Continent-wide genomic signatures of adaptation to urbanisation in a songbird across Europe

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Urbanisation is increasing worldwide, and there is now ample evidence of phenotypic changes in wild organisms in response to this novel environment. Yet, the genetic changes and genomic architecture underlying these adaptations are poorly understood. Here, we genotype 192 great tits (Parus major) from nine European cities, each paired with an adjacent rural site, to address this major knowledge gap in our understanding of wildlife urban adaptation. We find that a combination of polygenic allele frequency shifts and recurrent selective sweeps are associated with the adaptation of great tits to urban environments. While haplotypes under selection are rarely shared across urban populations, selective sweeps occur within the same genes, mostly linked to neural function and development. Collectively, we show that urban adaptation in a widespread songbird occurs through unique and shared selective sweeps in a core-set of behaviour-linked genes.
Urban development is rapidly expanding across the globe, and although urbanisation is regarded a major threat for wildlife\(^1\), its potential role as an evolutionary driver of adaptation has not been explored until recently\(^2\)–\(^5\). Many species show phenotypic adaptations to the multiple urban challenges, such as higher levels of noise, artificial light at night, air pollution, altered food sources or habitat fragmentation\(^6\). These adaptations include changes in behaviour, e.g., refs. \(^7\)–\(^9\), morphology and locomotion, e.g., refs. \(^10\)–\(^13\) or toxin tolerance\(^1\)\(^4\). Indeed, there is now evidence that the phenotypic divergence between urban and rural populations may have a genetic basis in some species\(^4\)\(^,\)\(^15\)\(^,\)\(^16\), in line with the finding that micro-evolutionary adaptations in natural populations can occur within short timescales, particularly in response to human activities\(^16\)–\(^18\). The study of the genetic signals of adaptation could provide important insights into the magnitude of the evolutionary change induced by urbanisation on wildlife. However, the short evolutionary timescale, the dependence of evolution on local factors, and the polygenic nature of many phenotypic traits, make detecting the genomic signals of adaptation difficult\(^17\)\(^,\)\(^19\).

The majority of studies on the genomic basis of urban adaptation have either focused on a limited number of markers and genes\(^2\)\(^0\) or on a narrow geographical scale, e.g., refs. \(^4\)\(^,\)\(^1\)\(^5\)\(^,\)\(^2\)\(^1\)\(^2\)\(^2\), thereby limiting the inferences that can be made on the consistency of genomic responses to urbanisation\(^5\). Conversely, the study of multiple populations on a broad geographical scale provides a powerful framework to identify the evolutionary forces shaping the genomic responses and to test the repeatability of urban adaptation\(^1\)\(^5\)\(^,\)\(^2\)\(^3\)\(^,\)\(^2\)\(^4\). In particular, comparing across populations enables the distinction between random demographic non-adaptive processes, such as genetic drift, and signatures of natural selection, as selection might be expected to affect the same genomic regions (nucleotides or genes) and/or functional pathways across cities\(^2\)\(^3\)\(^,\)\(^2\)\(^5\). The current evidence on the genomic basis of urban adaptations ranges from high parallelism, involving a single or few genes\(^1\)\(^5\)\(^,\)\(^2\)\(^3\), to polygenic, and to putative genetic redundancy, e.g., ref. \(^2\)\(^2\). This discrepancy might be due to the variable and sometimes local urban selection pressures together with the polygenic nature of many adaptive phenotypic traits, which could lessen the likelihood for shared genetic changes at the nucleotide level\(^2\)\(^6\).

In this study, we present a multi-location analysis of the evolutionary response to urbanisation, using the great tit (Parus major), to identify the genetic basis of urban adaptation. The great tit is a widely distributed songbird and a model species in urban, evolutionary and ecological research, e.g., refs. \(^2\)\(^7\)–\(^3\)\(^6\), with demonstrated phenotypic changes in response to urban environments in several populations\(^3\)\(^9\)\(^,\)\(^3\)\(^4\)\(^,\)\(^3\)\(^5\)\(^,\)\(^3\)\(^7\). In addition, genomic resources are well developed for this species\(^3\)\(^8\) and it is known that across its European range, the species presents low genetic differentiation\(^2\)\(^1\)\(^9\)–\(^2\)\(^1\)\(^1\). In order to examine genomic responses to urbanisation on a broad geographical scale, we analyse pairs of urban and rural great tit populations from nine localities across Europe (Fig. 1 and Supplementary Table 1). We combine several complementary approaches to: (1) detect allele frequency shifts across many loci, which will facilitate the exploration of the polygenic aspect in the adaptation to urbanisation; (2) search for selective sweeps in urban populations, which will provide information on population-specific signatures of selection; and (3) identify enriched functional pathways associated with genes putatively under selection in urban populations, which will help us to infer which particular phenotypes, known and unknown, underlie urban adaptation in great tits. Overall, the present study deepens our understanding of the evolutionary drivers and forces shaping the genomic landscape of urban adaptation on a continental scale.

Results and discussion

Genetic diversity and population structure across European urban and rural populations. A total of 192 great tits from the nine paired urban–rural populations were genotyped at 517,603 filtered SNPs, with 10–16 individuals per sampling site (Supplementary Table 1). We quantified the relative degree of urbanisation for each site (urbanisation score: PC\(_{\text{urb}}\) from principal component analysis, PCA; see “Methods”, Fig. 1b, Supplementary Fig. 1 and Supplementary Table 1) to inform our genetic downstream analyses. Population structuring based on 314,351 LD (linkage-disequilibrium)-pruned SNPs (excluding small linkage groups and the Z-chromosome) was overall low across the 18 studied sites (Supplementary Fig. 2), with each of the first two principal components explaining <3% of the overall variation across populations (Fig. 2a and Supplementary Fig. 3a). Thus, we used a UMAP (Uniform Manifold Approximation and Projection) approach to summarise the genetic variation along the first 20 PC axes. The UMAP analysis revealed the presence of distinct genetic clusters for some of the localities, although there was still a strong clustering of individuals from Gothenburg, Munich, Milan and Paris (Fig. 2a and Supplementary Fig. 3b); results were comparable when including the Z-chromosome, Supplementary Fig. 3c). This analysis also suggested that the levels of divergence differ strongly between each urban and adjacent rural population (Fig. 2a and Supplementary Fig. 3b). Furthermore, the population pairs from Glasgow and Lisbon showed the highest levels of divergence, with Lisbon and Glasgow separating along PC1 and PC2, respectively (Supplementary Fig. 3a), and also separating strongly in the UMAP plot (Fig. 2a). This increased divergence of Glasgow and Lisbon, both located in the range edge of the great tit distribution, could be explained by their slightly reduced heterozygosity, particularly in the urban populations (Supplementary Table 1). Indeed, population tree analyses using TreeMix supported the presence of increased drift in both urban populations (Fig. 2b). However, overall, we did not find lower heterozygosity levels in any of the nine-urban compared to the rural populations, which suggests that urban colonisation was in general not associated with significant bottlenecks (see Supplementary Table 1 for details; Wilcoxon test: \(W = 30, P = 0.377\)).

Interestingly, the population structure analysis indicated that multiple urban populations, namely Glasgow, Lisbon, Madrid, Malmö, Milan, and to weaker extent Barcelona and Gothenburg, formed distinct genetic clusters and independent drift units together, in some cases, with their adjacent rural counterparts (Fig. 2a, b). Nonetheless, the TreeMix analysis also suggests the presence of migration events across some populations, including long distance migration events, e.g., from Lisbon to Glasgow (Supplementary Fig. 4b). Although, there was no clear pattern in relation to predominant gene flow between urban populations (Supplementary Fig. 4). In contrast to the mentioned population clusters, we did not detect any particular signs of genetic divergence between populations from Munich and Paris (neither PC or UMAP axis see, e.g., Fig. 2a and Supplementary Fig. 3). Likewise, we detected significant genetic differentiation between all pairwise comparisons (\(P < 0.05; F_{ST}\) permutation test; see “Methods”) except for the urban populations in Munich and Paris (Fig. 2e and Supplementary Table 2). Moreover, the genetic population differentiation (\(F_{ST}\) within population pairs (urban vs adjacent rural population), as well as between urban populations (urban vs urban), was on average higher than the differentiation between rural populations (rural vs rural) across Europe (Fig. 2c). These results support the idea that gene flow is generally stronger between rural compared to urban habitats in the nine population pairs, and indicates reduced gene flow between urban and adjacent rural populations. This is further supported by the observed isolation-by-distance patterns, which showed slightly
stronger IBD for urban (Mantel’s $r = 0.46, P = 0.008$) compared to rural populations ($r = 0.43, P = 0.007$; Fig. 2d). Lastly, analysis of the effective migration surfaces confirmed reductions in gene flow in many parts of Europe, compared to neutral expectations, including those in close proximity, e.g., Gothenburg and Malmö, and simultaneously highlighted strong gene flow in Central/Western Europe, i.e., Munich and Paris (Fig. 2e).

Overall, our results reveal weak population structuring across Europe, with slightly increased genetic differentiation between urban populations compared to the more admixed rural populations. Previous results in another European songbird, the blackbird ($Turdus merula$) showed that urban birds likely independently colonised multiple times European cities from forest sources. In our study, the finding of independent clusters for some urban populations also point towards a similar scenario, i.e., an independent and repeated colonisation of urban habitats from largely admixed rural populations. However, the overall weak genetic divergence and the detection of significant migration events across distant populations suggests a possible role of gene flow in the facilitation of urban adaptation, via the spread of adaptive alleles. Still, in our subsequent analyses we treated all our urban–rural population pairs as independent, including Munich and Paris, as selection pressures might differ across cities and result in idiosyncratic genomic responses to selection despite any ongoing gene flow.

Identification of urbanisation-associated allele frequency shifts. To identify genomic regions with consistent allele frequency shifts associated with urbanisation, we used two complementary genotype–environment association (GEA) approaches, LFMM (latent-factor mixed models) and an additional Bayesian approach (using BayPass). Testing for GEAs with LFMM (using $PC_{urb}$ as a continuous habitat descriptor, Fig. 1b) revealed 2758 SNPs associated with urbanisation (0.52% of the full SNP dataset, false-discovery rate (FDR) < 1%; Fig. 3a). Urbanisation-associated SNPs were widely distributed across the genome and did not cluster in specific regions. Larger chromosomes ($R^2 = 0.97$) and those with more genes ($R^2 = 0.90$; Fig. 3b) contained more urbanisation-associated SNPs, highlighting the polygenic nature of urban adaptation. A PCA ($PC_{GEA}$), based on all urban-associated SNPs detected by LFMM, clearly separated urban and rural populations along $PC_{GEA}$ (proportion of variance explained—PVE—by $PC1 = 1.98$%; Supplementary Fig. 5c), suggesting consistent allele frequencies in those loci across European cities.
**Fig. 2 Great tit population structure.**

a) UMAP reduction of the first 20 principal component axes for an LD-pruned SNP dataset, excluding the Z-chromosome and small linkage groups. The insert shows the percent of variance explained (PVE) by each of the first 50 PC axes. The corresponding principal component plots can be found in Supplementary Fig. 2.

b) Population tree generated with TreeMix showing the relationship of urban (open circles) and rural (closed circles) populations from all sampling locations.

c) Comparison of $F_{ST}$ value distributions (mean ± SD) across population and habitats. The effect size plots (mean ± 95% bootstrap CI) show that overall rural populations (rural vs rural) display lower differentiation than the studied population pairs (urban vs rural), but not lower than urban populations (urban vs urban).

d) Isolation-by-distance analyses (Mantel’s test) for urban (open circles, light shaded, $n = 72$) and rural (closed circles, dark shaded, $n = 72$) populations separately. Shaded area denotes the 95% CI.

e) Effective migration surfaces for great tits across Europe. Negative log migration rates [log(m)] depict areas with less gene flow than expected under an isolation-by-distance model, whereas positive migration rates indicate stronger gene flow. Note that migration rates outside the central part of the sampling distribution are generally low, also between closely related cities. BCN Barcelona, GLA Glasgow, GOT Gothenburg, LIS Lisbon, MAD Madrid, MAL Malmö, MIL Milan, MUC Munich, PAR Paris. Colour code is given in panel a. Source data are provided as a Source data file.
SNPs
SNP density across the genome. The red and blue dotted lines show the 0.1 and 1% FDR significance thresholds, respectively. Red dots highlight “core urbanisation SNPs” that were also identified as urbanisation associated using BayPass (Supplementary Fig. 6). The heatmap below the Manhattan plot highlights the SNP density across the genome. b) Correlation between the number of urbanisation-associated SNPs (from LFMM) and the number of genes per chromosome (linear model: \( F_{1,96} = 284.60, P < 2.2 \times 10^{-16} \)). Shaded area denotes the 95% CI. c) Main axis of variation in a PCA based on “core urbanisation SNPs”. Grey circles show individuals, and yellow dots and lines show the mean ± SD for urban and rural populations. \( n_{\text{urban}} = 96 \) and \( n_{\text{rural}} = 96 \) independent birds. d) Effect sizes (partial \( \eta^2 \)) for the effects of “habitat” (urban vs rural) and “habitat × locality” interaction on urban-rural allele frequency, using linear model are shown for all significant LFMM SNPs on the x-axis and y-axis, respectively. “Core urbanisation SNPs” are highlighted in yellow circles. SNPs that lie above the dashed line show strong consistent shifts in allele frequencies across localities, whereas SNPs below the line show more variable allele frequency shifts across localities. Source data are provided as a Source data file.

In contrast, the results obtained by BayPass only identified 70 SNPs strongly associated with urbanisation (Bayes factor ≥ 20; Supplementary Fig. 6a). The lower number of identified SNPs might respond to the stronger population structure correction applied by BayPass, which correlates to some extent with the direction of local adaptation. Of these significant SNPs, 34 were shared with the LFMM analysis (more than expected by chance: \( \chi^2 = 31.20, P = 2.35 \times 10^{-10} \); Fig. 3a and Supplementary Fig. 6b). These shared SNPs, which we term “core urbanisation SNPs” (Supplementary Table 3), are likely involved in the local adaptation of great tits to urban habitats, and indeed, they more strongly discriminated urban and rural individuals across Europe (PVE by PC1\(_{\text{GSEA-shared}}\) = 11.7%; Fig. 3c and Supplementary Fig. 7a).

In order to gain a better understanding of the importance of habitat (i.e., urban vs rural) on allele frequency shifts in the detected SNPs, we assessed using univariate linear models, the explanatory power of habitat, locality (city), and their interaction on the direction and strength of allele frequency shifts per-SNP across all paired populations. A significant “habitat” term indicates consistent allele frequency shifts across cities, particularly when the effect size (partial \( \eta^2 \)) is higher compared to the effect size of a significant “habitat × locality” interaction term, which describes differences in the direction and magnitude of the allele frequency change between cities. Applying these models to each urbanisation-associated SNP from the LFMM model (2758 SNPs), we detected large variation in allele frequency shifts across localities (Fig. 3d and Supplementary Fig. 7b), with a slightly larger proportion of SNPs showing differences in allele frequency shifts across localities (“habitat × locality” \( \eta^2 > \text{“habitat” } \eta^2 \)). In contrast to this, most of the “core urbanisation SNPs” showed similar allele frequency differences across localities, with 76% of them showing a main effect of the urban habitat (Fig. 3d), and with the same allele increased in frequency in seven or more urban populations (Supplementary Fig. 7c; see Supplementary Fig. 8 for the minor allele frequency trajectory between habitats). We refined this analysis by accounting for the effect of allele frequency correlations between SNPs through the use of the principal component axis from all the LFMM urbanisation-associated SNPs (PC1\(_{\text{GSEA}}\)) and the “core urbanisation SNPs”
(PC1|GEA-shared) Supplementary Fig. 7d). In both cases, the "habitat" term explained the majority of the total variation in allele frequency divergence (\(\eta^2_{PC1|GEA, \text{habitat}} = 0.87, P < 0.001; \eta^2_{PC1|GEA-shared, \text{habitat}} = 0.73, P < 0.001\)). In comparison, both the effect of "locality", which corresponds to the distinct evolutionary history of the local populations (\(\eta^2_{PC1|GEA, \text{locality}} = 0.59, P < 0.001; \eta^2_{PC1|GEA-shared, \text{locality}} = 0.20, P < 0.001\)), and the interaction of "habitat \times locality" (\(\eta^2_{PC1|GEA, \text{habitat} \times \text{locality}} = 0.13, P = 0.002; \eta^2_{PC1|GEA-shared, \text{habitat} \times \text{locality}} = 0.13, P = 0.001\)), explained much smaller proportions of the total variation (smaller effect size) than the habitat term by itself. In contrast, genetic variation along PC2 and PC3 was mostly inconsistent across localities and likely specific for each city (Supplementary Fig. 7d). Overall, the PCA-based results are in line with those obtained by the per-SNP results, reinforcing the idea of consistent continent-wide responses to urbanisation, in particular regarding the "core urbanisation SNPs".

Together, we show that there is local adaptation to urban habitats in great tits, and that this occurred across Europe through shifts in allele frequency of the same loci. This might have occurred via the standing genetic variation observed for the species, as putatively adaptive alleles were shared across large parts of the species' European distribution, or the exchange of adaptive alleles through gene flow across urban populations. Nonetheless, in line with a polygenic basis of adaptation via subtle allele frequency shifts in several loci, the detected urban–rural differences were generally small (Supplementary Fig. 9) with only a few SNPs showing relatively strong allele frequency shifts with \(\Delta A F\) (allele frequencies difference between urban and rural) >0.5.

**Signatures of selection in urban populations.** While GEA approaches are powerful tools for identifying even subtle allele frequency shifts associated with local adaptation, they have difficulty detecting selective sweeps unique to one or few populations or sweeps of different haplotypes associated with the same gene. Selective sweeps have been associated with urban adaptation in other species, for example, in the case of New York City brown rats (Rattus norvegicus) and white-footed mice (Peromyscus leucopus). Therefore, we further performed genome-wide scans of differentiation and selective sweep analyses in each of our studied populations to test if these contributed to the urban adaptation in great tits and, importantly, to explore if selective sweeps were population specific or widely shared across Europe.

Since the exchange of genetic material between urban and rural populations, and the selection pressures are likely recent and ongoing in great tits, we opted to use a cross-population statistic that can identify ongoing and recently completed hard and soft selective sweeps (XP-nSL). Using this approach, we found that between 436 and 700 genomic windows (200 kb sliding windows with 50 kb steps, see "Methods"), which clustered into 127–173 wider genomic regions, showed signatures of selection in urban populations (Fig. 4a). Genomic outlier regions had an average size of 355.8 kb \(\pm\) 251.6 SD, with the largest region (3.05 Mb) detected on chromosome 1 in Glasgow and were widely distributed across the genome with a few distinct population-specific peaks (Fig. 4a). The large number of genomic outlier regions are in line with the high number of significant GEAAs across the genome and confirms the inferred polygenic nature of urban adaptation in great tits (Fig. 3a). However, in contrast to the highly concordant allele frequency shifts of GEA loci, XP-nSL values were in general weakly correlated across populations (Fig. 4b), and outlier windows were shared across a maximum of five populations (Supplementary Fig. 10). The top three shared windows clustered in a 300 kb region on chromosome 11 (16.05–16.35 Mb) and were detected in Gothenburg, Munich and the Iberian Peninsula (Barcelona, Madrid and Lisbon), with one partially overlapping outlier window detected in Glasgow. In general, the most pronounced sharing of outlier windows did not seem to occur between the geographically closest populations, as expected for shared genetic variation or adaptive introgression (see Supplementary Fig. 10). For example, Gothenburg and Paris, and Lisbon and Glasgow, showed the highest number of shared outlier windows (53 and 47, respectively). Indeed, the number of shared outlier windows between urban populations was not correlated with their genetic (Mantel’s \(r = 0.28, P = 0.150\)) or geographic distance (Mantel’s \(r = 0.05, P = 0.400\)), suggesting that shared adaptive genetic variation through introgression and gene flow between neighbouring urban populations likely did not facilitate urban adaptation. However, we cannot fully exclude the role of gene flow as migration was generally strong, even across large distances (Supplementary Fig. 4).

The observed landscape of variation in XP-nSL is in agreement with the genomic landscape of genetic differentiation (standardised Z-transformed \(F_{ST}[Z_{ST}]\)) between each adjacent urban and rural pair across the same windows (Fig. 4b, Supplementary Figs. 11 and 12). \(Z_{ST}\) outlier windows (\(Z_{ST} > 4\), equivalent to four SD) were largely population specific (Supplementary Fig. 12), suggesting that population-specific selective and demographic processes have partly shaped the genomic landscape. To strengthen our inference of the selective sweeps landscape associated with urban adaptation in our species, we additionally searched for older and completed selective sweeps using the haplootype-based \(R_{sb}\) statistic. Similarly to the results obtained using the XP-nSL statistic and \(Z_{ST}\), window-based \(R_{sb}\) scores were weakly and inconsistently correlated across populations (Fig. 4b and Supplementary Fig. 13). We detected fewer \(R_{sb}\) outlier windows (209–538) and clustered genomic outlier regions (39–98; average size: 396.9 kb \(\pm\) 329.2 SD; \(max = 3.45\) Mb on chromosome 3 in Gothenburg) compared to XP-nSL. This result suggests that ongoing/recent sweeps outnumber older and completed selective sweeps in urban great tits. Similar to XP-nSL, the \(R_{sb}\) outlier windows were only shared across a maximum of five populations and in general widely distributed across the genome (Supplementary Fig. 14).

Overall, the selection analyses showed that selective sweeps, and in particular those that are recent and ongoing (XP-nSL), were pervasive across the genomes of urban great tits, supporting our inference of a highly redundant polygenic adaptation to urban environments. The underpinning genomic changes associated with urban adaptation were largely population specific, likely the result of differential selective pressures, adaptation through complex multivariate phenotypic changes and/or selection on standing genetic variation. Nonetheless, we still detected shared selective sweeps across urban populations, suggesting the presence of common genomic responses to the urban selective pressures, either through repeated selective sweeps or sharing of adaptive genetic variants.

**Evolutionary drivers of genetic differentiation and signatures of selection.** The largely population-specific landscapes of differentiation are in line with observations in other young divergences, and have been previously explained by the weaker effect of linked selection at early stages of the divergence process. However, to exclude a driving role of non-adaptive processes, we corroborated that the detected signatures of selection were indeed caused by divergent selection and not by genetic drift or linked selection in low-recombination regions. To achieve this, we evaluated the correlation between signatures of selection (XP-nSL and \(R_{sb}\) and differentiation (\(F_{ST}\)) with two proxies of genomic recombination rate, i.e., LD and intronic GC-content (both in
LD is correlated with population-specific recombination rates and the strength of selection, while intronic GC-content is a surrogate for the long-term recombination rate variation across the great tit genome, i.e., a higher GC-content will indicate a higher recombination rate. To confirm that both proxies provide similar pictures of the recombination landscape, we tested their correlation across all population pairs. Indeed, LD and GC-content were (negatively) correlated in all populations, suggesting that the general effect of recombination on the genomic diversity landscape is broadly consistent.

Under a scenario of linked selection in low-recombination regions, we would expect strong and consistent positive correlations between GC-content or LD with our estimates of genetic differentiation and selection, i.e., \( F_{ST} \), \( XP-nSL \) and \( R_{sb} \). Yet, this was not the case, which indicates that processes other than linked selection are the main driver for population differentiation in our study. Indeed, the overall patterns are more in line with divergent selection, as e.g., the population-specific selection peak on chromosome 1 in Glasgow is not associated with low-recombination regions.
content) or a pronounced peak in LD (Fig. 4d). However, it is important to note that these correlations are positive in some populations, see e.g., XP-nSL and GC-content (Fig. 4c), and that some population-specific peaks are located on low-recombination regions, and likely caused by linked selection or other non-adaptive processes (Fig. 4d). While the contribution of non-adaptive processes likely differed across populations, we did not detect a general signature of linked selection as a major driver of divergence between urban and rural populations.

Divergent selection has been suggested to be the main driver in the early stages of differentiation in other avian systems, and it is not surprising that a similar incipient pattern is observed between urban–rural populations. Although other non-adaptive processes, such as stochasticity via population-specific drift, could also generate variable genomic landscapes in an urban context, that scenario would be characterised by a decreased gene flow and increased genetic differentiation with geographical distance between adjacent urban and rural populations. However, genetic differentiation between paired urban and rural populations did not significantly increase with geographic distance ($R^2 = −0.11, P = 0.645$; Fig. 4e), further excluding drift as the main process underneath the variability in the genomic and the genetic differentiation landscape between urban and rural populations. Nonetheless, genetic drift potentially led to increased genetic differentiation in some urban populations, i.e., Glasgow and Lisbon, which both showed reduced heterozygosity.

Hence, we conclude that the mosaic of genomic signatures of differentiation between urban and rural populations and selective sweeps in the studied populations are most likely driven by genomic responses to selection on standing genetic variation, with potentially minor contributions of linked selection in low-recombination regions or other idiosyncratic non-adaptive processes.

Genes and pathways associated with adaptation to urbanisation. While responses to selection at the haplotype level were highly variable across urban populations, selective sweeps might affect the same genes or different genes with similar functional impacts (e.g., same pathway). Such consistent genomic responses on the gene or functional level could explain the similarity in phenotypic responses to environmental variation despite a large variation on the haplotype level. Following this rationale, we identified genes associated with signatures of selective sweeps in each urban population, but without requiring outlier windows to overlap with each other. Between 976 and 1366 genes were associated with signatures of ongoing or recent selective sweeps (XP-nSL), and 362–923 with completed selective sweeps (Rsb). Of these genes, 64–207, and 5–76, were detected in at least two urban populations (based on XP-nSL or Rsb, respectively; Supplementary Figs. 15 and 16). The higher number of genes associated with recent and ongoing selective sweeps compared to complete selective sweeps is in line with the colonisation pattern of urban habitats, under ongoing or recent gene flow.

Although it is statistically unlikely to detect selective sweeps in the same gene in more than three urban populations by chance alone (permutation test; $\chi^2 = 77.95, P < 2.2 \times 10^{-16}$), we conservatively focused our functional interpretation only on those genes that were associated with signatures of selection (XP-nSL or Rsb) in more than half of all urban populations (≥5 urban populations), as these likely play more important roles in urban adaptation. In total, we detected 42 genes associated with recent or ongoing selective sweeps in at least five populations (XP-nSL: max. six populations), and 15 genes associated with older completed selective sweeps (Rsb: max. seven populations; Fig. 5a and Supplementary Table 4), with none of the genes detected by both selection statistics. It is noteworthy that we did not detect an increase in the number of shared genes under selection in relation to geographical (Mantel’s $r = 0.27, P = 0.080$) or genetic distance (Mantel’s $r = 0.05, P = 0.480$), which is in accordance with the results regarding shared outlier windows and further supports that many selective sweeps likely occurred independently in multiple urban populations. In addition, 12 of the 57 shared genes under selection were also associated with urbanisation in the LFMN analysis (Supplementary Table 4). Interestingly, two of these, $GMDS$ and $SLC6A15$, were not only associated with complete selective sweeps (Rsb scores) in seven and six populations, respectively, but also previously shown to be differentially expressed in blood and/or liver between urban and rural birds from Malmo, among others. Moreover, following Malmo as an example, one gene found to be differentially expressed in Watson et al., $VPS13A$, was highly differentiated ($ZFS_T$) and associated with selective sweeps in that particular population, but importantly, also under selection in Madrid (Rsb) and Lisbon (XP-nSL) and in general, associated with urbanisation (LFMN analysis). This exemplifies that some genes show signatures of urban adaptation on a continental scale, making them strong candidates for adaptation in these urban centres.

It is noteworthy that despite finding signatures of selection overlapping the same gene in five or more urban populations (e.g., $GMDS$, $HTR7$ or $CDH18$), the exact locations of these signatures were not consistent (Fig. 5b, c). Under a shared selective sweep or adaptive introgression scenario, we would have
and HRT7 was plotted in R using ggplot with the maps package. The frequency in urban populations across Europe, which is in line suggesting that different adaptive haplotypes swept to high frequencies in at least six urban populations. For example, signatures of selection around HTR7 were downstream under selection in urban populations across Europe, detected in at least six urban populations. CDH18, marked with an asterisk, was also detected in the GEA analysis. Genes in bold font were associated with ongoing selection (XP-nSL), and those with grey font with older completed sweeps (Rsb). The map was plotted in R using ggplot with the maps package. Haplotypic stripes showing haplotype structure around two representative candidate genes (GMDS and HRT7) on chromosome 2 and 7, respectively. Individuals (rows) are ordered by haplotype similarity and colour-coded by habitat of origin. Note that not always the same regions (upstream, downstream or genic) are under selection in urban populations (positive scores) around the candidate genes. Red shades depict SNPs with the strongest positive selection scores. Gene boundaries are shown by red dotted lines and grey background box. Note that not always the same regions (upstream, downstream or genic) are under selection in urban populations (positive scores) around the candidate genes. Enrichment of associated genes in significantly enriched gene ontology (GO) groups by GO category in the LFMM and BayPass analysis (see Supplementary Table 4 for a detailed list). The colour of the bars represents the false-expectation discovery rate (FDR) from the gene ontology overrepresentation analyses. BCN Barcelona, GLA Glasgow, GOT Gothenburg, LIS Lisbon, MAD Madrid, MAL Malmö, MIL Milan, MUC Munich, PAR Paris.

expected the sharing of urban haplotypes between populations. However, haplotype plots do not show any noticeable clustering across urban individuals (e.g., GMDS or HRT7, Fig. 5b), suggesting that different adaptive haplotypes swept to high frequency in urban populations across Europe, which is in line with soft selective sweeps from standing genetic variation. Furthermore, we find that the strongest selective sweep signatures are, in some cases, not within the genes but up- or downstream and without a consistent pattern among populations. For example, signatures of selection around HTR7 were downstream in Glasgow and Munich, but upstream in Malmö and Lisbon (Fig. 5c). This might suggest that regulatory changes rather than protein-coding ones are associated with urban adaptation and the nature of regulatory variation likely differs across populations. However, high-resolution genomic studies based on whole-genome resequencing coupled with multi-tissue functional genomic analyses are needed to resolve this question, which is beyond the scope of this study.

Many of the genes associated with urbanisation have previously been linked to behavioural divergence, and cognitive and learning
functions, suggesting adaptive phenotypic shifts related to behaviour. In particular, HTR7 (Chr 7) codes for a receptor of the neurotransmitter serotonin, a pathway consistently identified as target of changes linked to urbanisation. Indeed, two previous studies in birds, one on European blackbirds using a candidate gene approach and another on burrowing owls (Athene noctua), using full-genome resequencing, also described changes in this pathway as the main difference between urban and rural populations. Both species also show consistent urban–rural differences in two behaviours associated with serotonin: down-regulation of the stress axis and altered phenotypic plasticity was to a large extent still unknown. Our studies in birds, one on European blackbirds using a candidate gene approach and another on burrowing owls, were also previously linked to urban divergence in burrowing owls.

Thus, our selection analyses suggest that natural selection repeatedly acts on behavioural traits and sensory and cognitive performance, all previously shown to be among the most widespread differences between urban and rural wildlife populations. These findings were furthermore supported by gene ontology (GO) analysis of the 2758 urbanisation-associated SNPs (LFMM analysis), linked to 984 genes (1501 SNPs in genic regions). Accordingly, most of the GO terms were related to neural functioning and development (e.g., GO:00016358, FDR = 4.30 × 10⁻⁶), cell adhesion (e.g., GO:0098742, FDR = 3.43 × 10⁻⁶) and sensory perception (e.g., GO:0050954, FDR = 3.42 × 10⁻³; Fig. 5d and Supplementary Table 5). These GO terms were mainly clustered into two interacting networks, one related to sensory recognition and the other to neural development and cell adhesion (Supplementary Fig. 17). These findings reinforce the previous idea on cognitive and behavioural changes as key putatively urban adaptive traits and sensory and cognitive performance, all previously shown to be among the most widespread differences between urban and rural wildlife populations. Nonetheless, whether this is the result of a genetic response to selection or phenotypic plasticity was to a large extent still unknown.

Our present study suggests a strong genetic component for these putatively urban adaptive phenotypes in great tits across Europe. Detailed genomic and phenotypic analyses are now needed to understand the role of these genes and pathways in the adaptive divergence of urban and rural great tits and other songbirds.

Our study demonstrates clear genomic signals of local adaptation to urban habitats in a common songbird on a continent-wide scale across Europe. We found that a combination of polygenic allele frequency shifts and spatially varying selective sweeps are associated with adaptation to urban environments. Our results strongly suggest that a few genes, which have known neural developmental and behavioural functions, experienced selective sweeps in urban populations. This suggests a strong consistency in the functional processes associated with urbanisation, despite the fact that the underlying haplotypes are often not shared. Thus, our study exemplifies evolutionary adaptation to urban environments on a European scale, and highlights behavioural and neurosensory adjustments as important phenotypic adaptations in urban habitats.

Methods
Sample collection and DNA extraction. During the years 2013–2015, 20 or more individual great tits were sampled at paired urban–rural sites from nine European cities (Fig. 1a and Supplementary Table 1). We sampled a total of 192 individuals (aged >1 year old) with 10–16 individuals per site (Supplementary Table 1). Sexes were balanced between pairs (urban–rural) in the dataset (GLMM; sex: *χ² = 1.33, P = 0.280). Each of the paired sampling sites (urban or rural, hereinafter populations) was sampled within the same season. Barcelona and Munich were sampled only two years, however, in both cases only known birds (recaptures) were included in the study, thus, all birds can be considered resident. All urban populations were located within the city boundaries, the areas are characterised with significant proportion of human-built structures, such as houses and roads with managed parks as the only green space (Supplementary Fig. 1). Rural populations were chosen to contrast the urban locations regarding degree of urbanisation and were always natural/semi-natural forests, and contained only a few isolated houses. All urban and rural populations were separated by a distance above the mean adult and natal dispersal distance of this species (i.e., see Supplementary Table 1). Blood samples (~25 μl) were obtained either from the jugular or brachial vein and were kept in 1 ml EDTA buffer and subsequently frozen at −20 °C. In each case, procedures were identical for the paired urban and rural populations. All procedures employed during field work were approved by national Ethical Committees and authorisations to collect samples were delivered by the Generalitat de Catalunya (SAGM/001739/2014), the Institute for Environmental Protection and Research (ISPRA, license no.15510 and 15944) and the Lombardy Region (permit no. 3462), the Tiereschutzgesetz (TierSchG, German animal protection law) and the Regierung von Oberbayern (permit nos. 52.2-1-54.2522.2-7.07 and 52.2-1-54.2522.2-7.09) and the Ministry of Higher Education, Research and Innovation (Ethics Committee for Animal Experimental Licence no. 8305 and permit no. APAIS199491-2019032516270525), the Prefect of Paris and the Prefect of Seine et Marne (permit no. DRJEE-2012-31 and DRJEE-2012-32) and the CRBPO (National Museum of Natural History, permit no. 537 and licence no. 1454). DNA was extracted from ~5 μl samples of red blood cells in 195 μl of phosphate-buffered saline, using Macherey-Nagel Nucleospin Blood Kits (Bethylan, PA, USA), and following the manufacturer’s instructions or manual salt extraction (ammonium acetate). The quantity and purity of the extracted genomic DNA was high as measured using a Nanodrop 2000 Spectrophotometer (Thermo Fisher Scientific) and Qubit 2.0 Fluorometer (Thermo Fisher Scientific).

Urbanisation score. To quantify the degree of urbanisation at each site, we used the UrbanisationScore image analysis software, based on aerial images from Google Maps (Google Maps 2017), and following the methods described in different studies assessing the effect of urbanisation on wild bird populations. Briefly, each sampling site was represented by a 1 × 1 km rectangular area around the capture locations. We opted to use this spatial resolution around the sampling sites to approximately cover individuals’ territory, but also to ensure that we considered the heterogeneity within each site. The content in each rectangle was evaluated dividing the image in 100 × 100 m cells and considering three land-cover categories in each: proportion of buildings, vegetation (including cultivated fields) and paved surfaces (Supplementary Fig. 1). The different land-cover measures obtained per site were used in a PCA to estimate an urbanisation score variable (PC_u) for each of the urban or rural populations per locality, see Table S1. The PC_u values were transformed to obtain negative values in the less urbanised and positive values in the more urbanised sites. We used the average of the urbanisation estimates if birds were captured in more than one location within each site (>2 km apart, mean SD: 531.22 ± 1005.26 m). All quantifications were done in triplicates by the same person (P.S.) and the estimates were highly repeatable (intra class correlation coefficient, ICC = 0.993, 95% confidence interval (CI): 0.997–0.987, P < 0.001).

SNP genotyping. All 192 individuals were successfully genotyped using a custom made Aflymetrix® great tit 65K SNP chip at Edinburgh Genomics (Edinburgh, United Kingdom). The Aflymetrix® SNP chip was developed based on whole-genome sequencing data from great tits sampled across multiple European populations, largely corresponding to our sample sites. Thus, this SNP chip allowed us to genotype a large number of individuals on a genome-wide basis at high SNP density without a strong ascertainment bias. SNP calling was done following the “Best Practices Workflow” in the software Axiom Analysis Suite.
Genetic diversity and population structure. We calculated the genome-wide genetic diversity as expected heterozygosity (\(H_e\)) for each population using Plink v.1.9 (ref. 78), and tested if genetic diversity significantly differed between urban–rural populations from the same location using \(t\) tests in R, and overall across urban–rural pairings using a Wilcoxon rank-sum test in R v.3.6.1 (ref. 79). Furthermore, we estimated pairwise \(F_{ST}\) between all population pairs (urban–rural per locality), using VCFTools v.0.1.15 (ref. 80). Mean \(F_{ST}\) was computed across all comparisons after setting negative values to zero. For analyses of population structure, we pruned the SNP dataset based on LD in Plink v.1.9 (ref. 79), using a variance inflation factor threshold of 2 (“\(\text{indp} 50 52\)”), retaining 358,149 SNPs. Using this LD-pruned dataset, we performed a PCA using Plink. We performed two different PCAs, (i) only with autosomes and excluding all small linkage groups and the Z-chromosome, and (ii) including the Z-chromosome but excluding all small linkage groups. Subsequently, we performed additional dimensionality reduction for each PCA dataset using the first 20 principal components, using the UMAP v.0.2.7.0 R-package with default settings81. A total of 555,610 SNPs were then filtered and assigned to chromosomes using the P. major reference genome build 1.1 (annotation release ID 101; “GCA_001522545.2”). A total of 155 SNPs were not found in the used assembly and 26,852 SNPs were not in chromosome regions; thus, these SNPs were removed from further analysis leaving a total of 517,603 SNPs.

Environment-associated SNPs. We used two different approaches to identify SNPs associated with the degree of urbanisation, based on the “urbanisation score”. First, we used a univariate latent-factor linear mixed model implemented in LFMM v.1.5 for examining allele frequency–environment associations85. Based on the number of ancestry clusters (K) inferred with fastStructure v.1.0, we fitted a LFMM with two and four latent factors, respectively. Each model was run five times for 10,000 iterations with a 5000-iteration burn-in. We calculated the median \(z\)-score for each locus across all ten runs, and selected SNPs with a FDR < 1% to be associated with urbanisation. The results with two or four latent factors were highly replicated runs and considered all extended haplotype homozygosity (EHHS) between urban and rural populations, the cross-population comparative nature of the \(Rsb\) statistic provides an internal control that cancels out the effect of heterogeneous recombination across the genome86.

To determine if the extent of genetic differentiation between all possible population pairs was significantly different from zero, we estimated \(P\) values using a permutation analysis in Genodive v.3 (ref. 89). For computational reasons, the analysis was performed on a thinned SNP dataset (21,062 SNPs), for which we randomly selected one SNP every 50 kb.

Haplotype-based selection analyses. To identify genomic regions showing signs of selective sweeps in urban–rural population pairs, we used two haplotype-based selection scans. First, we scanned the genome for regions showing differences in extended haplotype homozygosity (EHHS) between urban and rural populations (see \(Rsb\) score below) to identify genomic regions showing signs of older and completed selective sweeps. We used fastPHASE v.1.4 (ref. 85) to reconstruct haplotypic phase and impute missing data independently for each chromosome using the default parameters, except that each individual was classified by its population (“\(\text{urb}\)” option). We used ten random start of the EM algorithm (“\(\text{T}\)” option) and 100 haplotypes (“\(\text{H}\)” option). The fastPHASE output files were analysed using reh v.2.0 (ref. 85) to calculate \(Rsb\) statistics for focal SNP. The \(Rsb\) score is the \(\chi^2\) statistic of integrated haplotype scores (IHS), which is a statistic between two populations48,91. This statistic measures the extent of haplotype homozygosity between two populations and follows the rationale that if a SNP is under selection in one population compared to the other, the region around this locus will show an unusually high level of haplotype homozygosity compared to the neutral distribution. Following Fuller et al.86 we determined a significance cut-off based on the \(\chi^2\) distribution of \(Rsb\) values >4 compared to the total number of SNPs per window85. Because recombination rates can be assumed to be conserved between closely related urban and rural populations, the cross-population comparative nature of the \(Rsb\) statistic provides an internal control that cancels out the effect of heterogeneous recombination across the genome86.

To determine if the extent of genetic differentiation between all possible population pairs, the cross-population comparative nature of the \(Rsb\) statistic provides an internal control that cancels out the effect of heterogeneous recombination across the genome86.

To determine the consistency of selection across urban centres, we implemented a resampling approach to assess the likelihood of genes showing signs of selection in two, three, four or more populations. We resampled with replacement \(n\) genes \((n = \text{number of genes with signatures of selection in each urban population})\) for each population from the list of all SNP-linked genes using the “\(\text{resample}\)” function in R, assessed the amount of overlap between populations (from two to eight populations) and repeated the sampling 100,000 times for each comparison. We then calculated the mean and 95% CI for each comparison and compared the number of observed shared candidate genes to the expected number of candidate genes. The expected number of genes showing signs of selection in three or more populations was zero, thus we focused on genes showing signs of selection (\(Rsb\) and \(XP\)-\(n\)-\(SL\)) in three or more populations.
Patterns of linkage disequilibrium and intrinsic GC-content across the genome. To estimate the impact of variation in recombination rate and linked selection in low-recombination regions on patterns of divergence (i.e., regions showing signs of selective sweeps), we estimated the correlations of LD and intrinsic GC-content in 200 kb windows with measures of selection/differentiation (Rb, XP-nSL, and ZF0).

First, we estimated patterns of LD (r2) across the genome using Plink 1.9 (ref. 78) for pairs of SNPs located up to 200 kb apart for each urban population (<−2|d-window−r2| 0−d-window−kb 200.000). LD estimates were averaged in 200 non-sliding windows using the WindowScan v.0.1 R-package.

Second, we estimated the GC-content for all introns across the great tit (P. major) reference genome build 1.1 (annotation release ID 101; “GCA_001522545.2”). We used all genes containing SNPs associated with urbanisation (LFMM and BayPass, n = 1501 SNPs within genes). To analyse the enrichment of functional classes, we identified over-represented GOs (biological processes, molecular functions, and cellular components), using the WebGestalt software tool (ref. 102). The gene background was set using annotated great tit genes (annotation release ID 101) containing SNPs from the SNP chip and with Homo sapiens orthologues. H. sapiens genes were used as a reference set as human genes are better annotated with GO terms than those of any avian system (e.g., Gallus gallus) (ref. 16, 39). We focused on non-redundant GO terms to account for correlations across the GO graph topology and GO terms as implemented in WebGestalt (ref. 102). An EFR < 0.05 was used as a threshold for significantly enriched GO terms. Furthermore, we searched the public record for functions of individual candidate genes. We also used GOtilla (ref. 103) to visualise the connections of GO terms associated with LFMM candidate genes and Cytoscape v.3.6.1 (ref. 104) to visualise the GO network and identify all enriched GO terms (biological processes, P < 0.001, including redundant terms).

Candidate genes associated with signatures of selection were those that overlapped with significant XP-nSL or Rb outlier windows. Overlaps between outlier windows and annotated genes were assessed using the pylranges R-package v.3.12.

Reporting summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

The genotyping data that support the findings of this study is available in variant call format (VCF) via the European Variation Archive (EVA) with the accession number PRERB44069. Source data are provided with this paper.

Code availability

No custom code or mathematical algorithm was developed for this project or is considered crucial to the conclusions. All relevant software and R-functions that are used are referred to in the “Methods” section.

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References

1. Hendry, A. P., Gotanda, K. M. & Svensson, E. I. Human influences on evolution, and the ecological and societal consequences. Philos. Trans. R. Soc. Lond. B Biol. Sci. 372, 20160028 (2017).
2. Johnson, M. T. J. & Munshi-South, J. Evolution of life in urban environments. Science 358, eaam8327 (2017).
3. Mueller, J. C., Partecke, J., Hatchwell, B. J., Gaston, K. J. & Evans, K. L. Candidate gene polymorphisms for behavioural adaptations during urbanization in blackbirds. Mol. Ecol. 22, 3629–3637 (2013).
4. Harris, S. E. & Munshi-South, J. Signatures of positive selection and local adaptation to urbanization in white-footed mice (Peromyscus leucopus). Mol. Ecol. 26, 6336–6350 (2017).
5. Rivkin, L. R. et al. A roadmap for urban evolutionary ecology. Evol. Appl. 12, 384–398 (2019).
6. Alberti, M. et al. Global urban signatures of phenotypic change in animal and plant populations. Proc. Natl Acad. Sci. USA 114, 8951–8956 (2017).
7. Møller, A. P. & Báez-Alamo, J. D. Escape behaviour of birds provides evidence of predation being involved in urbanization. Anim. Behav. 84, 338–348 (2012).
8. Audet, J.-N., Ducatez, S. & Lefebvre, L. The town bird and the country bird: problem solving and immunocompetence vary with urbanization. Behav. Ecol. 27, 637–644 (2016).
9. Carrete, M. & Tell, J. L. Behavioral correlations associated with fear of humans differ between rural and urban burrowing owls. Front. Ecol. Evol. 5, 1–10 (2017).
10. Winchell, K. M., Reynolds, R. G., Prado-Irwin, S. R., Puente-Rolón, A. R. & Revell, L. J. Phenotypic shifts in urban areas in the tropical lizard Anolis cristatellus. Evolution 70, 1009–1022 (2016).
11. Winchell, K. M., Maayani, I., Fredette, J. R. & Revell, L. J. Linking locomotor performance to morphological shifts in urban lizards. Proc. R. Soc. B Biol. Sci. 285, 20180229 (2018).
12. Kern, E. M. A. & Langerhans, R. B. Urbanization drives contemporary evolution in stream fish. Glob. Change Biol. 24, 3791–3803 (2018).
13. Parsons, K. J. et al. Skull morphology diverges between urban and rural populations of red foxes mirroring patterns of domestication and macroevolution. Proc. R. Soc. B Biol. Sci. 287, 20200763 (2020).
14. Reid, N. M. et al. The genomic landscape of rapid repeated evolutionary adaptation to toxic pollution in wild fish. Science 354, 1305–1308 (2016).
15. Campbell-Stanton, S. C. et al. Parallel selection on thermal physiology facilitates repeated adaptation of city lizards to urban heat islands. Nat. Ecol. Evol. 4, 652–658 (2020).
16. Bosse, M. et al. Recent natural selection causes adaptive evolution of an avian polygenic trait. Science 358, 365–368 (2017).
17. Hendry, A. P., Farrugia, T. J. & Kinnison, M. T. Human influences on rates of phenotypic change in wild animal populations. Mol. Ecol. 17, 20–29 (2008).
18. Jacobs, A. et al. Rapid niche expansion by selection on functional genomic variation after ecosystem recovery. Nat. Ecol. Evol. 3, 77–86 (2019).
19. Pritchard, J. K., Pickrell, J. K. & Coop, G. The genetics of human adaptation: hard sweeps, soft sweeps, and polygenic adaptation. Curr. Biol. 20, R208–R215 (2010).
20. Thompson, K. A., Renaudin, M. & Johnson, M. T. J. Urbanization drives the evolution of parallel clines in plant populations. Proc. R. Soc. B Biol. Sci. 283, 20162186 (2016).
21. Perrier, C. et al. Great tits and the city: distribution of genomic diversity and gene–environment associations along an urbanization gradient. Evolut. Appl. 11, 593–613 (2018).
22. Mueller, J. C. et al. Genes acting in synapses and neuron projections are early targets of selection during urban colonization. Mol. Ecol. 29, 3403–3412 (2020).
23. Johnson, M. T. J., Prashad, C. M., Lavoginat, M. & Saini, H. S. Contrasting the effects of natural selection, genetic drift and gene flow on urban evolution in white clover (Trifolium repens). Proc. R. Soc. B Biol. Sci. 285, 20181019 (2018).
24. Elmer, K. R. & Meyer, A. Adaptation in the age of ecological genomics: insights from parallelism and convergence. Trends Ecol. Evol. 26, 298–306 (2011).
25. Miles, L. S., Rivkin, L. R., Johnson, M. T. J., Munshi-South, J. & Verrelli, B. C. Gene flow and genetic drift in urban environments. Mol. Ecol. 28, 4138–4151 (2019).
26. Barghi, N., Hermisson, J. & Schlötterer, C. Polygenic adaptation: a unifying framework to understand positive selection. Nat. Rev. Genet. 21, 769–781 (2020).
27. Boyce, M. S. & Perrins, C. M. Optimizing great tit clutch size in a fluctuating environment. Ecology 68, 142–153 (1987).
28. Charmantier, A. et al. Adaptive phenotypic plasticity in response to climate change in a wild bird population. Science 320, 800–803 (2008).
29. Charmantier, A., Demeyrier, Y., Lombrechts, M., Perret, S. & Grégoire, A. Urbanization is associated with divergence in pace-of-life in great tits. Front. Ecol. Evol. 5, 53 (2017).
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