

Does urbanisation lead to parallel demographic shifts across the world in a cosmopolitan plant?

Aude E. Caizergues^{1,2}  | James S. Santangelo³  | Rob W. Ness^{1,2} | Fabio Angeoletto⁴ | Daniel N. Anstett⁵ | Julia Anstett^{6,7} | Fernanda Baena-Diaz⁸  | Elizabeth J. Carlen⁹ | Jaime A. Chaves^{10,11} | Mattheau S. Comerford¹² | Karen Dyson¹³ | Mohsen Falahati-Anbaran¹⁴ | Mark D. E. Fellowes¹⁵ | Kathryn A. Hodgins¹⁶ | Glen Ray Hood¹⁷ | Carlos Iñiguez-Armijos¹⁸  | Nicholas J. Kooyers¹⁹ | Adrián Lázaro-Lobo²⁰ | Angela T. Moles²¹ | Jason Munshi-South²²  | Juraj Paule²³ | Ilga M. Porth²⁴ | Luis Y. Santiago-Rosario²⁵ | Kaitlin Stack Whitney²⁶ | Ayko J. M. Tack²⁷ | Marc T. J. Johnson^{1,2}

Correspondence

Aude E. Caizergues, Centre for Urban Environments, University of Toronto Mississauga, Mississauga, Ontario, Canada.

Email: audeemiliecaizergues@gmail.com

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Abstract

Urbanisation is occurring globally, leading to dramatic environmental changes that are altering the ecology and evolution of species. In particular, the expansion of human infrastructure and the loss and fragmentation of natural habitats in cities is predicted to increase genetic drift and reduce gene flow by reducing the size and connectivity of populations. Alternatively, the 'urban facilitation model' suggests that some species will have greater gene flow into and within cities leading to higher diversity and lower differentiation in urban populations. These alternative hypotheses have not been contrasted across multiple cities. Here, we used the genomic data from the GLocal Urban Evolution project (GLUE), to study the effects of urbanisation on non-adaptive evolutionary processes of white clover (*Trifolium repens*) at a global scale. We found that white clover populations presented high genetic diversity and no evidence of reduced N_e linked to urbanisation. On the contrary, we found that urban populations were less likely to experience a recent decrease in effective population size than rural ones. In addition, we found little genetic structure among populations both globally and between urban and rural populations, which showed extensive gene flow between habitats. Interestingly, white clover displayed overall higher gene flow within urban areas than within rural habitats. Our study provides the largest comprehensive test of the demographic effects of urbanisation. Our results contrast with the common perception that heavily altered and fragmented urban environments will reduce the effective population size and genetic diversity of populations and contribute to their isolation.

Aude E. Caizergues, James S. Santangelo, Rob W. Ness and Marc T.J. Johnson are members of the lead team.

For Affiliation refer page on 10

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KEYWORDS

effective population size, gene flow, genetic diversity, neutral evolution, urbanisation

1 | INTRODUCTION

In the past century, urbanisation has rapidly modified landscapes throughout the world. Urbanisation replaces natural and rural habitats with highly disturbed, human-modified habitats, characterised by a high density of humans, buildings and roads, and consequently more impervious surfaces, increased pollution, and elevated habitat loss and fragmentation (Grimm et al., 2008; Liu et al., 2020). These landscape alterations influence multiple evolutionary processes, including natural selection, mutation, gene flow and genetic drift (Johnson & Munshi-South, 2017; Miles et al., 2019; Schmidt et al., 2020; Somers et al., 2004; Yauk et al., 2008). For example, we know that cities influence natural selection, yet not all species in urban habitats have adapted to these new environments (Lambert et al., 2021). The potential of a population to adapt to a new environment is heavily influenced by the availability of genetic diversity, which is in part driven by demographic changes in population size and the connections among populations. Hence, the demographic history of a population can play a major role in its past and present evolutionary trajectory, which raises concerns about the future of urban dwelling species and their conservation. How urbanisation influences the evolutionary demography of populations remains poorly understood and is the focus of our study.

Habitat fragmentation and degradation can influence population demographic processes, which in turn can affect genetic drift within populations and gene flow between populations (Hanski, 1998). Fragmentation occurs when habitats are divided into patches with limited corridors for dispersal among remaining habitat fragments. For instance, when non-urban habitats are converted into buildings and roads, it splits and potentially isolates natural patches. This phenomenon can reduce the size, increase the isolation (i.e. by limiting dispersal), and influence the persistence of populations. One predicted evolutionary outcome to urban habitat fragmentation is increased genetic drift, which can reduce fitness when there is a loss of genetic diversity, which is often associated with elevated inbreeding, accumulation of deleterious mutations, and reduced ability to adapt to environmental changes. The reduced gene flow that ensues from the isolation of populations is also expected to lead to increased genetic differentiation between populations (Beninde et al., 2018; Lourenço et al., 2017; Munshi-South et al., 2016). The hypothesis of increased drift and divergence of urban populations is referred to as the 'urban fragmentation model', and it is widely thought to be the most prevalent outcome of urbanisation on the non-adaptive evolution of populations (Miles et al., 2019).

While the urban fragmentation model has the most empirical support (Miles et al., 2019), urbanisation can also facilitate the ecological success and evolutionary potential of some species. In particular, human commensals may thrive in urban habitats (Carlen &

Munshi-South, 2021; Medina et al., 2018; Rochat et al., 2017). In such cases, urban features and human behaviour can facilitate individual movement between populations and create corridors of dispersal and gene flow, leading to higher genetic diversity within urban populations and decreased divergence between urban populations (Miles et al., 2018). Such observations have led to an alternative hypothesis – the 'urban facilitation model' (Miles et al., 2019). Understanding which of these two scenarios is likely to occur for any given species is of major importance to understanding how urbanisation shapes evolution, because demographic processes such as changes in population size and dispersal influence the amount of genetic variation within and between populations, and thus the evolutionary potential of populations.

Urbanisation is expected to cause similar evolutionary processes and patterns across cities (Santangelo, Rivkin, et al., 2020), since urbanisation frequently leads to similar environmental changes (McKinney, 2006; Santangelo et al., 2022). While there has been a focus on studying whether urbanisation causes parallel adaptive evolution to different cities (Caizergues et al., 2022; Salmón et al., 2021), there has not been the same focus on whether there could be parallel non-adaptive processes. This leads to the question: Does urbanisation consistently lead to increased genetic drift within populations and divergence between populations? Some have predicted that urbanisation can drive parallel non-adaptive processes (Lambert et al., 2021; Santangelo, Miles, et al., 2020), but existing tests of this prediction are inconclusive and limited in spatial scale (Beninde et al., 2018; Combs et al., 2018; Mueller et al., 2018; Theodorou et al., 2018). Hence, understanding if urbanisation causes parallel evolutionary and demographic patterns among cities throughout the globe remains unresolved.

We have been using white clover (*Trifolium repens* L., *Fabaceae*) as a model to understand how global urbanisation affects evolution as part of the GLobal Urban Evolution (GLUE) project (www.globalurbanevolution.com). Here we combine new data with the large-scale dataset generated as part of GLUE. We previously reported that white clover frequently exhibited urban–rural clines in the production of an antiherbivore defence trait, and this repeated evolution was attributed to adaptive evolution. Previous results also revealed that populations of clover consistently showed high genetic diversity and low worldwide genetic structure (Johnson et al., 2018; Santangelo et al., 2022; Wu et al., 2021), but we did not explicitly investigate how urbanisation impacts genetic drift and gene flow, or the potential for parallel non-adaptive evolution across the world.

Here, we seek to understand how urbanisation impacts non-adaptive evolutionary processes, including demography and gene flow, of white clover in cities throughout the world. We addressed the following questions: (1) Does urbanisation repeatedly cause changes in effective population size across cities, thus influencing

genetic diversity and inbreeding? (2) Does urbanisation influence gene flow, differentiation and structure among populations? To answer these questions, we sampled and performed whole genome sequencing of over 2000 plants from urban and rural populations of white clover in 24 cities across the world, and used both population genomic analyses and demographic modelling to reconstruct urban and rural population histories. Given that the 24 cities sampled are geographically distant from each other, and since the urbanisation process occurred independently in each city, we can expect the evolutionary trajectories of white clover populations to be sufficiently independent among cities to treat them as independent tests of evolutionary changes, and thus suitable for the study of parallel evolution. Based on white clovers' strong positive association with human modified habitats, we predicted demographic processes and gene flow would better reflect the urban facilitation model than the urban fragmentation model. Specifically, we predicted that *T. repens* populations thrive more in urban areas than rural areas, which would be seen as higher effective population size, higher genetic diversity, lower inbreeding, and lower population structure due to high gene flow among populations compared to rural areas.

2 | METHODS

2.1 | Study system and data sampling

White clover (*Trifolium repens* L., Fabaceae) is an herbaceous perennial plant native to Eurasia. It can reproduce clonally via stolons that spread horizontally on the soil surface or through sexual reproduction via outcrossing (Burdon, 1983). Each plant produces inflorescences with numerous hermaphroditic flowers that are pollinated by a diversity of bee species (Kakes, 1997). White clover is an allotetraploid (Griffiths et al., 2019) with disomic inheritance (Williams et al., 1998). Because of its ability to fix atmospheric nitrogen, white clover has been introduced to all inhabited continents in the past several hundred years as livestock fodder and as a cover crop (Kjærgaard, 2003). Its global distribution covers a wide range of climates, and the fact that it grows in anthropogenically modified habitats (e.g. mowed grass, pastures), makes it an ideal model to study urban evolutionary biology at a global scale.

Our study expands upon the GLUE project that aimed to examine how global urbanisation affects parallel adaptation (Santangelo et al., 2022). As a brief background, scientists from around the world sampled 20–50 populations from each city along urban–rural transects. In total, 110,019 plants from 6169 populations were collected in 160 cities from 27 countries. Among these, a subset of 24 cities, chosen to capture variation in geography and climate, were subject to whole genome sequencing. For each of these selected cities, ~42 individuals from the five subpopulations closest to the city centre and an equivalent number of individuals from the five furthest rural subpopulations were selected for subsequent genomic analyses (mean $\pm$ SD = 83.8 $\pm$ 14.6 individuals/city, see Table S1).

While the initial analyses focused on adaptive parallel evolution of a single trait (i.e. ability to produce hydrogen cyanide) and the loci controlling it (*CYP79D15* and *Li*), here we specifically examined how urbanisation affects non-adaptive genomic evolution. We used an improved and larger dataset including 19 cities from the initial GLUE project for which good quality genomic data were available (removing cities where more than 50% of the sampled individuals had a coverage $<0.5\times$) and five newly sequenced cities (Palmerston North, New Zealand; Punta Arenas, Chile; Sapporo, Japan; Vancouver, Canada and Warsaw, Poland), for a worldwide sample across all inhabited continents (Figure 1). The final whole genome sequence dataset included six European, three Asian, one African, seven North American, four South American and three Oceanian cities, for a total of 1013 individuals.

2.2 | Molecular biology and genome sequencing

To obtain our genomic dataset, we used the sequencing protocol from GLUE (Santangelo et al., 2022) when sequencing the five cities newly added to the original genomic dataset. Briefly, we extracted genomic DNA from freeze-dried samples using a modified phenol–chloroform extraction protocol (detailed in Santangelo et al., 2022), and quantified DNA after extraction using the dsDNA HS Assay Kit (Fisher Scientific, Mississauga, Canada). We generated dual-indexed genomic DNA libraries following Santangelo et al. (2022) and Glenn et al. (2019), and sequenced genomic libraries with a concentration of DNA ≥ 0.8 ng/ μ L. The genomes of 1922



FIGURE 1 Cities sampled for rural and urban populations of white clover *Trifolium repens*. Each circle represents a city in which we sequenced the genomes of 80 plants on average from urban and rural habitats. The colour scale represents nucleotide diversity, measured as π .

individuals from 23 cities (all except Toronto) were sequenced on a Novaseq 6000 S4 platform using 150bp paired-end reads at low coverage (1.05× on average). Ninety plants from Toronto were sequenced at ~13× as a part of another project, and downsampled using SAMtools (v1.10) to ~2.5× to be included in this study. Seven cities from Santangelo et al. (2022) were removed from the dataset because of low sequence quality and/or low sample size (Bogota and Medellin, Colombia; Canberra and Melbourne, Australia; Hiroshima and Kyoto, Japan; and Paris, France), leaving us with data from 24 cities (Figure 1).

2.3 | Sequence alignment and genotype likelihoods

Beginning with fastq sequence data, we first used *fastp* V0.20.1 to trim raw reads with the *-trim_poly_g* argument to remove polyG tails commonly generated by the Novaseq platform and performed quality checks on both raw and trimmed reads of every sample using *FastQC* v0.11.9. We then mapped trimmed reads to the *T. repens* reference genome (NCBI BioProject number PRJNA523044, Griffiths et al., 2019) with *BWA MEM* v0.7.17. Although white clover is an allotetraploid, it exhibits disomic inheritance, all loci are functionally diploid. For this reason, reads could be mapped independently to each of the two ancestral genomes. We used *SAMtools* to sort, index and mark duplicates in BAM files. We performed a quality check of mapped reads with *Qualimap* v2.2.2, *Bamtools* v2.5.1, *BamUtil* v1.0.14 and *multiQC* v1.11. Since calling individual genotypes from low coverage data can lead to bias in variant detection (Han et al., 2014; Nielsen et al., 2012), we computed genotype likelihoods with *ANGSD* v0.933 (Korneliussen et al., 2014) on all four-fold degenerate sites (see below) using the *SAMtools* genotype likelihood model. Previous evidence demonstrated that allele frequencies and population genetic estimates can be robustly estimated with sample sizes and sequence coverage levels similar to those implemented in our study (Lou et al., 2021). As demographic inference can be biased by sites under selection, we focused our analyses on four-fold degenerate sites that are largely considered to evolve neutrally. We filtered for a minimum phred-scaled base quality score of 20 and minimum mapping quality of 30, keeping both variant and invariant sites present in a minimum of 50% of sequenced individuals (405,118 total sites passed the quality filtering criteria and were common to all populations). While the framework of our bioinformatic pipeline is built upon the earlier Santangelo et al. (2022) pipeline (https://github.com/James-S-Santangelo/glue_pc), all analyses and results are new, using a larger and improved dataset to specifically focus on the evolutionary signatures of population demographic change in response to urbanisation. A diagram summarising all the bioinformatic steps is available in Figure S1 and all analyses were integrated into a reproducible *Snakemake* (Mölder et al., 2021) pipeline (https://github.com/AudeCaizergues/glue_demography/).

The site frequency spectra (SFS) was the basis for most of the analyses performed in *ANGSD* and other software. We estimated

the SFS at four-fold degenerate sites in the *T. repens* reference genome using the 'degeneracy' pipeline (github.com/tvcent/Degeneracy). We retained an average of 2,110,100 four-fold degenerate sites that were present in at least 60% of individuals per population per city per habitat after filtering (see below). Since related individuals can bias population genomic analyses, and clover has the ability to grow clonally, we identified closely related individuals using *NgsRelate* (Hanghøj et al., 2019) with default parameters, and removed individuals with a pairwise relatedness $r_{xy} > .5$, where $r_{xy} = .5$ corresponds to a parent-offspring or full-sibling relationship; a total of 28 individuals were removed from subsequent analyses. After removing related individuals, we estimated the folded SFS for each urban and rural population. We also estimated the folded two-dimensional (2D) SFS for each urban-rural pair per city using the *realSFS* *ANGSD* function.

2.4 | Data analyses

2.4.1 | Genetic diversity estimates and demographic inference

To investigate the effects of urbanisation on neutral evolutionary processes within populations, our first step was to estimate genetic diversity, effective population size, gene flow and levels of relatedness within each habitat (urban and rural) of a city. To characterise population genomic parameters of diversity, we estimated nucleotide diversity based on π (pairwise nucleotide diversity), Watterson's theta (θ_w) (Nei, 1975; Tajima, 1983) and Tajima's D (Tajima, 1989), from the SFS using the *thetaStat* function of *ANGSD*. These three estimators were estimated both at the population level (all sub-populations of a habitat pooled together) and at the sub-population level. Inbreeding levels within subpopulations were estimated as mean r_{xy} (i.e. the pairwise relatedness, Hedrick & Lacy, 2015), computed with *NgsRelate* using default parameters. To understand whether these diversity parameters differed between environments we included them in linear mixed models with habitat (urban vs. non-urban) as an explanatory variable and city as a random effect. We then estimated the effective population size (N_e) in each habitat. Since urban populations are unlikely to be at mutation-drift equilibrium, statistics like π and θ_w will not capture recent changes in N_e . We therefore used a model-based approach to estimate the dynamics of contemporary N_e . In each city, we reconstructed variation of N_e through time using *EPOS* (Lynch et al., 2020), with 1000 bootstrap iterations and a mutation rate of 1.8×10^{-8} (Griffiths et al., 2019). Raw outputs of *EPOS* were converted to a plottable format using the *epos2plot* function. These analyses gave us a detailed understanding of how urbanisation affects genetic diversity and effective population size within populations.

In addition, to explore the potential role of city age on clover's evolution, we investigated if population genetic estimates (π , θ_w and Tajima's D) and N_e were correlated with the age of each

city. Following Santangelo, Rivkin, et al. (2020), we defined a city's age as the number of years before 2020 that each city reached a population of 150,000. We log-transformed city age to normalise the data and performed Pearson correlation tests with each of the predictors.

2.4.2 | Genetic structure

We next investigated how urbanisation influenced population genetic structure and gene flow of *T. repens* within and between urban and rural habitats. First, since linkage disequilibrium can bias genetic structure analyses, we identified linked positions among the four-fold degenerate SNPs with a minor allele frequency $\geq .05$ using *ngsLD* (Fox et al., 2019), and pruned the linked SNPs within 20kb using a r^2 cut-off of 0.2, resulting in a dataset of 41,543 sites. The pruned dataset was used for subsequent principal component analysis (PCA) and admixture analyses only. To describe the genetic structure both between habitats and cities, we performed a PCA with *PCAngsd* (Meisner & Albrechtsen, 2018) that estimates a variance-covariance matrix of allele frequencies directly from genotype likelihoods. We then estimated population differentiation using Hudson's F_{ST} separately for each city (Hudson et al., 1992). We computed F_{ST} with the *realSFS fst index* function from the two-dimensional SFS. First, to understand genetic structure between habitats, we computed F_{ST} between each urban-rural pair of populations, and similarly with diversity estimators, investigated if it was correlated with city age. Second, to further analyse patterns of differentiation within habitats, we computed F_{ST} between all pairs of subpopulations per city, and obtained an average F_{ST} for urban-urban, urban-rural or rural-rural comparisons separately. As a complementary analysis of urban-rural differentiation, we estimated admixture proportions for each pair of urban-rural populations with *NGSadmix* (Skotte et al., 2013). We ran the analysis for cluster numbers (K) from 2 to 10, with 10 iterations per K and selected the best K for each city using the method of Evanno et al. (2005) implemented in *CLUMPAK* (Kopelman et al., 2015). Finally, we used *GADMA* (with *dadi* engine) to estimate migration rates between urban and rural populations (Gutenkunst et al., 2010; Noskova et al., 2020). *GADMA* implements methods for automatic inference of the joint demographic history of multiple populations. It therefore eliminates the need to test for multiple demographic models in parallel as its algorithm automatically models a multitude of scenarios and parameter values and selects the best supported ones. We modelled each city separately, and set the initial structure to [1,1] (indicating one epoch before the population split), final structure to [2,1] (allowing two epochs after the split to model any change in population size), mutation rate to 1.8×10^{-8} , generation time to 1 year, sequence length to the size of the SFS (number of sites) per city, and other parameters were set to their default mode. To have a more comprehensive understanding of gene flow we also estimated migration rate (m) using Wright's equation $N_e m = (1/F_{ST} - 1)/4$ (Wright, 1984).

3 | RESULTS

3.1 | Does urbanisation repeatedly affect genetic diversity, relatedness within populations and effective population size?

Demographic processes frequently differed among cities, yet genetic diversity and effective population size were, on average, similar between urban and rural populations. While some cities displayed higher nucleotide diversity than others within cities (e.g. see Linköping, Sweden, Figure 2a), both urban and rural clover populations had high nucleotide diversity (mean $\pm$ SD $\pi = 0.025 \pm 0.004$, Figure 2a; mean $\pm$ SD $\theta_w = 0.028 \pm 0.006$, Figure S2), which did not differ between urban and rural habitats (habitat effect for π : $F_{1,23} = 0.505$, $p = .485$; habitat effect for θ_w : $F_{1,23} = 1.303$, $p = .265$). Tajima's D varied among cities from -0.682 to 0.318 (Figure 2b), and was negative on average, which is consistent with recent population expansion. Tajima's D also varied within cities, but there was no consistent difference between urban and rural habitats ($F_{1,23} = 2.935$, $p = .100$). Such high levels of genetic diversity are consistent with large effective population sizes. None of the π , θ_w or Tajima's D per sub-population (within habitat) estimates revealed any clear small-scale pattern (Figures S3–S5).

Overall, N_e was high but varied substantially between cities and was more likely to decline in rural than urban habitats. N_e varied among cities by four orders of magnitude, ranging from 1750 to 40,800,000 (Table S2; Figure S6). Comparing urban and rural habitats, N_e was twice as likely to be higher in the urban habitat; there were seven occurrences of $N_{e \text{ urban}} > N_{e \text{ rural}}$ (e.g. Munich, Germany, Figure 3a), three occurrences of $N_{e \text{ urban}} < N_{e \text{ rural}}$ (e.g. Toronto, Figure 3b) and 14 occurrences of $N_{e \text{ urban}} = N_{e \text{ rural}}$ (e.g. Kunming, Figure 3c). When N_e was compared between habitats with parametric (LMER) and non-parametric (Wilcoxon rank test) analyses, we found no consistent effect of urban/rural habitat on N_e (LMER: $\chi^2_1 = 1.181$, $p = .277$; Wilcoxon rank test: $p = .107$). We found low levels of relatedness between individuals, and relatedness did not differ between urban and rural habitats (Figure 2c, Wilcoxon rank test: $p = .303$), suggesting urbanisation did not affect the propensity of inbreeding. Using the whole genome dataset, we modelled recent changes in N_e through time in both habitats. Although N_e did not consistently differ between habitats, urban populations were less likely to show a recent decrease in N_e in the last 500 years compared to rural populations. Specifically, N_e decreased in four urban populations, whereas it decreased in 11 rural habitats, and the remaining populations were stable between habitats (Figure S6; $\chi^2 = 4.751$, $p = .029$).

Finally, we found no link between the age of a city and any of the diversity estimators or N_e (π : $r = .040$, $p = .874$; θ_w : $r = .051$, $p = .840$; Tajima's D : $r = -.103$, $p = .690$; N_e : $r = .022$, $p = .932$).

Taken together, our results suggest that while clover shows overall high N_e that maintains substantial genetic diversity within populations, urban habitats are more likely to maintain large and stable populations than rural areas.

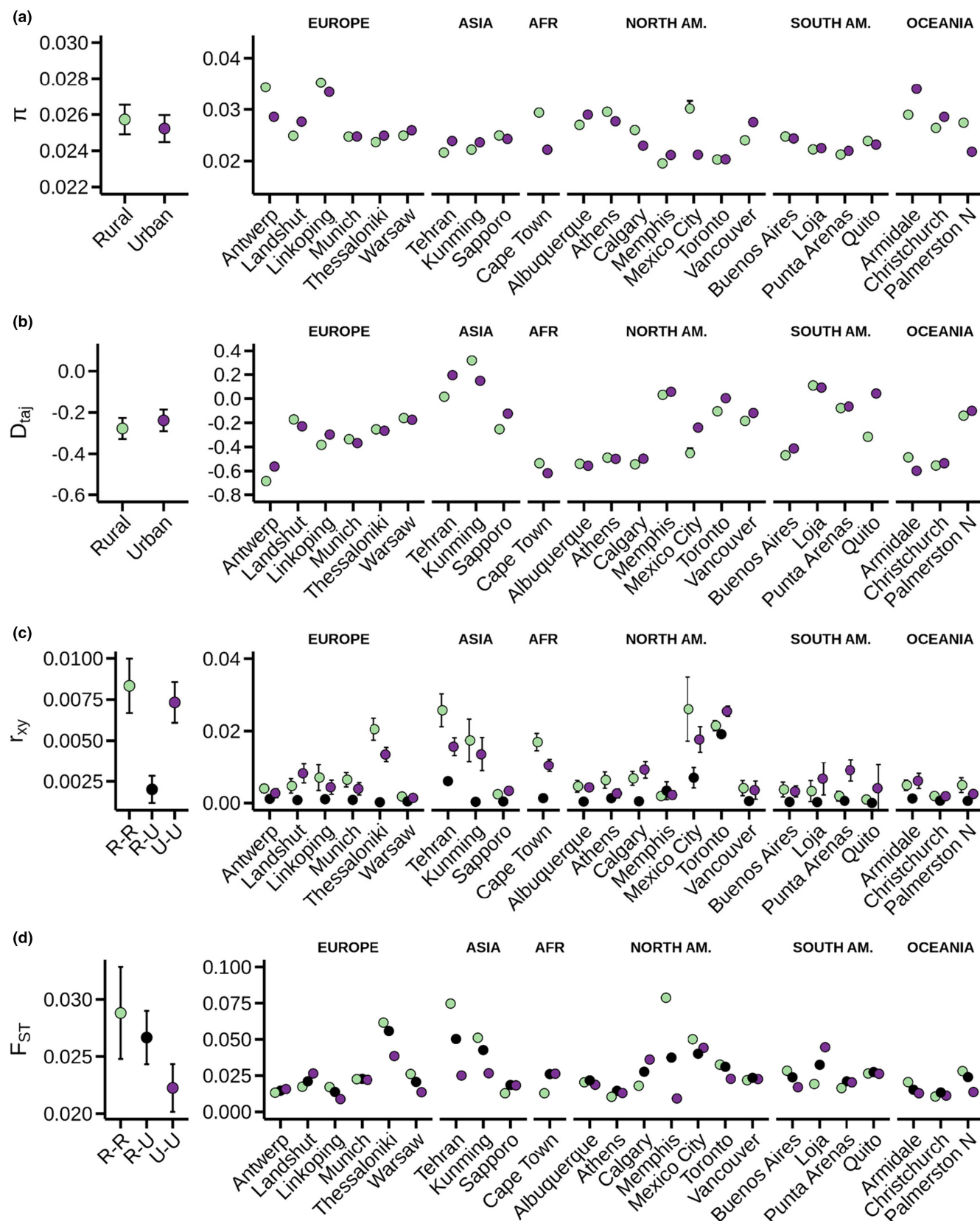


FIGURE 2 Summary of population genetic parameters averaged over all cities (left, mean $\pm$ 95% CI) and detailed per city (right, mean $\pm$ 95% CI) for: (a) pairwise nucleotide diversity, (b) Tajima's D, (c) averaged relatedness between individuals and (d) F_{ST} averaged between pairs of sub populations. Left graphs represent averaged values per habitat for F_{ST} and r_{xy} and genome wide averages for π and Tajima's D. In (a, b), green represents rural habitats and purple represents urban habitats. In (c, d), green, black and purple respectively represent rural-rural, rural-urban and urban-urban for F_{ST} and r_{xy} .

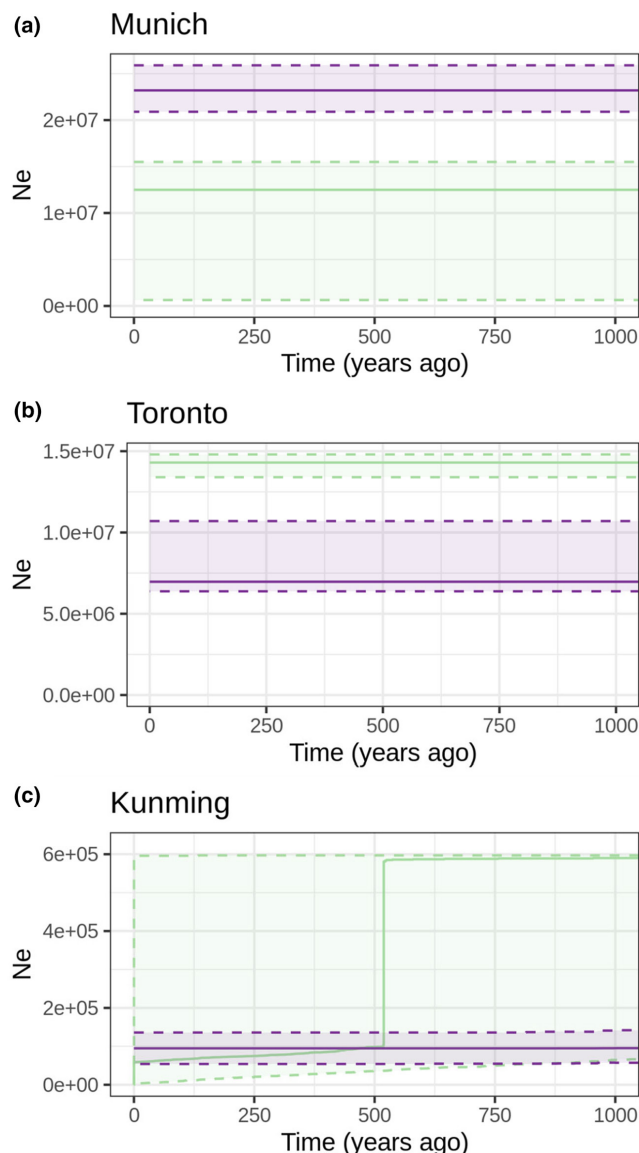


FIGURE 3 Effective population size (N_e) in the past 1000 years for three representative pairs of urban (purple lines) and rural (green lines) populations in Munich (Germany), Toronto (Canada) and Kunming (China), showing the diversity of patterns of recent N_e variations across the dataset, where we find no consistent effect of urbanisation on N_e . Solid lines represent the median and dashed line the 5% and 95% quantiles.

3.2 | Does urbanisation influence gene flow, differentiation and genetic structure?

Analysis of population structure shows that while cities can be genetically differentiated from one another on a global scale, urban and rural populations show limited structure within a given city. At a global scale, PCA analyses revealed that many cities cluster close to each other if they are geographically closer to one another. For instance, North and South American cities were typically more genetically similar to one another than they were to European cities (Figure 4a,b). By contrast, several cities were genetically distinct, such as Cape Town, South Africa, the only African city sampled, and

Tehran, Iran, and Thessaloniki, Greece, which are geographically isolated from other sampled points (Figure 4a,b). Within a city, PCA revealed no strong pattern of differentiation between urban and rural populations (Figure S7).

When population differentiation was quantified with F_{ST} , we observed that overall between-population F_{ST} was low (Figure 2d) and not correlated with city age ($r = .11$, $p = .678$), but there was evidence of fine-scale differences when moving from between- to within-habitat comparisons. Specifically, while the average F_{ST} between urban and rural populations was similar to F_{ST} within rural habitats (mean $\pm$ SD $F_{ST \text{ urb-rur}} = 0.029 \pm 0.028$, $F_{ST \text{ rur-rur}} = 0.026 \pm 0.016$, Wilcoxon rank test: $p = .931$), F_{ST} within urban habitats was 19% lower than F_{ST} within rural habitat and 27% lower than F_{ST} between habitats (mean $\pm$ SD $F_{ST \text{ urb-urb}} = 0.021 \pm 0.013$; Wilcoxon rank test $F_{ST \text{ urb-urb}} - F_{ST \text{ rur-rur}}$: $p = .031$, Wilcoxon rank test $F_{ST \text{ urb-urb}} - F_{ST \text{ urb-rur}}$: $p < .001$). These differences in population differentiation between habitats are consistent with more extensive gene flow among sites within urban habitats compared to the gene flow among sites within rural habitats, or between urban and rural habitats.

Estimates of historical and recent demographic processes reveal extensive gene flow between urban and rural populations. Admixture analyses identified admixture between urban and rural populations, with two to six populations (best supported 'K') found within cities that were broadly shared between urban and rural individuals. While we detected small scale structure between populations, the proportion of each assumed ancestral cluster did not differ between urban and rural habitats (Figure 5; per city G-test on average admixture proportions per habitat, all $p > .05$). More than half of the demographic models estimated with GADMA showed bi- or unidirectional gene flow between habitats (13 of 24). We caution that in numerous cities many demographic models explained similar amounts of variation and these models often included substantial gene flow. There was no difference in urban-to-rural and rural-to-urban migration rates based on estimates from the best GADMA models (Figure S8). Similarly, estimates of $N_e m$ (Wright, 1931), showed no clear variation between habitats (Figure S9), however for low levels of F_{ST} such as observed here, $N_e m$ can vary widely (see Figure 2 of Whitlock & McCauley, 1999), so these results should be interpreted with caution. In any case, when combining these migration rates results with PCA, F_{ST} and admixture results, we find a clear pattern of high ongoing gene flow between habitats.

4 | DISCUSSION

We tested whether global urbanisation leads to parallel non-adaptive evolutionary processes and patterns in the cosmopolitan plant white clover (*T. repens*). Specifically, we sought to understand whether urban and rural habitats consistently differed in genetic diversity, inbreeding, effective population size, genetic structure and gene flow. Our genome wide analyses consisting of 1922 individuals from 24 locations around the world revealed high levels of diversity in both urban and rural populations of white clover. Almost all urban

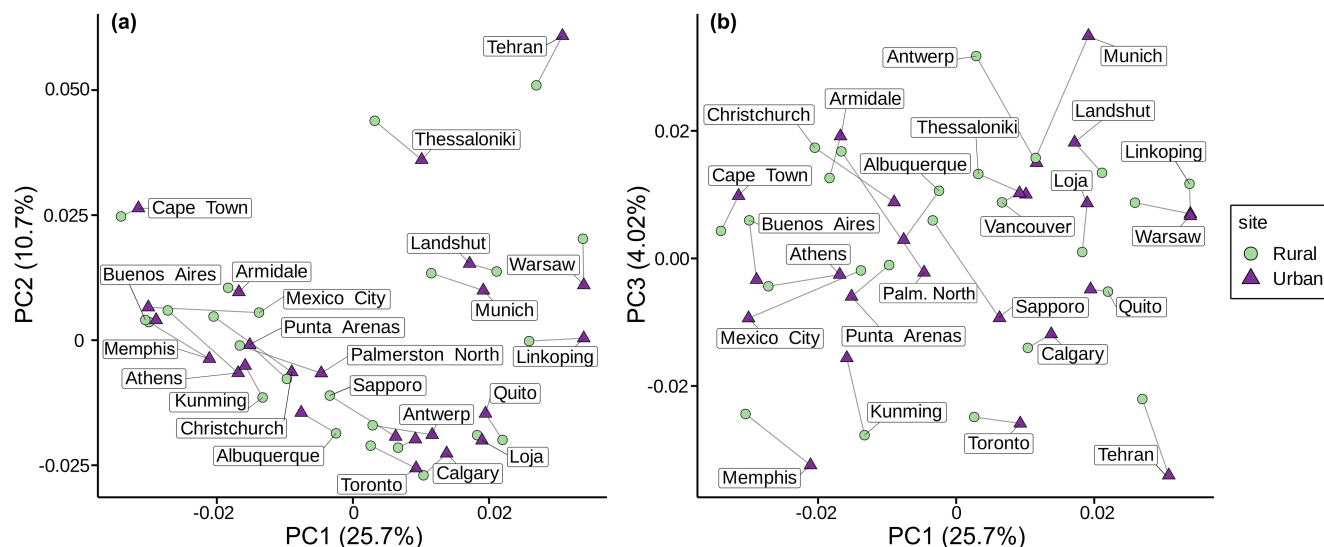


FIGURE 4 Genetic structure among *T. repens* populations revealed by a principal component analysis (PCA) on genotype likelihoods of 41,543 four-fold positions, (a) on PC1 and PC2, and (b) on PC1 and PC3. Each point represents the centroid of rural (green round) and urban (purple triangle) populations for each city sampled.

populations had high stable N_e , which was not the case for some rural populations, which disproportionately showed decreasing effective population sizes. We also detected low genetic structure among urban and rural populations and high admixture, consistent with frequent gene flow both within and among habitats. Gene flow appears to be more prevalent among urban than rural populations, leading to greater genetic homogenisation in urban habitats. Our results suggest that white clover demography aligns with the urban facilitation model more closely than the urban fragmentation model, in that white clover thrives in urban environments and maintains high evolutionary potential of populations.

4.1 | Effects of urbanisation on non-adaptive evolutionary processes within populations

Urbanisation frequently leads to habitat fragmentation, which often causes a decrease in effective population size and genetic diversity within populations (i.e. the Urban Fragmentation Model). This prediction has been supported by a broad diversity of studies. For example, fragmented forest habitats lead to a decrease in genetic diversity in red-backed salamanders (*Plethodon cinereus*) in the city of Montreal (Noël et al., 2007). Similarly, white footed mice (*Peromyscus leucopus*) showed reduced diversity along an urbanisation gradient in New York (Munshi-South et al., 2016). Our results do not match this expected pattern of the urban fragmentation model.

Consistent with the urban facilitation model, we found that urban white clover was able to maintain high levels of genetic diversity and high effective population size throughout the sampling range of our study. Our results of high genetic diversity in urban and rural populations are consistent with previous smaller scale studies of white clover that used microsatellite markers, which showed that genetic diversity did not vary along urbanisation gradients in

multiple cities in Ontario, Canada (Johnson et al., 2018). These findings may also help explain how white clover has been able to rapidly adapt to repeated urbanisation gradients throughout the world (Santangelo et al., 2022). Adaptive evolution is expected to be more efficient when N_e is high and selection is strong. We confirmed large N_e in most cities, which is consistent with the observation that white clover is frequently abundant and widespread throughout cities. A novel result of the current study is that urban habitats are more likely to maintain high and stable population sizes than rural areas. Given that urban habitats frequently undergo rapid environmental change, we may expect urban populations to experience stronger selection than rural habitats.

There is mounting evidence that the urban facilitation model, supported by our study, may apply to many species. For instance, brown rats display high genetic diversity in urban areas that can be attributed to large population sizes (Combs et al., 2018). Similarly, urban great tits (*Parus major*) showed higher genetic diversity in cities than in the associated forests (Björklund et al., 2009). In a broad meta-analysis, Schmidt et al. (2020) suggested that human commensal species could be more likely to display higher diversity in urban compared to rural areas, especially in birds that are not affected by habitat fragmentation (but not necessarily true for mammals). White clover has many traits (e.g. clonal growth, prostrate growth habit, ability to regrow following cutting, polyploidy) that make it well-adapted to human-dominated landscapes like lawns and well-grazed pastures (Burdon, 1983; Van Drunen & Johnson, 2022). In particular, clover is a low-growing plant making it a poor competitor for sunlight, but it has the ability to quickly regrow following damage, and might thus benefit from mowing. Urban areas display a multitude of well-mowed green spaces (parks, lawns, private backyards) where clover can grow. In addition, clover is sensitive to drought and benefits from irrigation provided to many urban spaces. By contrast, rural areas are frequently unmowed and unwatered, which can lead to



FIGURE 5 Admixture proportions between urban and rural habitats for each city estimated with *NGSadmix*. The best supported K value for each city was selected with the method of Evanno et al. (2005). Each vertical bar represents an individual and the height of the colour indicates the proportion of an individual's genome derived from each of the K ancestral populations within a given city. Individuals are grouped by sub-population.

clover being outcompeted and experience low fitness. Finally, urban white clover's ability to maintain high population sizes compared to rural ones might also benefit from frequent introductions of clover to cities (via seeds in sod and turfgrass) from diverse seed stocks,

leading to elevated diversity and low genetic structure. Hence, both the life-history traits of clover and its close link with intense human activities make it a species well adapted to performing best in anthropogenic habitats.

4.2 | Effects of urbanisation on genetic structure and gene flow between populations

Consistent with the urban facilitation model, we found that white clover displays high gene flow between urban and rural areas, as suggested by low levels of differentiation, limited genetic structure, high admixture proportions and high estimates of dispersal. High levels of gene flow between populations are consistent with high genetic diversity and large population sizes, as connectivity between populations enhances allele movement and reduces genetic drift. A major element of white clover history is that it was often intentionally planted by humans as a fodder crop, to enrich soil nitrogen in rotation farming, unintentionally introduced via sod or turfgrass, or intentionally planted as clover lawns (Kjærgaard, 2003). Thus, white clover was potentially introduced multiple times in each area and moved among locations by people. Such repeated introductions have likely played a major role in the low genetic structure observed between habitats and evidence of substantial admixture.

While urban and rural clover display similar low levels of relatedness, we found the lowest differentiation among urban populations (i.e. lower within urban F_{ST} than within rural F_{ST}), suggesting the urban landscape facilitates gene flow. As an obligate outcrosser, white clover depends on pollinators for its reproduction. Cities can harbour more pollinator diversity than rural areas (Wenzel et al., 2020), and since movement of pollinators is not necessarily constrained by the urban landscape (Theodorou et al., 2018), higher gene flow within cities might be driven by pollinators. For instance, in Toronto, Canada, previous research showed that pollinator visitation rate to white clover was higher in urban sites than in rural sites (Santangelo, Rivkin, et al., 2020). Additionally, clover is often moved around by humans in urban areas, for instance via turfgrass, which can in turn increase gene flow and reduce genetic differentiation between urban subpopulations. This novel result of high gene flow within urban versus within rural habitats, also suggests that any adaptations that do arise (e.g. HCN production, Santangelo et al., 2022) are likely to spread more quickly among urban than rural populations.

Recent literature suggests that several species may exhibit extensive gene flow in urban areas. For instance, highly mobile species such as the feral pigeons (*Columba livia*) can display high gene flow in urban areas (Carlen & Munshi-South, 2021). In small mammals, Combs et al. (2018) found high genetic diversity in brown rats (*Rattus norvegicus*) in four cities, likely due to high gene flow and large population sizes. However, high gene flow in urban habitats is not limited to highly mobile species. In fact, human commensals that are not necessarily good dispersers, like the western black widows (*Latrodectus hesperus*), can directly benefit from human movement and transportation networks for their dispersal (Miles et al., 2018). As a result, species that are intentionally or unintentionally moved by humans are likely to display higher levels of gene flow. Our results on white clover contribute to the growing evidence that urbanisation can facilitate gene flow and reveal that such processes can be repeated across cities worldwide.

4.3 | Future directions for the study of parallel genomic evolution in cities

Cities offer a great opportunity to study how anthropogenic disturbances affect evolutionary processes at a global scale. In line with our study, there has been a recent focus studying whether similar environmental changes caused by urbanisation lead to independent, parallel evolutionary trajectories. While evidence of repeated phenotypic shifts in urban areas is growing, they do not necessarily arise from parallel independent adaptive evolution. For example, a common garden experiment using the Virginia pepperweed (*Lepidium virginicum*) showed genetically based convergence in the urban phenotype of earlier bolting, larger size, producing fewer leaves and more seeds. However, population genetic analyses revealed that urban populations were likely derived from the same inbred haplotype, demonstrating that such parallelism in urban phenotype was facilitated by extensive gene flow among cities combined with lineage sorting within habitats, as opposed to multiple independent evolutionary events (Yakub & Tiffin, 2017). Similarly, in the Gulf killifish (*Fundulus grandis*), adaptive resistance to industrial pollutants was found in four populations, which was the result of de novo mutation in only one of the populations, whereas the other three populations likely evolved from standing genetic variation or introgression (Oziolor et al., 2019). In both cases, the adaptive parallelism was facilitated by demographic events, highlighting the need for combined analyses that explore both adaptive and non-adaptive evolution to investigate how urbanisation shapes evolutionary trajectories. The parallelism of non-adaptive processes has been particularly ignored in studies, even though theory suggests that genetic drift and gene flow can lead to parallel evolutionary patterns (e.g. Santangelo et al., 2018). Our results suggest that contrary to the prevailing hypothesis, cities can facilitate the demographic spread, growth and adaptation of species, especially for those with a cosmopolitan distribution.

AUTHOR CONTRIBUTIONS

AEC, JSS, RWN and MTJJ conceptualised the study. JSS and MTJJ developed lab protocols. RWN, JSS developed analytical methods. AEC carried out the analysis. All authors collected samples and contributed to the writing of the manuscript.

AFFILIATIONS

¹Centre for Urban Environments, University of Toronto Mississauga, Mississauga, Ontario, Canada

²Department of Biology, University of Toronto Mississauga, Mississauga, Ontario, Canada

³Department of Integrative Biology, University of California Berkeley, Berkeley, California, USA

⁴Programa de Pós-Graduação em Gestão e Tecnologia Ambiental da Universidade Federal de Rondonópolis, Rondonópolis, Brasil

⁵Department of Plant Biology, Department of Entomology, Plant Resilience Institute, Michigan State University, East Lansing, Michigan, USA

⁶Genomic Sciences and Technology Program, University of British Columbia, Vancouver, British Columbia, Canada

⁷Department of Microbiology and Immunology, University of British Columbia, Vancouver, British Columbia, Canada

⁸Red de Ecoetología, Instituto de Ecología A.C., Xalapa, Mexico

⁹Living Earth Collaborative, Washington University in St. Louis, St. Louis, Missouri, USA

¹⁰Universidad San Francisco de Quito, Ecuador, Quito

¹¹San Francisco State University, San Francisco, California, USA

¹²Department of Biology, University of Massachusetts Boston, Boston, Massachusetts, USA

¹³Dendrolytics, Seattle, Washington, USA

¹⁴NTNU University Museum, Norwegian University of Science and Technology, Trondheim, Norway

¹⁵School of Biological Sciences, University of Reading, Reading, UK

¹⁶School of Biological Sciences, Monash University, Melbourne, Victoria, Australia

¹⁷Department of Biological Sciences, Wayne State University, Detroit, Michigan, USA

¹⁸Laboratorio de Ecología Tropical y Servicios Ecosistémicos (EcoSs-Lab), Universidad Técnica Particular de Loja, Loja, Ecuador

¹⁹University of Louisiana, Lafayette, Louisiana, USA

²⁰Biodiversity Research Institute (IMIB), CSIC-University of Oviedo-Principality of Asturias, Mieres, Spain

²¹Evolution & Ecology Research Centre, UNSW-University of New South Wales, Sydney, New South Wales, Australia

²²Department of Biology and Louis Calder Center, Fordham University, New York City, New York, USA

²³Botanischer Garten und Botanisches Museum Berlin, Freie Universität Berlin, Berlin, Germany

²⁴Institut de biologie intégrative et des systèmes, Université Laval, Quebec City, Quebec, Canada

²⁵Department of Ecology, Evolution and Behavior, University of Minnesota, St. Paul, Minnesota, USA

²⁶Science, Technology & Society Department, Rochester Institute of Technology, Rochester, New York, USA

²⁷Department of Ecology, Environment and Plant Sciences, Stockholm University, Stockholm, Sweden

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DATA AVAILABILITY STATEMENT

All code is available on https://github.com/AudeCaizergues/glue_demography. BAM files are available on the European Nucleotide Archive (ENA BioProjects PRJEB48967 & PRJEB72257).

ORCID

Aude E. Caizergues  <https://orcid.org/0000-0003-4467-3912>

James S. Santangelo  <https://orcid.org/0000-0002-5921-2548>

Fernanda Baena-Díaz  <https://orcid.org/0000-0002-0660-0627>

Carlos Iñiguez-Armijos  <https://orcid.org/0000-0002-9787-3451>

Jason Munshi-South  <https://orcid.org/0000-0002-8067-4341>

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SUPPORTING INFORMATION

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