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Population genetics

# Urbanization without isolation: the absence of genetic structure among cities and forests in the tiny acorn ant *Temnothorax nylanderi*

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Urban alteration of neutral and adaptive evolutionary processes is still underexplored. Using a genome-wide SNP dataset, we investigated (i) urban-induced modifications of population demography, genetic diversity and population structure and (ii) signature of divergent selection between urban and forest populations in the ant species, *Temnothorax nylanderi*. Our results did not reveal an impact of urbanization on neutral processes since we observed: (i) analogous genetic diversity among paired urban/forest sites and two control populations; (ii) weak population genetic structure explained neither by habitat (urban versus forest) nor by geography; (iii) a remarkably similar demographic history across populations with an ancestral growth followed by a recent decline, regardless of their current habitat or geographical location. The micro-geographical home range of ants may explain their resilience to urbanization. Finally, we detected 19 candidate loci discriminating urban/forest populations and associated with core cellular components, molecular function or biological process. Two of these loci were associated with a gene ontology term that was previously found to belong to a module of co-expressed genes related to caste phenotype. These results call for transcriptomics analyses to identify genes associated with ant social traits and to infer their potential role in urban adaptation.

## 1. Background

Urbanization alters natural habitats [1–3], leading to the extirpation of native species and the establishment of non-native species, and promoting biotic homogenization [4–6]. Nevertheless, some species are able to persist in urban landscapes providing the unique opportunity to assess the consequences of urbanization on both neutral and adaptive evolutionary processes [7]. Habitat modification resulting from urbanization involves habitat loss and fragmentation. These changes may induce a rapid decline of genetic diversity and an increase of population differentiation in species with small effective population size whose dispersal is hampered by the new habitat configuration [8–10].

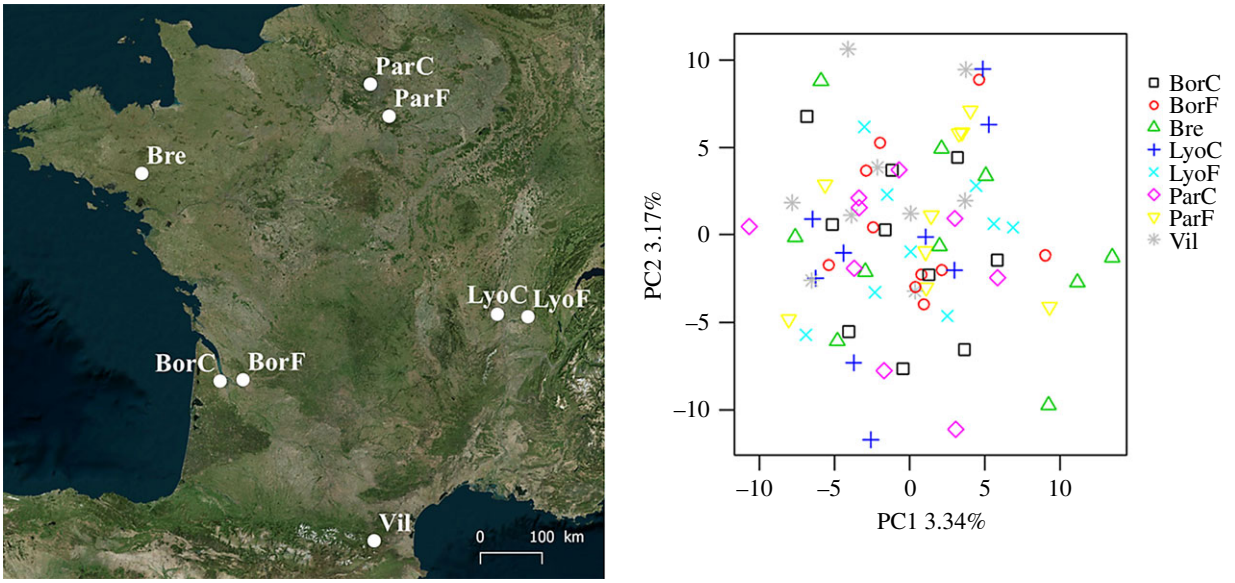


**Figure 1.** Sampled localities and individual genotypes plotted in the space of the first two axes of a principal coordinate axis (PCA). Colonies were sampled in wooded parts of urban parks (BorC, LyoC and ParC) and in forest localities (BorF, LyoF, ParF, Bre and Vil). (Online version in colour.)

Environmental changes resulting from urbanization may also impose new selective pressures, ultimately leading to genotypic and phenotypic differences between urban and non-urban populations [11–13]. Several studies reported urban-induced shifts in phenotypic traits, including life history, morphology, behaviour, physiology and reproductive traits. Although mechanisms underlying such phenotypic changes are unknown for most organisms, some common garden experiments disentangled phenotypic plasticity from genetic adaptation to urban-induced environmental changes [14,15]. Because such an approach allows study of only a restricted number of traits that are relatively straightforward to measure, difficult-to-measure but potentially informative traits may be overlooked [16]. Genomic data may help to overcome this limitation and to identify genomic signatures of local adaptation. Only a small number of studies have profited from genome-wide data to search for the footprints of divergent selection between urban and non-urban populations (but see [17–19]). In addition, they focused on a single urban area although replication over multiple urban areas is required to understanding urban adaptation [7,20].

Ants are frequently used as bio-indicators in ecological studies and monitoring programmes: they respond rapidly to environmental changes and have extensively adapted to a wide range of environments [21,22]. Studies on the consequences of urbanization on the genetic variability of ant species are rare (but see [23,24]), generally investigating the community level and reporting contrasted effects on species assemblages [25]. Here, using a replicated design over three cities, we explored (i) urban-induced modifications of genetic diversity, population structure and demography; (ii) signature of divergent selection between urban and forest populations in *Temnothorax nylanderi*, a forest leaf litter-dwelling, cavity nesting, small-sized predatory ant found in small colonies of 50–200 workers. An absence of genetic structure in Western Europe was previously suggested for this species based on one allozyme and two mitochondrial genes [26]. However, single-locus (such as mtDNA) inference of spatial genetic differentiation is not reliable [27] and further instigation based on genome-wide loci is warranted.

To this end, we generated a genome-wide SNP dataset for 96 colonies belonging to eight localities (12 colonies per locality). Colonies were sampled in wooded parts of historical urban parks (e.g. the Jardin des Plantes of Paris acquired its present form in the seventeenth century, around 400 generations before present considering a generation time of 1 year) of three highly populated French cities: Bordeaux (BorC), Lyon (LyoC) and Paris (ParC). Forest localities (BorF, LyoF and ParF) were chosen to achieve a paired study design including three replicates, each consisting of one urban and one forest locality in close geographical proximity (30–50 km) but functionally disconnected. Two additional forest localities (Bretagne, Bre and Villefranche, Vil) were included to provide a better coverage of the *T. nylanderi* geographical distribution (figure 1). Because of the tiny size of *T. nylanderi* (*ca* 3 mm), we randomly pooled 50 workers per colony to obtain a suitable amount of DNA. We followed a single-digestion RADseq protocol [28], individually barcoding each colony. *Temnothorax nylanderi* colonies are predominantly monogynous and monoandrous [29]: pooling several workers from a colony results in sequencing three alleles per locus, since workers carry the allele from the male and one of the two alleles from the queen. Barcoding a colony is therefore analogous to barcoding a triploid genotype. Popular pipelines performing de novo assembly and SNP calling for RADseq data cannot handle triploid individuals. To circumvent this problem, we split the bioinformatics analysis of raw fastq reads into two steps: (i) we first built loci using the *denovo_map* pipeline implemented in STACKS [30]; (ii) we artificially built a reference sequence using the assembled loci and mapped reads back using BWA v0.7.15 [31]. Triploid genotypes were called after local realignment using GATK v3.8-1-0 [32].

To test the hypothesis of urban-induced reductions of population size, genetic diversity and gene flow, we estimated within-population diversity, in all sampled localities, by calculating the observed heterozygosity ($H_O$) using the gametic homozygosity concept of Moody *et al.* [33], the expected heterozygosity ($H_E$) and multilocus $F_{IS}$ values corrected for the ploidy level following Hardy [34]. We also

**Table 1.** Estimates of genetic diversity. For each site, geographical coordinates, the number of sampled colonies ($N$), expected ($H_E$) and observed ($H_O$) heterozygosity and $F_{IS}$ values are provided.

| locality | site | latitude | longitude | $N$ | $H_E$ | $H_O$ | $F_{IS}$ |
|---|---|---|---|---|---|---|---|
| Bor | BorC | 44°51′16.60″ N | 0°35′17.21″ O | 10 | 0.224 | 0.231 | 0 |
| | BorF | 44°52′23.61″ N | 0° 8′15.25″ O | 11 | 0.216 | 0.217 | 0.028 |
| Lyo | LyoC | 45°46′43.87″ N | 4°51′20.35″ E | 9 | 0.229 | 0.230 | 0.034 |
| | LyoF | 45°44′40.28″ N | 5°27′23.36″ E | 9 | 0.230 | 0.228 | 0.046 |
| Par | ParC | 48°50′39.39″ N | 2°21′43.45″ E | 9 | 0.235 | 0.243 | 0.003 |
| | ParF | 48°25′42.14″ N | 2°43′50.81″ E | 9 | 0.235 | 0.237 | 0.029 |
| Bre | Bre | 47°40′37.84″ N | 2° 7′30.27″ O | 9 | 0.230 | 0.233 | 0.027 |
| Vil | Vil | 42°35′9.77″ N | 2°26′13.07″ E | 9 | 0.233 | 0.234 | 0.032 |

investigated the genetic structure by: (i) a principal component analysis (PCA) on triploid genotype frequencies; (ii) a non-metric multidimensional scaling (NMDS) on the matrix of $F_{ST}$ pairwise distances. Finally, for each locality, we investigated the variation of the scaled mutation rate ($\Theta$, the product of the effective population size $Ne$ and the mutation rate per generation $\mu$) using the *stairwayplot* software [35] and the *abc-skyline* method of [36]. To investigate the potential genomic signature of local adaptation to urban landscape in *T. nylanderi*, we applied two complementary random forest (RF) variable selection procedures, implemented in the packages *VSURF* [37] and *Boruta* [38], by feeding genotypes as discriminating variables for each pair of urban/forest localities.

Full details on the methodology for this study are in electronic supplementary material, S1.

## 2. Results and discussion

### (a) Locus assembly and data filtering

Following [39], the optimal set of STACKS's parameters resulted in m3, M3 and n3 (electronic supplementary material, S2). After implementing a newly developed pipeline for building triploid genotypes in non-model organisms, we obtained the final filtered dataset composed of 5728 polymorphic loci and 10 723 SNP for 75 colonies, with an average coverage of 47X and 4.9% of missing data.

### (b) Genetic diversity and population structure

Expected ($H_E$) and observed heterozygosity ($H_O$) averaged across loci ranged, respectively, from 0.216 to 0.235 and from 0.217 to 0.243 (table 1). We observed similar values between each pair of forest (F) and urban (C) sites. Multilocus $F_{IS}$ corrected for the ploidy level were slightly positive within each sampled site and ranged from 0 (BorC) to 0.046 (LyoF), suggesting that populations within each site are at Hardy–Weinberg equilibrium.

Principal component analyses revealed a lack of structure (figure 1), as colonies were not separated on the basis of their origin for any of the first 20 axes (representing 48% of the total variance). Consistently, both the global $F_{ST}$ ($F_{ST}$ = 0.028) and the pairwise $F_{ST}$ values suggest that most of the observed genetic variance is partitioned within sites. Indeed, none of the pairwise $F_{ST}$ values exceeded 0.039 (LyoF versus Bre), despite many comparisons being significant at the 0.05 level (electronic supplementary material, S3). The lack of genetic structure driven by habitat type was confirmed in two out of three forest versus urban comparisons by the NDMS (electronic supplementary material, S4), the only exception being Lyon (in agreement with the pairwise $F_{ST}$, electronic supplementary material, S3). An absence of genetic structure in Western Europe was previously suggested for this species based on one allozyme and two mitochondrial genes [26]. Here we confirmed this finding, strengthened not only by genome-wide data, but also by the finer spatial sampling. The dispersal flight of sexuals over large distances and/or the passive transport of established colonies in acorns and hazelnuts by vertebrates including humans [40] could allow high level of gene flow and may explain this absence of contemporary genetic structure.

### (c) Demographic history and adaptation

We inferred the demographic history of the eight populations using *stairwayplot* (figure 2) and the *abc-skyline*. After removing missing data, an average of 2800 polymorphic loci were available for each population, the majority of which have one SNP only (approx. 90% of the loci). The inferred variation of $\Theta$ through time was substantially unchanged whether the singletons were included or not in the case of the *stairwayplot* (figure 2). The results reveal an initial growth followed by a recent decline for all populations analysed, regardless of their current habitat. This suggests that the observed demographic history is the result of events independent from the contemporary urbanization process. The lack of a robust estimation of the genome-wide molecular rate hampers a flawless dating of the observed demography. We therefore preferred to report estimates of $\Theta$ and scaled time. However, we note that using the evolutionary rate proposed for the whole ant group by Romiguier *et al*. [41], the expansion and the following decline (figure 2) would have occurred around approximately 50 000 and approximately 20 000 years B.P. respectively, further strengthening the idea that the historical demography of *T. nylanderi* was not affected by urbanization. We further check the robustness of the *stairwayplot*, the performance of which has been recently questioned for datasets of less than approximately 10 000 SNPs [42], by running the *abc-skyline* (electronic supplementary material, S4). Results were in agreement, suggesting

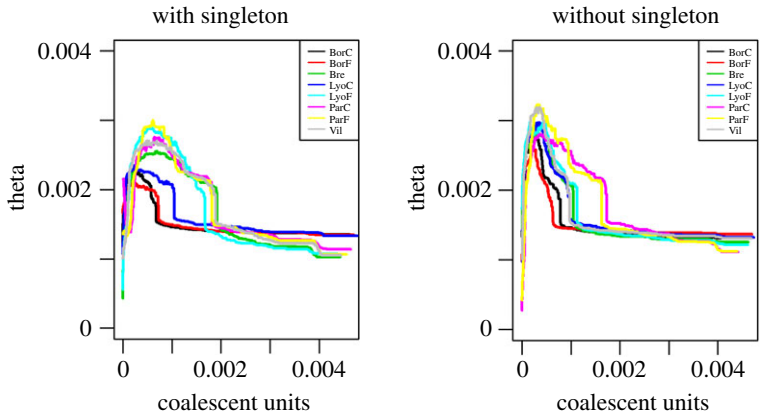


**Figure 2.** Maximized composite likelihood of $\Theta$ through time obtained through the *stairwayplot* method on the folded SFS. The inferred effective size $\Theta$ is plotted, from left to right, from present time (0) to the past (in scaled units of time). (Online version in colour.)

**Table 2.** Candidate loci discriminating urban and forest sites and corresponding functional annotation (Gene Ontology GO).

| paired urban–forest sites | SNP IDs | BLASTn E-values | description of significant hits | GO IDs | GO names |
|---|---|---|---|---|---|
| BorC–BorF | 775198 | no significant similarity found | — | — | — |
| BorC–BorF | 782672 | $1.00 \times 10^{-7}$ | protein kinase C | GO:0004697 | protein kinase C activity |
| BorC–BorF | 855369 | no significant similarity found | — | — | — |
| BorC–BorF | 1416915 | no significant similarity found | — | — | — |
| BorC–BorF | 1471596 | no significant similarity found | — | — | — |
| BorC–BorF | 1640863 | no significant similarity found | — | — | — |
| BorC–BorF | 2343564 | $6.00 \times 10^{-20}$ | ultra-conserved locus | no GO found | no GO found |
| BorC–BorF | 2627927 | no significant similarity found | — | — | — |
| LyoC–LyoF | 280520 | no significant similarity found | — | — | — |
| LyoC–LyoF | 395140 | no significant similarity found | — | — | — |
| LyoC–LyoF | 813323 | $4.00 \times 10^{-22}$ | protein kinase C | GO:0004697 | protein kinase C activity |
| LyoC–LyoF | 827337 | $4.00 \times 10^{-56}$ | serine/arginine repetitive matrix protein | GO:0006397 | mRNA processing |
| LyoC–LyoF | 1567225 | no significant similarity found | — | — | — |
| LyoC–LyoF | 2136348 | $3.00 \times 10^{-58}$ | uncharacterized protein | GO:0016021 | integral component of membrane |
| LyoC–LyoF | 2602998 | no significant similarity found | — | — | — |
| LyoC–LyoF | 3297929 | no significant similarity found | — | — | — |
| ParC–ParF | 808830 | no significant similarity found | — | — | — |
| ParC–ParF | 865740 | no significant similarity found | — | — | — |
| ParC–ParF | 2841848 | no significant similarity found | — | — | — |

that the recent decline is not an artefact of the inferential methods but the result of real biological processes shared among all sampled sites regardless of the habitat type.

The demographic signature and the absence of contemporary genetic structure are compatible with the scenario of rapid post-glacial recolonization from southern refuges hosting genetically homogeneous populations previously invoked in this species [26]. However, the causes of the decline observed in all populations remain to be further explored. A recent increase in connectivity may result in an apparent decrease of $\Theta$ in populations analysed with unstructured models such as the *stairwayplot* [43]. This explanation would be consistent with the low population differentiation found, but the causes of such a recent increase in connectivity remain to be elucidated.

The two selection procedures identified 19 common SNPs that discriminate urban and forest paired sites (three for Par and eight for both Bor and Lyo, electronic supplementary material, S5). To validate RF results, following [44], we further computed $F_{ST}$ at the selected SNPs between urban and forest paired sites and found an approximately 10 times increase compared to values based on the whole SNP dataset, with all three comparisons becoming significant. These SNPs are therefore efficient in discriminating forest versus urban landscape and could be considered candidates for being under divergent selection. We recorded five loci with significant homologies after BLASTn searches (table 2). The Blat2GO annotation tool identified a protein kinase C, involved in catalysis of a molecular reaction, from two different candidate loci found in Bor and Lyo, strongly suggesting

the role of this protein family in urban adaptation. An uncharacterized protein recognized as an integral component of membrane and a serine/arginine repetitive matrix protein involved in biological processes related to mRNA processing were also identified (table 2). The mRNA processing GO term associated with the locus carrying the SNP 827337 (table 2) was previously found to belong to a module of co-expressed genes correlated with caste phenotype [45]. The genetic toolkit hypothesis posits that conserved sets of genes and gene pathways involved in core physiological processes have been repeatedly used in the evolution of complex social behaviour [46]. Recent studies identified modules of co-expressed genes whose up- and downregulation is associated with phenotypic traits related to social behaviour (e.g. caste, worker sterility) and species ecology (e.g. invasiveness) [45,47]. Further transcriptomics analyses will help to identify genes related to social traits associated with adaptation to urban habitat.

## 3. Conclusion

Recent empirical research on urban dweller species has shown that urbanization may increase genetic differentiation among populations through a reduction of functional connectivity and an increase of genetic drift [8,9,18,48,49]. Our results, substantially strengthened by the congruence among the replicated landscapes, support a different scenario and call for a species-specific null model in urbanization studies. Despite living in fragmented patches of habitat, colonies of *T. nylanderi* are apparently genetically not isolated. The demographic reconstruction suggests that this is the consequence of past demographic events rather than urbanization. Moreover, a recent increase in connectivity is possibly ongoing, contributing to further hinder the effects of urbanization on genetic patterns. Indeed, with the intensification of transport networks, human-mediated dispersal contributes, in some species like ants, to gene flow by transgressing dispersal barriers [50,51].

Discrepancies between observed and expected consequences of urbanization on neutral evolutionary processes were previously described in studies on ant communities structure, suggesting that biotic homogenization has not taken place in this group at the European scale [52].

Models of natural habitat islands isolated within an urban matrix that reduces functional connectivity are often used to describe patterns of vertebrate diversity. Our results provide evidence, at another organizational level of biodiversity (within-species genetic diversity), that these models may not be relevant for small species exploiting micro-habitats [22,25]. Such ecological and/or life-history requirements drive species-specific patterns of landscape functional connectivity. Species-centred approaches offer considerable promise to predict species sensitivity to human-induced landscape alteration [53,54].

Despite the predominant influence of the demographic history, we found evidence of divergent selection at 19 loci; among them, four are known to be involved in core cellular components, molecular functions or biological processes. Although recent transcriptomics studies in ants (e.g. [45,47]) identified conserved functional genomic units involved in social behaviour traits, their potential role in adaptation to urban habitats was not investigated. Group living, by allowing workers to manipulate the environment of the developing larvae, could buffer environmental variations between urban and forest environments [55,56]. Nevertheless, we found signatures of divergent selection between urban and forest sites, potentially linked to sociality (table 2). This is in agreement with recent phenotypic studies that suggest rapid phenotypic differentiation in urban populations in response to heavy metal pollution in *T. nylanderi* [57], rapid temperature increases in *T. curvispinosus* [14,58] and human food inputs [59], indicating that further transcriptomic studies under controlled conditions are warranted.

Ethics. All applicable national and/or institutional guidelines for the collection of animals were followed.

Data accessibility. Home-made code to reproduce the analysis of population structure (PCA, Fst and bootstrap confidence interval, NMDS) and the filtered vcf with one SNP per locus are deposited in Dryad and they are accessible with this temporary review link: https://datadryad.org/stash/share/9y3WVtfMO1YPoszoIvaoHlOGFiaaW7U6EPqJIUvq3Zs.

Authors' contributions. C.D. and M.M. designed the study; A.K., R.P., C.D., M.M., B.K. and P.A.E. performed fieldwork; A.K., S.M. and C.D. analysed the data; A.K., S.M. and C.D. drafted the manuscript and all authors contributed to the final version. All authors agree to be held accountable for the content therein and approve the final version of the manuscript.

Competing interests. We declare we have no competing interests.

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