
60 of ecosystems including urban areas and can deeply affect the functioning of ecosystems (44–46). This  
61 ecological success partly originates from their social lifestyle. Ants live in colonies made of few tens to  
62 millions of individuals where labor is divided among individuals according to their caste. Eusociality  
63 could enhance robustness against environmental changes. For example, eusocial species of bees are less  
64 negatively impacted by neocotinoids than solitary species (47), and models suggest that, within species,  
65 larger colonies resist better to pesticides (48). In ants, social life enhances resistance to parasites and  
66 starvation (49,50). In spite of their ecological interest and their social life, few studies investigating the  
67 effect of urbanization have been conducted on ants (5,51–53) and even fewer at the species level (54).  
68 How sociality could help ants resist anthropic stressors is an open question.

69 The aim of this study was to test whether there is a general effect of urbanization on resistance to trace  
70 metals (cadmium) in the small acorn ant *Temnothorax nylanderi*. Ants are a good model to study the  
71 effects of trace metals because they live in long-lived colonies and they forage locally around their nest,  
72 exposing themselves to bioaccumulation (55). We tested whether colonies of *T. nylanderi* from city and  
73 forest populations exhibit a differential response to pollution by feeding them with food enriched with  
74 cadmium or not, in a common garden laboratory setup, and by measuring several traits of the newly  
75 produced workers. We expected urban colonies to resist cadmium better because of their long history  
76 (several generations) of exposure to trace metal pollution relative to non-urban colonies. Moreover, this  
77 species offers the opportunity to assess the effects of social life on cadmium resistance, as colony size  
78 varies from less than ten to a few hundred individuals. The common-garden setup allowed to test for a

79 causal link between the stressor and the observed shift in traits (mortality of adult workers, and  
80 emergence rate and size of newly produced workers).

## 81 **Material and methods**

### 82 *Colony collection and rearing*

83 *Temnothorax nylanderi* is a small ant with small colonies (up to few hundred workers) that nests in  
84 acorns and twigs on the ground. Colonies are easy to collect and rear in the laboratory, and they produce  
85 new workers in two months at 22-26°C cycle (56). Two hundred and thirty-eight colonies of *T. nylanderi*  
86 were collected from eight sites at three locations in France (Paris, Bordeaux, Lyon). Each location had  
87 one pair of collection sites: a central urban park (city habitat) and a forest located between 23 and 52  
88 km from the city (forest habitat). Two pairs were collected in Paris location from two different parks  
89 and forest sites and in two different years, 2017 and 2018 (see table 1). They are subsequently called  
90 “Paris’17” for colonies collected in March 2017 and “Paris’18” for colonies collected in March 2018.  
91 Colonies from Lyon and Bordeaux were collected in February and March 2018. After collection,  
92 colonies were brought back to the laboratory and workers were counted. We discarded colonies with no  
93 queen or more than one queen (72 colonies) as well as colonies parasitized by cestodes (9 colonies,  
94 identified by the pale orange colour of workers). A total of 157 queenright colonies were used for the  
95 experiment and reared in foraging plastic boxes (11.5 x 11.5 x 5.5 cm) containing an artificial nest made  
96 of two microscope slides separated by a thin layer of plastic foam with hollow chambers. The top slide  
97 was covered with dark plastic to protect the colony from the light. Plastic boxes were kept in  
98 temperature-controlled chambers (reference CTS TP10/600) at 22°C from 7pm to 7am and 27°C from  
99 7am to 7pm. These stressful temperatures were chosen to speed up development and to amplify the  
100 effects of cadmium (57).

### 101 *Experimental design*

102 Our feeding treatment included two conditions: cadmium and control, applied to all locations and all  
103 pairs of habitats. In order to avoid any bias in colony size between control and cadmium conditions, we  
104 evenly assigned colonies to cadmium and control based on their colony size for each habitat within each

105 location. Colonies were randomly distributed between the two chambers independently of their location,  
106 habitat and treatment. All original brood were removed from the colonies except for about 30 second  
107 instar larvae. When colonies had less than 30 second instar larvae, we left all of them in the colony.  
108 Newly laid eggs were removed every week to avoid the hatching of extra larvae. We fed colonies for 61  
109 days every other day with a mixture of diluted honey and dried crickets containing 100µg/g cadmium  
110 (cadmium condition) or not (control condition). This cadmium concentration corresponds to the LC<sub>50</sub>  
111 (concentration at which half of the treated individuals die) of *Spodoptera littoralis* (M.A. Pottier  
112 personal communication). The mortality rate of the adult workers present in the colony from the  
113 beginning of the experiment (i.e. workers born in the field, subsequently called field workers) was  
114 computed based on the frequent removal of corpses and defined as the total number of dead field workers  
115 at the end of the experiment divided by the total number of field workers at the start of the experiment.

116 The experiment was performed over two different years: 2017 for Paris'17, and 2018 for  
117 Bordeaux, Lyon and Paris'18. For both years, colonies were acclimated for 14 days in laboratory before  
118 the experiment started. The experiment lasted for 61 days, so any larva that would have escaped the egg  
119 removal protocol and hatched could not have reached adulthood before the end of the experiment. The  
120 emerged workers (subsequently called lab workers) were identified based on their pale color  
121 (*Temnothorax* larvae do not spin a cocoon) and collected once they started to move. They were  
122 anesthetized by cooling down and stored in ethanol for subsequent morphological measurements. We  
123 computed the emergence rate of larvae as the ratio between the number of emerged adults (both workers  
124 and sexuals) and the initial number of larvae. The date of emergence was recorded in order to compute  
125 development duration for each lab worker, i.e. the duration from the second instar larva to the first  
126 movements of the newly emerged lab worker. Nine queens died during the experiment (one from  
127 Bordeaux-city-cadmium, one from Lyon-city-control, three from Paris'17-city-control, one from  
128 Paris'17-city-cadmium, two from Paris'17-forest-control and one from Paris'18-forest-cadmium). We  
129 excluded these colonies from the analyses, so 148 colonies were left in.

130

### 131 ***Morphological measurements***

132 At the end of the experiment, the heads of lab and field workers were stuck on double-sided tape and  
133 photographed at a x5 magnification under a stereomicroscope (Zeiss Discovery.V12) connected to a  
134 computer via a camera (Zeiss AxioCamICc). Head width was measured from eye to eye (just posterior  
135 to the eyes) with ImageJ (<https://imagej.nih.gov/ij/>; (58) and used as a proxy of body size (56).

136

### 137 ***Statistical analyses***

138 We used R v1.1.383 (R Core Team, 2015) to conduct our statistical analyses. We tested for the effect of  
139 treatment (control, ie fed without cadmium, and cadmium, ie fed with cadmium), habitat (city or forest)  
140 and location (Paris'17, Paris'18, Bordeaux, Lyon), and their two level interactions on three dependent  
141 variables (mortality of field workers; emergence rate and size of lab workers). We considered the two  
142 collections in Paris as two independent locations (Paris'17 and Paris'18) as they were carried out during  
143 two different years and at two different pairs of sites. We also tested for the effect of two covariates  
144 (initial colony size and mean size of field workers) on each dependent variable to investigate the effects  
145 of these social parameters. To test for a buffering effect of colony size or field worker size, we included  
146 in the models the interaction between treatment and colony size or field worker size. For the two colony-  
147 level dependent variables (mortality rate of field workers and emergence rate of lab workers), we used  
148 generalized linear models (GLMs). Because mortality and emergence rates were around 50%, using a  
149 quasibinomial model was not better than using a Gaussian model with log-transformed data, and both  
150 models provided the same qualitative results. We therefore presented data from the Gaussian model for  
151 simplicity and because this allows to get AIC information. Regarding the individual-level dependent  
152 variable (size of lab workers), we used a linear mixed-effect model (*lmer*) with the package *nlme* (59),  
153 including colony as a random effect. We visually checked the homogeneity of variance and normality  
154 of residuals. The minimal adequate model was chosen based on the AIC scores (*stepmodel*)(SI). The *P*-  
155 values for the explanatory variables and interactions were obtained by removing or adding the variable  
156 of interest from/to this minimal adequate model (60). *P*-values were obtained by comparing the model  
157 fitted with maximum likelihood method with and without the variable of interest using a log likelihood

158 ratio test (*anova*). When necessary, we used *lsmeans* to compare means among the different levels of  
159 the explanatory variables.

## 160 **Results**

161 The 148 colonies produced a total of 1269 lab workers (922 for the control condition and 347 for the  
162 cadmium condition, Table 1), 215 males (176 for the control condition and 39 for the cadmium  
163 condition, Table 1), and no gynes in 61 days. Because only few males (39) emerged under cadmium  
164 condition, statistical power was lacking and we chose not to present results on male traits.

165

### 166 ***Field workers***

167 Only nine queens died during the experiment. There was no differential queen mortality between control  
168 and cadmium conditions (Fisher's exact test,  $P = 0.49$ ) nor between city and forest colonies (Fisher's  
169 exact test,  $P = 0.32$ ).

170 Colony size (the number of field workers by colony) was only affected by the location ( $F = 4.90$ ,  $P <$   
171  $0.001$ ), with larger colonies found in Paris'18 ( $P$ -adjusted=0.041 for Paris'18-Bordeaux,  $P$ -  
172 adjusted=0.014 for Paris'18-Lyon and  $P$ -adjusted = 0.002 for Paris'17-Paris'18). The size of field  
173 workers also differed among locations ( $F = 17.58$ ;  $P < 0.001$ ) with the bigger field workers found in  
174 Paris'17 and Paris'18 ( $P$ -adjusted Paris'17-Paris'18=0.95,  $P$ -adjusted Paris'17-Bordeaux and Paris'18-  
175 Bordeaux  $< 10^{-6}$ ,  $P$ -adjusted Paris'17- Lyon and Paris-18-Lyon  $< 10^{-3}$ ), and it was positively correlated  
176 with colony size ( $F = 13.10$ ,  $P < 0.001$ ; slope estimate =  $1.285e-04$ ). However, there was no difference  
177 in the size of field workers ( $p = 0.187$ ,  $F = 1.76$ ) and in colony size ( $P = 0.87$ ,  $F = 0.024$ ) between habitats.

178 At all locations, the mortality rate of field workers was significantly higher in cadmium condition than  
179 in control condition ( $F = 115$ ,  $P < 10^{-16}$ ) but the magnitude of the effect varied among locations (Treatment-  
180 Location interaction,  $P = 0.00037$ ,  $F = 6.5$ , Figure 1, Table S2). Mortality rate significantly differed  
181 among locations ( $F = 2.9$ ,  $P = 0.037$ ) with a higher mortality rate in Lyon ( $P = 0.07$ ,  $t = 1.824$ , figure 1).

182 Under the hypothesis that urban colonies resist better to stressors than forest colonies, we were expecting  
183 to find a differential effect of cadmium on mortality rate depending on habitat (Treatment: Habitat  
184 interaction). However we did not find this effect (Treatment: Habitat interaction,  $P = 0.91$ ,  $F = 0.1$ ) and



185 there was no effect of habitat on mortality rate ( $P=0.54$ ,  $F=0.36$ ). Regarding social parameters, colony  
186 size and size of field workers as well as their interactions with treatment did not affect mortality rates  
187 (Table S2).

188

189

### 190 *Laboratory workers*

191 In all locations, the emergence rate was significantly lower in cadmium condition than in control  
192 condition ( $F=104$ ,  $P<10^{-16}$ , figure 2A) even though the magnitude of the effect differed significantly  
193 among locations (Treatment: Location interaction,  $F=2.7$ ,  $P = 0.045$ , Table S2). The emergence rate  
194 significantly differed among locations ( $F=21$ ,  $P<10^{-10}$ ). Interestingly, when treated with cadmium, forest  
195 colonies experienced a stronger decrease in emergence rate than city colonies for all location (Treatment:  
196 Habitat interaction,  $F=4.22$ ,  $P = 0.04$ , figure 2A, Table S2). This was associated with an increase in the  
197 number of larvae that apparently paused their development at early stages (15% in control vs 30% in  
198 cadmium condition,  $t=3.76$ ,  $df=134$ ,  $P=0.00024$ ). Emergence rate in control condition was the same  
199 between city and forest habitats at each location (Paris'17:  $t=1.8$ ,  $df=16$ ,  $P=0.08$ ; Lyon:  $t=-0.07$ ,  $df=5.9$ ,  
200  $P=0.9$ ; Paris'18:  $t=-0.44$ ,  $df=18.9$ ,  $P= 0.65$ ) except for Bordeaux ( $t = -2.99$ ,  $df=10.3$ ,  $P = 0.01$ ), so the  
201 observed Treatment: Habitat interaction was not caused by an initial differential emergence rate between  
202 forest and city colonies when exposed to a stressful temperature. The only social parameters affecting  
203 the emergence rate was the mean size of field worker (Table S2), with colonies containing larger field  
204 workers having lower emergence rate ( $F=9.7$ ,  $P = 0.0022$ , slope estimate =  $-2.4$ ) whatever the treatment  
205 (no significant interaction treatment and size of field workers ( $P=0.62$ ,  $F=0.24$ )).

206 Colonies treated with cadmium produced significantly smaller lab workers in all locations but as  
207 previously, the effect significantly differed in magnitude among locations (Treatment: Location  
208 interaction,  $X_3=9.87$ ,  $P = 0.0197$ , Figure 2B,  $P<10^{-2}$ , Table S3). Interestingly, this decrease in the size  
209 of lab workers was stronger in forest colonies than in city colonies for all location (Treatment: Habitat  
210 interaction,  $X_1=5.03$ ,  $P=0.02485$ , table S3, Figure 2B). The size of lab workers under control condition  
211 did not differ between the two types of habitats for all location (Paris'17:  $t=1$ ,  $P=0.29$ , Lyon:  $t=0.8$ ,

212  $P=0.4$ , Paris'18:  $t=-1.7$ ,  $P=0.09$ ) except for Bordeaux ( $t = -3.2$ ,  $P = 0.002$ ), so the observed Treatment:  
213 Habitat interaction was not caused by an initial difference in the size of lab workers between forest and  
214 city colonies when exposed to a stressful temperature. Regarding social parameters, only colony size  
215 affected significantly and positively the size of lab workers ( $X_1=12.52$ ,  $P= 0.00040$ , slope estimate  
216  $=1.7e-04$ ) whatever the treatment (no significant interaction between treatment and colony size) (Table  
217 S3 to see all statistical results).

218

## 219 **Discussion**

220 The aim of our study was to assess whether urban colonies of the small acorn ant *Temnothorax nylanderi*  
221 are more resistant to a trace metal than forest colonies. We exposed them to food with a very high  
222 cadmium concentration. As expected, this caused an increase in adult mortality rates, and a decrease in  
223 size and emergence rate of lab workers relative to the control condition, confirming that cadmium  
224 condition has a negative effect on life history traits in this species as found in other insects (61,62). More  
225 interestingly, our experiment showed that city colonies were less affected by cadmium than the forest  
226 ones and this was observed in the four city/forest pairs studied, strongly suggesting a general effect of  
227 urbanization in term of cadmium resistance. Whether this effect of urbanization could be considered as  
228 an adaptive response is discussed below.

229 Different hypotheses could explain the lower vulnerability of city colonies to cadmium. First, because  
230 urban soils often contain more cadmium than non-urban soils (on average, 2.45mg/kg for urban wood  
231 soil in Paris, and 0.3 mg/kg in rural wood soils of Ile-de-France (35)), we can hypothesize that urban  
232 colonies have acquired a resistance to cadmium following exposure. Such adaptation has already been  
233 documented in the collembola *Orchesella cincta*, where individuals from populations of heavily  
234 cadmium-contaminated disused mining sites are more resistant to cadmium (63). Second, the better  
235 resistance to cadmium of city colonies observed in our experiment could reflect a general resistance to  
236 other trace elements such as arsenic, zinc, lead or mercury that could be found at a higher concentration  
237 in urban leaf litter because of intense industrial activity and proximity with traffic. Accordingly, urban

238 ants may have evolved general detoxification systems that can cope with various trace elements. Third,  
239 more generally, the lower sensitivity to cadmium of city colonies could reflect a better tolerance to  
240 various stressors. Indeed, it is important to remind that our rearing temperature was stressful for ants  
241 (22-27 °C as opposed to the typical 15-20 °C experienced in nature, (56)). Because of the Urban Heat  
242 Island effect, populations living in urban centers frequently experience higher temperature than their  
243 non-urban counterparts and could therefore be less stressed by heat (64) allowing them to resist better  
244 to cadmium. City colonies did not perform better than forest colonies under control conditions, but this  
245 may be due to the ability of forest colonies to buffer stressors. When exposed to several stressing factors  
246 (heat + cadmium), forest colonies may however collapse whereas urban colonies would resist.  
247 Interestingly, studies performed on European black birds reared under common garden experiments  
248 showed that urban populations have a lower stress response to human handling than their rural  
249 counterparts (19), suggesting that urban populations can evolve a general tolerance to stressors. These  
250 conclusions should be confirmed by further experiments to assess whether the observed lower sensitivity  
251 to cadmium of city colonies in *T. nylanderi* is due to a general resistance to stress or to a more efficient  
252 detoxification system.

253 An unexpected effect of cadmium was the increased frequency of larvae stopping their development.  
254 These larvae did not grow over the entire course of the experiment, but they still looked alive and were  
255 kept in the nest with the rest of the brood and cared for by workers, unlike dead larvae that were typically  
256 discarded outside. In insects, environmental factors such as cold temperature, can induce a pause in  
257 development (65). In ants, diapause seems to be endogenous, even if external factors such as photoperiod  
258 and temperature can secondarily affect its occurrence and duration (66). However diapause can be  
259 induced by other non-seasonal (thus unpredictable) environmental factors, such as trace metals in adult  
260 earthworms or hypoxia in *C. elegans* embryos (67,68). Diapausing individuals usually survive longer in  
261 response to a stress, for example flesh-fly larvae exposed to anoxic conditions (69). Thus, diapause can  
262 be interpreted as a stress-avoidance mechanism(67). The higher proportion of paused *T. nylanderi* larvae  
263 under cadmium condition may be a general defense mechanism to face a wide range of stressors. It is  
264 unknown whether paused larvae can resume development or are just barely surviving and are doomed

265 to death. This and the potential impact of paused development on the life history traits of workers that  
266 would manage to resume development deserves further investigation.

267 The differential response to cadmium between urban and non-urban colonies of *T. nylanderi* could have  
268 genetic or environmental determination. In some hexapodes, populations that are tolerant to heavy metal  
269 have duplicated metallothionein genes (*Drosophila melanogaster*, (70)) or an increased expression of a  
270 single metallothionein gene (*Orchesella cincta*, (71)). A recent genomic analysis (Rad-seq) based on  
271 our *T. nylanderi* colonies revealed a lack of genetic differentiation between habitats (Khimoun et al,  
272 submitted). However, the authors found that 19 loci are under divergent selection between urban and  
273 non-urban populations, but none of them corresponding to metallothionein genes. Interestingly, two of  
274 these loci (one from the Lyon pair of populations and one from the Bordeaux pair of populations) code  
275 for kinase C proteins involved in signalization pathways (72). Two other loci (both from the Lyon pair  
276 of populations) code for a protein involved in mRNA processing and for a membrane protein. These  
277 results support the idea that, in spite of a high gene flow among populations, adaptive genetic  
278 differentiation can occur between urban and forest populations. Similarly, Schrader et al (73) found that  
279 the invasive ant *Cardiocondyla obscurior* could rapidly adapt to environmental changes because of  
280 transposable elements (TE) that generate genetic diversity through transpositions. Given that stressors  
281 could trigger transposition events (74), it would be interesting to test whether TE could trigger genetic  
282 adaptation in urban populations exposed to various stressors in *T. nylanderi*. Phenotypic differences  
283 found after a single laboratory generation could also result from maternal effects and/or epigenetic  
284 effects. For example, queens experiencing stressful conditions like pollution may undergo epigenetic  
285 modifications of their genome that would be transmitted and help workers to face stressors. In that case,  
286 urban queens could resist better to cadmium; however we did not find differential queen mortality  
287 between city and forest colonies. This may be due to a lack of statistical power as very few queens died  
288 over the experiment. Moreover, there was no differential queen mortality between control and cadmium  
289 colonies despite a strong differential worker mortality. Some studies suggested that workers act as a  
290 filter, limiting queen exposure to toxic compounds such as cadmium (75). It is possible that queens are

291 exposed to pollution during colony foundation only, and not after, limiting the potential involvement of  
292 maternal effects.

293 In social groups, the phenotype of an individual is also determined by the surrounding social  
294 environment. The social environment includes colony size (number of individuals), the phenotype of  
295 other individuals (caste, size, morphology, physiology, behaviour), the proportion of individual types,  
296 and complex emerging group properties including communication. In this study, we tested for a potential  
297 buffering effect of colony size and size of field workers on the response to cadmium. We found two  
298 effects of social factors on the phenotype of lab workers but they were similar with and without  
299 cadmium. 1/ Larger colonies produced larger lab workers, a typical phenomenon in ants (56,76). 2/  
300 Colonies with larger field workers had a lower emergence rate, which may be due to the longer  
301 development duration of large workers that were then exposed to cadmium for longer. In contrast, in  
302 bees, larger colonies were found to be more resistant to cadmium (47,48). Even though we did not detect  
303 any effect of the social environment on colony sensitivity to cadmium, workers from urban colonies  
304 could still be more used to polluted food or stressors. Workers could change their behavior when facing  
305 cadmium, either through direct induction by cadmium or indirectly through the higher mortality  
306 triggered by cadmium. The relative contribution of intrinsic factors within larvae and social factors (i.e.  
307 nestmates that limit or enhance environmental variations experienced by larvae) deserves further  
308 investigation through cross-fostering experiments. Social life could deeply impact the ability of ants to  
309 face environmental changes. On one hand, it may help species to cope with environmental changes to  
310 an extent that cannot be achieved by solitary species. The social filtering of the environment can for  
311 instance protect the brood from temperature fluctuations, drought, starvation or pathogens. On the other  
312 hand, consequences of social life such as extreme task-specialization or obligatory collective behavior  
313 may make social species more vulnerable to environmental changes (77,78). Because of the ecologic  
314 and economic values of social species (46,75) it is crucial to understand how they react to environmental  
315 changes.

316 **Conclusion**

317 Our study provides a unique example of replicated common garden experiment highlighting a better  
 318 resistance to cadmium in urban colonies. The plastic response is convergent across all four urban/forest  
 319 pairs tested. This differential resistance can be genetically determined, due to the social environment, or  
 320 a combination of both. The social factors that we assessed had no effect on colony resistance to  
 321 cadmium, in contrast with what was found in bees. To our knowledge, the role of sociality in the  
 322 adaptation to environmental changes has barely been investigated. The relative contribution of  
 323 individual and social factors to the final adult phenotype could be assessed through cross fostering  
 324 experiments.

### 325 **Figures Legend**

326 **Figure 1: Boxplot of the mortality rate of field workers by habitat, location and treatment.**

327 Colonies fed with cadmium are in red, colonies fed with control are in blue. Boxes show median,  
 328 quartiles and extreme values. Statistics are presented in Table S2.

329 **Figure 2: Boxplot of worker traits by habitat, location and treatment.** Colonies fed with cadmium  
 330 are in red, colonies fed with control are in blue. (A) Emergence rate of lab workers. (B) Head width of  
 331 lab workers (mm). Boxes show median, quartiles and extreme values

332

### 333 **Tables**

	Control	Cadmium	Site	GPS	
Paris'17	City	13	14	Jardin	48°50'59.684"N
		247W, 21M	206W, 8M	Ecologique	2° 21' 40.385"E
	Forest	13	13	Fontainebleau	48° 26' 2.595"N
		213W, 6M	87W, 1M		2°43' 52.842"E
	City	7	7	Jardin public	44°51'16.9"N
		39W, 12M	12W, 1M		0°35'16.5"W

Bordeaux	Forest	12	11	Monfaucon	44°52'23.6"N
		130W, 29M	11W, 11M		0°08'14.7"W
Lyon	City	4	5	Tête d'Or	45°46'44.2"N
		18W, 22M	10W, 1M		4°52'54.3"E
	Forest	4	4	Arandon	45°43'07.5"N
		28W, 4M	7W, 3M		5°26'44.5"E
Paris'18	City	10	11	Buttes	48°52'47.1"N
		107W, 64M	7W, 7M	Chaumont	2°23'45.6"E
	Forest	10	10	Chantilly	49°10'59.8"N
		140W, 23M	7W, 2M		2°28'43.6"E

334 Table 1: Number of *Temnothorax nylanderi* colonies used and number of workers (W) and males (M)  
 335 collected for each location, treatment and habitat type.

336

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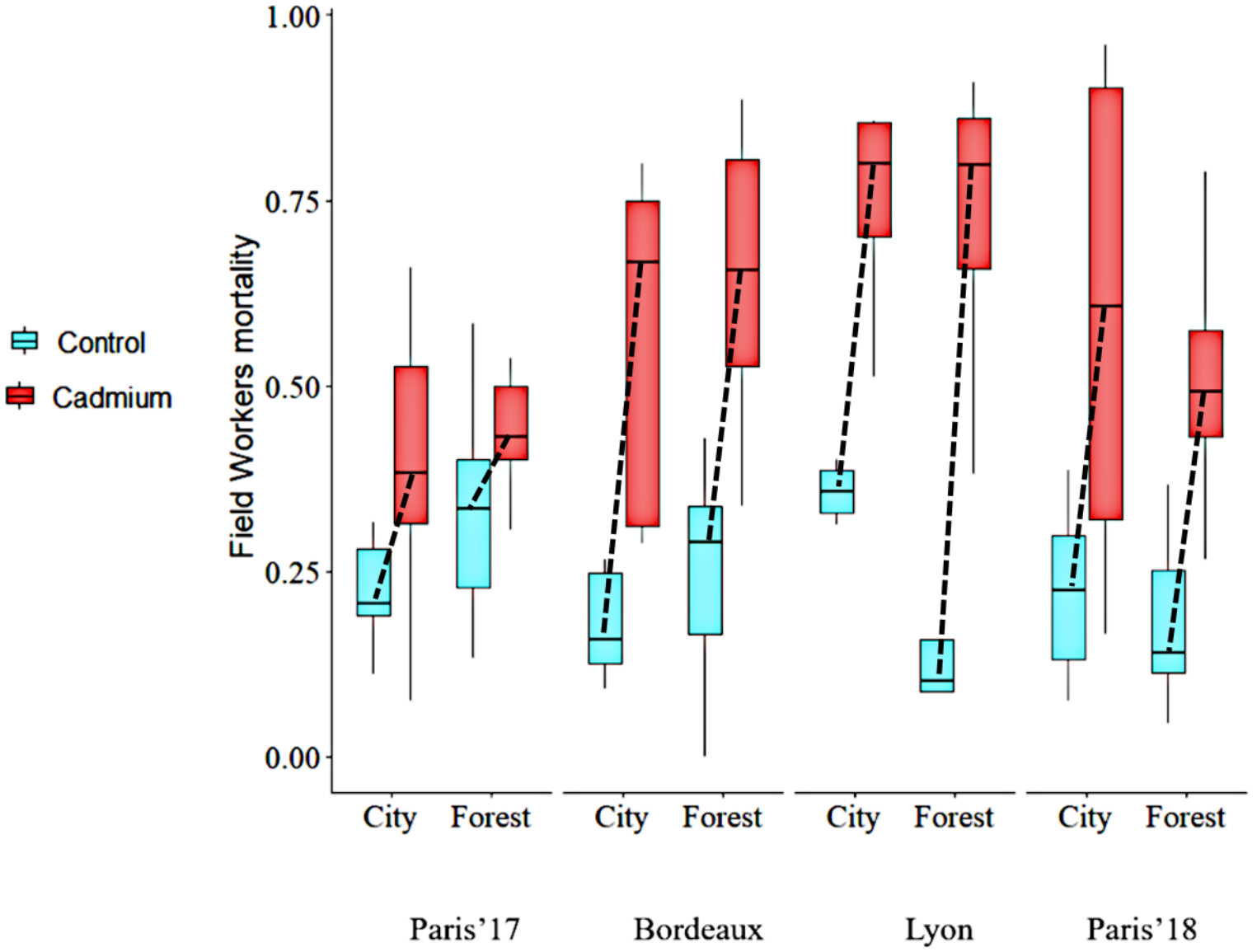


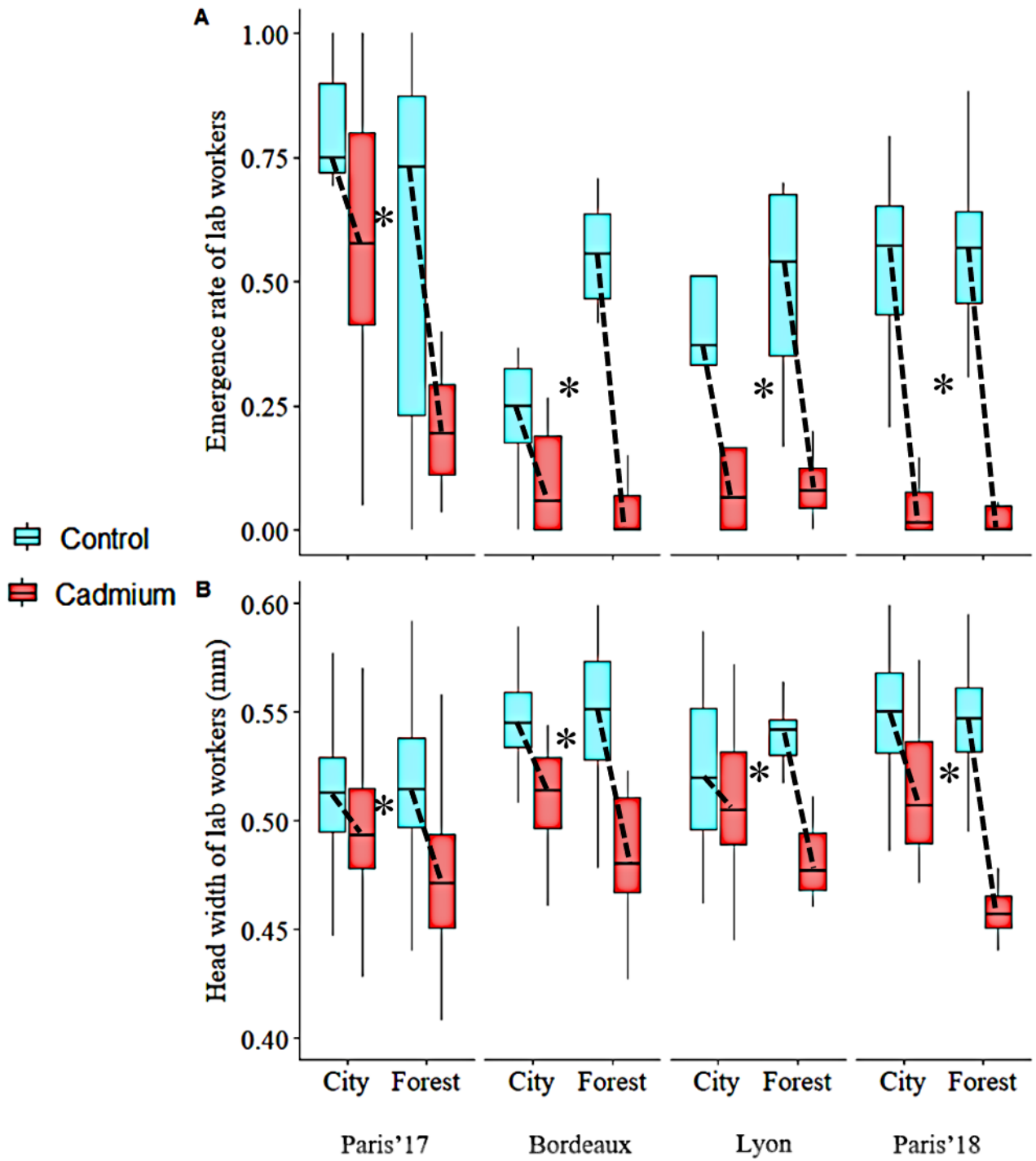
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1 Urban colonies are more resistant to a trace metal than their forest counterparts in the ant *Temnothorax*  
 2 *nylanderi*

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## 9 **Supplementary Information**

### 10 ***Statistical models***

11 For each dependent variable, we built the following complete model:

12 *Dependent variable* ~ *Treatment:Habitat* + *Treatment:Location* + *Treatment* + *Habitat* + *Location* +  
 13 *Colony size:Treatment* + *Mean field worker size:Treatment* + *Colony size* + *Mean field worker size*  
 14 *Treatment(+ random Colony effect)*.

15 We then chose the minimal adequate model based on AIC scores. Finally, we tested the effect of each  
 16 factor as follows: (1) Colony size:Treatment; (2) Mean field worker size:Treatment (3) Colony size; (4)  
 17 Mean field worker size; (5) Treatment:Habitat; (6) Treatment:Location; (7) Treatment; (8) Habitat and  
 18 (9) Location. When the Treatment:Habitat or the Treatment:Location interaction was significant, we  
 19 used lsmeans to estimate the effect of one factor depending on the level of the other.

	Colony size	Size of field workers
Colony size	NA	$F_1=13.10$ 0.00042
Treatment : Habitat	$F_1=2.13$ 0.15	$F_1=1.93$ 0.17
Treatment : Location	$F_3=0.61$	$F_3=0.93$

	0.61	0.43
Treatment	$F_1=0$	$F_1=1.49$
	0.99	0.22
Habitat	$F_1=0.02$	$F_1=1.76$
	0.8754	0.187
Location	$F_3=4.89$	$F_3=17.6$
	$2.85 \times 10^{-3}$	$<10^{-6}$

20 **Table S1: Effect of the different factors and their interactions on colony size (number of field**  
 21 **workers) and size of field workers.** Fisher scores and associated p-values are from ANOVAs between  
 22 generalized linear models including or excluding the factor or interaction.

	Mortality rate of field workers	Emergence rate of lab workers
Colony size	$F_1=1.8$ 0.17	$F_1=0.3$ 0.58
Size of field workers	$F_1=0.8$ 0.37	<b><math>F_1=8.8</math></b> <b>0.0034</b>
Colony size :	$F_1=0.072$	$F_1=0.74$
Treatment	0.93	0.38
Size of field workers :Treatment	$F=0.023$ 0.87	$F-1=0.24$ 0.62
Treatment : Habitat	$F_1=0.01$ 0.91	<b><math>F_1=4.22</math></b> <b>0.041</b>
Treatment : Location	<b><math>F_3=6.5</math></b> <b><math>&lt;10^{-3}</math></b>	<b><math>F_3=2.87</math></b> <b>0.038</b>
Treatment	<b><math>F_1=115</math></b> <b><math>&lt;10^{-16}</math></b>	<b><math>F_1=104</math></b> <b><math>&lt;10^{-16}</math></b>
Habitat	$F_1=0.36$	$F_1=5.8$



	<i>0.54</i>	<b>0.017</b>
Location	<b><i>F<sub>3</sub>=2.9</i></b>	<b><i>F<sub>3</sub>=21.3</i></b>
	<i>0.035</i>	<b><i>&lt;10<sup>-10</sup></i></b>
Lsmeans		<b>Control</b>
Habitat   Treatment		<b>0.74</b>
		<b>Cadmium</b>
		<b>0.011</b>
Lsmeans	Paris	Paris2
	Lyon	Bordeaux
Treatment   Location	<i>&lt;10<sup>-3</sup></i>	<i>&lt;10<sup>-3</sup></i>
	<i>&lt;10<sup>-4</sup></i>	<i>&lt;10<sup>-4</sup></i>
	<i>&lt;10<sup>-4</sup></i>	<i>&lt;10<sup>-4</sup></i>
	<i>&lt;10<sup>-4</sup></i>	<i>&lt;10<sup>-4</sup></i>

23 **Table S2: Effect of the different factors and their interactions on the mortality rate of field workers**  
 24 **and the emergence rate of lab workers.** Fisher scores and associated p-values are from ANOVAs  
 25 between generalized linear models including or excluding the factor or interaction.

	Size of lab workers
Colony size	<b><i>X<sub>I</sub>=12.52</i></b> <b><i>&lt;10<sup>-3</sup></i></b>
Size of field workers	<b><i>X<sub>I</sub>=1.42</i></b> <b><i>0.23</i></b>
Colony size : Treatment	<b><i>X<sub>I</sub>=0.08</i></b> <b><i>0.76</i></b>
Size of field workers :Treatment	<b><i>X<sub>I</sub>=0.23</i></b> <b><i>0.62</i></b>
Treatment : Location	<b><i>X<sub>3</sub>=9.87</i></b> <b><i>0.019</i></b>
Treatment : Habitat	<b><i>X<sub>I</sub>=5.03</i></b> <b><i>0.025</i></b>
Treatment	<b><i>X<sub>I</sub>=48</i></b> <b><i>4.10<sup>-12</sup></i></b>
Habitat	<b><i>X<sub>I</sub>=4.29</i></b>

	<b>0.038</b>			
Location	$X_3=42.13$			
	$<10^{-8}$			
Treatment   Habitat	City	Forest		
	$<10^{-4}$	$<10^{-4}$		
Lsmeans	Paris	Paris2	Lyon	Bordeaux
Treatment   Location	$<10^{-4}$	$<10^{-4}$	<b>0.0026</b>	$10^{-4}$

26 **Table S3: Effect of the different factors and their interactions on the size and the development**  
 27 **duration of lab workers.** Chi<sup>2</sup> scores and associated p-values are from ANOVAs between generalized  
 28 linear models including or excluding the factor or interaction.