

Genome-wide evidence for differential selection between urban and non-urban habitats in a bird of prey

Gianluca Damiani

gianlucadamianiph@gmail.com

University of Tuscia Department of Biological and Ecological Sciences: Università degli Studi della Tuscia Dipartimento di Scienze Ecologiche e Biologiche <https://orcid.org/0000-0001-6225-6309>

Giulia Gentile

University of Tuscia Department of Biological and Ecological Sciences: Università degli Studi della Tuscia Dipartimento di Scienze Ecologiche e Biologiche

Paolo Franchini

University of Tuscia Department of Biological and Ecological Sciences: Università degli Studi della Tuscia Dipartimento di Scienze Ecologiche e Biologiche

Simone Messina

University of Tuscia Department of Biological and Ecological Sciences: Università degli Studi della Tuscia Dipartimento di Scienze Ecologiche e Biologiche

Giacomo Dell'Omo

Ornis italica

David Costantini

University of Tuscia Department of Biological and Ecological Sciences: Università degli Studi della Tuscia Dipartimento di Scienze Ecologiche e Biologiche

Research Article

Keywords: genomics, genes, birds of prey, common kestrel, *Falco tinnunculus*, urbanisation

Posted Date: August 7th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-9995561/v1>

License:   This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Urbanisation represents one of the most powerful contemporary drivers of evolution. Yet, the genomic basis of adaptation to urban environments remains understudied, particularly in highly mobile vertebrates. We investigated population structure and genomic signatures of selection in common kestrels (*Falco tinnunculus*) in urban and non-urban environments. Using whole-genome sequencing data, we found limited genome-wide differentiation and high connectivity among habitats, indicating substantial gene flow. Nonetheless, composite selection scans revealed distinct habitat-specific genomic regions under selection. In urban kestrels, a strong signal on chromosome 1 included *ANKRD50*, a gene associated with viral response, suggesting pathogen-mediated selection in urban habitats, and *RELN* gene, associated with neuronal functions. Non-urban kestrels showed selection on genes involved in lipid metabolism (*CDS1*, *PIK3CB*), neuronal functions (*NAPEPLD*, *RELN*), DNA damage response (*BOD1L1*), circadian rhythm (*PER2*), and growth regulation (*FAM155A*). These results indicate that adaptive divergence can occur at specific loci despite extensive connectivity. Further studies involving other study areas will be important to assess the generality of our findings.

Introduction

Understanding how evolutionary forces operate across contrasting environments is particularly important in the Anthropocene, as human-driven modifications of habitats expose animals to novel and complex selective pressures. Urbanisation represents one of the most pervasive anthropogenic drivers of biodiversity change and loss (Schell, 2018). More than half of the global human population already lives in urban areas, a proportion expected to increase further in the near future (UN, 2024). Urban expansion causes extensive habitat modification, altering both abiotic and biotic conditions (Crooks & Sanjayan, 2006; Aronson et al., 2014; Alberti et al., 2017; Johnson & Munshi-South, 2017). These modifications can lead to physiological and behavioural changes (Audet et al., 2016; Costantini et al., 2025), and variation in life-history traits (Damiani et al., 2025). As a result, urbanisation is expected to influence animal evolution (Alberti, 2015; Johnson & Munshi-South, 2017). Understanding the mechanisms underlying these evolutionary responses is therefore essential to interpret how species persist in urban environments. A key question then is to what extent observed differences between co-specific urban and non-urban populations are due to phenotypic plasticity or genetic adaptation (Hendry et al., 2008).

Genomic technologies enable the identification of genomic regions under selection even in species with extensive connectivity and gene flow, conditions that obscure signals of local adaptation (Low et al., 2018). This is particularly relevant in urban environments, where conditions impose intense and often novel selective pressures that may accelerate adaptive responses (Fugère & Hendry, 2018; Perrier et al., 2018). Importantly, adaptive divergence can arise even in the absence of pronounced genome-wide differentiation, as selection targets specific genes and pathways linked to physiological or behavioural traits that confer advantages in distinct environments.

Previous studies revealed genomic differentiation between urban and non-urban populations in vertebrates. For instance, Munshi-South et al. (2016) reported a loss of genomic variation along an urban-to-rural gradient in white-footed mice (*Peromyscus leucopus*). Similarly, Perrier et al. (2017) found that urban great tits (*Parus major*) had slightly lower heterozygosity than non-urban great tits. Moreover, they found evidence for a possible polygenic response to selection in cities. Both studies involved species that are subject to intense predation, which can also vary across different environments resulting in selection independent from habitat quality. Studying a predator could provide further insight into the selective pressures of environmental urban conditions.

Birds of prey are among the most successful urban exploiters (Kettel et al., 2018). In particular, the common kestrel (*Falco tinnunculus*) is a generalist predator widely distributed in metropolitan areas (Village, 2010; Costantini & Dell’Omo, 2020). Its long history of nesting in cities (Village, 2010), makes it a valuable species for studying genomic responses to urbanisation. Previous studies on kestrels pointed out that different stressors are acting in urban and non-urban environments, leading to divergence in diet (Sumasgutner et al., 2014), home range (Damiani et al., 2022), exposure to pollutants (Dell’Omo et al., 2008), immune function (Giovanetti et al., 2024; Damiani et al., 2024), and laying date (Damiani et al., 2025). Moreover, Casagrande et al. (2006) found significant intra-population genetic differentiation, suggesting that fine-scale selection might operate even in panmictic systems. Together with recent transcriptomic evidence (Damiani et al., 2024), these results reinforce the idea that subtle but consistent genetic and regulatory shifts can occur despite extensive gene flow.

Previous studies found mixed evidence of genetic differentiation between urban and natural kestrel populations: while some studies found moderate divergence (Rutkowski et al., 2006, 2010 in Warsaw, Poland), other studies found little support for this genetic differentiation (Riegert et al., 2010). Similar patterns of genetic differentiation due to urbanisation were also observed in the American kestrel (*Falco sparverius*) (Ruegg et al., 2021). In our study region, common kestrels are largely sedentary, with limited dispersal around the nest (Damiani et al., 2022), and urban individuals reproduce earlier than their non-urban individuals (Damiani et al., 2025). This different reproductive phenology could limit gene flow, potentially promoting divergence (Hille et al., 2003; Casagrande et al., 2006). However, because of the recent advent of urbanisation, strong genome-wide differentiation is not expected (Negro et al., 2020; Salmón et al., 2021). Instead, adaptation to urban habitats might be reflected in signatures of selection on specific genes and pathways.

We investigated for the first time how urbanisation can drive selection on specific genes despite pervasive gene flow, providing insights into the genomic basis of adaptation in a widespread avian predator. Using whole-genome resequencing data, we assessed population structure, connectivity and signatures of selection. We combined genome-wide scans of differentiation with haplotype-based tests to identify candidate loci and pathways under selection in urban versus non-urban environments.

Materials and methods

Study area and data collection

We carried out the fieldwork in central Italy (Latium region), from April to July 2024. We considered three main habitat types: urban (URB), rural (RUR), and natural (NAT). This classification was based on land-use quantification, and previous studies identified differences in life-history traits, physiology, and immunology between kestrels living in these habitats (Damiani et al., 2024, 2025; Giovanetti et al., 2024). The urban site corresponded to the metropolitan area of Rome, characterised by a dense anthropogenic matrix of buildings, paved surfaces and roads, interspersed with small green patches such as parks and gardens. The rural site, located in the outskirts of the city, was dominated by agricultural fields and scattered Mediterranean trees (*Quercus* spp.). The natural site, situated in the Litorale Romano Nature Reserve and in northern Latium, mainly consisted of Mediterranean forests, scrublands and unmanaged grasslands (Fig. 1).

To identify the main habitat type surrounding each nest, we calculated an urbanisation index as the percentage of sealed surfaces, cultivated lands and natural habitats within a 500 m and 1 km buffer around each nest (Damiani et al., 2022, 2025). Threshold values of sealed surfaces were used to define categories: 0–3% (NAT), 5–10% (RUR), and > 15% (URB) (de Satgé et al., 2019). NAT and RUR were further distinguished according to the proportion of cultivated land (< 50% for NAT, > 50% for RUR). Land-use data were derived from CORINE Land Cover (levels 1–2) and analysed with QGIS v3.10.

We collected data from common kestrels breeding in wooden nest boxes (50 × 30 × 30 cm) installed on high-voltage power lines of Terna S.p.A. and Acea S.p.A. We collected blood samples (~ 0.1 ml) from nestlings (one individual per nest) when 25 days old (from April to July) using a 1 ml syringe (Owen, 2011; Klein & Sojka, 2012). Samples were immediately stored in pure ethanol to prevent DNA degradation and subsequently stored at 4°C until laboratory analyses. DNA extraction and downstream library preparation and whole-genome resequencing (WGRS) were performed at laboratories of Novogene (Germany), aiming at 13x coverage per individual.

Bioinformatic analysis

Raw sequencing reads were quality-controlled using Trimmomatic v0.39 (Bolger et al., 2014) to remove adapters and low-quality bases. For each individual, reads were aligned to the chromosome-level annotated genome of a congeneric species, the lesser kestrel (*Falco naumanni*; NCBI GenBank accession: GCA_017639645.1), using bwa-mem v0.7.17 (Li & Durbin, 2009). The resulting reads were indexed and sorted with Samtools v1.9 (Danecek et al., 2021). Variant calling was then performed using Freebayes v0.1.15 (Garrison & Marth., 2012), and the obtained VCF file was processed with VCFfilter, part of the VCFlib package v1.0.14 (Garrison & Marth, 2012), VT v0.5772 (Tan et al. 2015), and VCFtools v0.1.15 (Danecek et al., 2011). VCFfilter was first used to filter out poorly-supported or otherwise biased variant sites ("QUAL > 1 & QUAL / AO > 10 & SAF > 0 & SAR > 0 & RPR > 1 & RPL > 1"). The resulting variants were then normalized with VT, and then VCFtools was used to retain only biallelic SNPs (Single-Nucleotide Polymorphisms), excluding indels and filtering out variants with more than 0.9% missing data

across individuals. Additionally, variants with a mapping quality (MQ) > 30, a minimum read depth ≥ 5 , a maximum mean depth ≤ 29.2 and a minimum allele count ≥ 3 were retained. Finally, using PLINK v1.9 (Purcell et al., 2007), we generated a pruned SNP dataset, by excluding linked variants with an $r^2 \geq 0.15$ and applying a window size of 50 SNPs.

Population genomic analysis

Population genetic structure was assessed using the pruned SNP dataset through principal component analysis (PCA) carried out with PLINK. Individual admixture proportions were estimated using ADMIXTURE 1.3.0 (Alexander et al., 2009), with values of K ranging from 2 to 6. Based on the results of the population genetic structure analyses and the available sample sizes, instead of using the URB, RUR, and NAT urbanisation gradient, individuals were classified into only two groups: “urban” (URB) and “non-urban” (RUR and NAT). To compare genetic diversity between these two groups, mean heterozygosity values were estimated from the site frequency spectrum (SFS), which was derived directly from BAM alignment files using ANGSD v0.940 (Korneliussen et al., 2014). Finally, Hardy-Weinberg equilibrium and the inbreeding coefficient were calculated across all individuals using PLINK.

Signatures of selection

To perform whole-genome scans for selection signatures, we utilized indices based on Extended Haplotype Homozygosity (EHH) as formulated by Sabeti et al. (2002), along with a Composite Selection Signal (CSS) analysis (Randhawa et al., 2014). After splitting the VCF file into two populations, the subsets were phased using Beagle v5.1 (Browning et al., 2018) with default settings. Using the R package *rehh* (Gautier & Vitalis, 2012), we calculated the Integrated Haplotype Homozygosity (iHH) for all markers across pseudochromosomes of lesser kestrel. The standardized ratio of iHH values between ancestral and derived alleles (iHS) (Voight et al., 2006) was computed to detect selection signatures within populations. We then computed the CSS statistic by combining fractional ranks from three population genetic tests: the fixation index F_{st} (Weir & Cockerham, 1984), the selected allele frequency difference (ΔSAF) statistics (Grossman et al., 2010), and the cross-population extended haplotype homozygosity (XP-EHH) test (Sabeti et al., 2007). CSS scores were calculated according to the method described by Randhawa et al. (2014) and smoothed by averaging values within ± 500 kb sliding windows centred on each SNP. Genome-wide correlation analyses among the different tests (XP-EHH, ΔSAF , F_{st} and CSS scores) were visualized using pairwise correlation plots.

Control of false positive signals was achieved using the False Discovery Rate (FDR) method, applied to the empirical p-values from raw CSS scores for both populations with the R package *fdrtool* (Strimmer, 2008). To identify SNPs consistent with the null hypothesis of neutrality, differences in the density distribution of FDR (q-values) across SNPs were observed, and the proportion of null p-values (η_0) was estimated using the bootstrap approach proposed by Storey (2002). Candidate genomic regions were

identified by selecting SNPs falling within the top 0.1% of smoothed CSS scores ($-\log_{10}$ p-values) and further filtered by significance under the FDR threshold.

Candidate SNPs were mapped onto recombination windows inferred with ReLERNN, and summary statistics were calculated per window. Given the absence of clear genetic structure and evidence of gene flow between urban and non-urban habitats, we inferred recombination using all individuals jointly, treating the dataset as a single panmictic population.

We performed the analysis on the filtered variant VCF dataset including all autosomal chromosomes. We conducted ReLERNN simulations assuming a per-site mutation rate of 4.6×10^{-9} (Smeds et al., 2016) and a generation time of 3 years, consistent with estimates reported for avian species.

We retained only windows containing at least 50 SNPs to ensure robust estimates, and those with more than five candidate SNPs were defined as candidate windows. Recombination rates were averaged per window and classified into four categories based on quartiles of their distribution. Differences between candidate and non-candidate windows were assessed using a permutation test (10,000 permutations). The relationship between recombination rate and the proportion of candidate SNPs per window was evaluated using Spearman's rank correlation. To identify candidate genes potentially under selection, the genome was divided into non-overlapping windows of 100 kb. Windows containing at least one significant SNP were then intersected with the reference genome annotation (GFF format) using the *GenomicRanges* package in R (Lawrence et al., 2013), and the included genes were retrieved for the enrichment test. Loci annotated as "uncharacterized" in the GFF file were queried using BLASTx (Camacho et al., 2009) against the NCBI non-redundant protein (NR).

Results

Data sample

Whole-genome resequencing (WGRS) generated a total of 3,432.9 million (M) raw reads (514.8 Gbp) across the 23 individuals sampled (URB = 8; RUR = 11; NAT = 4), with per-sample yield ranging from 22.3 to 521.4 M reads. After quality filtering, 3254.3 M reads were retained (480.6 Gbp), corresponding to 21.2-482.4 M reads per sample. The reads showed high mapping rates to the lesser kestrel reference genome (99.499.7%), with a duplication rate from 10.4 to 30.0%. The aligned and duplicate-marked reads were genotyped using Freebayes, and variant filtering followed the procedures described in the Methods section. After filtering, all individuals were retained, resulting in a dataset of 4,474,028 SNPs, which was reduced to 466,115 SNPs after linkage disequilibrium pruning.

Population genomic analysis

The principal component analysis showed a partial separation between urban and non-urban individuals (Fig. 1). The first principal component (PC1) explained 5.95% of the variation, and separated urban

individuals from non-urban ones. The second principal component (PC2) explained 5.45% of the variation, and separated individuals within the “urban” habitat, despite the short geographical distances among them. The Admixture analysis for $K = 2$ generic clusters showed no clear separation between habitats (Figure S1), denoting gene flow across the whole population. Based on these results, we grouped NAT and RUR as a single group of non-urban birds for further analyses. Mean heterozygosity did not differ significantly between urban and non-urban birds. Individual inbreeding coefficients were slightly negative across all samples (Table S1), indicating no evidence of inbreeding. This pattern is consistent with a slight excess of heterozygosity across loci. Accordingly, a limited number of SNPs deviated from Hardy–Weinberg expectations ($p < 0.05$), indicating no substantial genome-wide departure from equilibrium.

Signature of selection

The iHS values, visualised through a Manhattan plot (Figure S2), did not reveal any clear patterns of selective sweeps within either habitat. By contrast, CSS analysis revealed genomic regions under selection in both habitats (Fig. 2) where $-\log_{10}(p\text{-values})$ of the smoothed CSS from the pairwise comparison between urban and non-urban populations are plotted along the genome. This pattern is supported by pairwise correlation plots among the three component tests (Figure S3), where CSS scores showed strong correlations with each of their component statistics (XP-EHH, ΔDAF , and F_{st}). Smoothed CSS scores were generally higher in the urban habitat compared to the non-urban habitat, consistent with the greater number of significant SNPs detected in urban group. False discovery rate (FDR) analysis revealed clusters of candidate SNPs with significant q-values in both habitats (Figure S4), identifying approximately 0.6% significant SNPs in the urban habitat and 0.2% in the non-urban habitat, respectively. Candidate SNPs were observed across all recombination classes (Table S2), with no evident enrichment in any specific category. The permutation test comparing recombination rates between candidate and non-candidate windows was not significant ($p = 0.30$). Similarly, Spearman’s rank correlation analysis showed no significant relationship between recombination rate and the proportion of candidate SNPs per window ($\rho = -0.01$, $p = 0.23$).

Candidate genomic regions under selection are listed in Table 1, along with their corresponding annotated genes. In the urban habitat, a signal of selection was detected on chromosome 1, including the gene *ANKRD50* and two uncharacterized loci (LOC121096262, LOC121096213) annotated as ncRNA, which remained uncharacterized after BLAST searches; and on chromosome 5, including *RELN*, common to both habitats, and *ORC5*. In non-urban habitats, candidate regions under selection were identified on chromosomes 1, 2, 5, and 13. These regions include several genes with diverse biological functions, such as lipid metabolism (*CDS1*, *PIK3CB*) neuronal functions (*NAPEPLD*, and *RELN*) DNA damage response (*BOD1L1*) and circadian rhythm (*PER2*) alongside additional loci listed in Table 1.

Table 1
Candidate genes located within significant selective regions in urban and non-urban habitat. Coordinates indicate the genomic intervals of significant SNPs.

chr	region_start	region_end	candidate_snps	gene	population
1	6000001	6100000	103	LOC121096213	urban
1	6100001	6300000	139	ANKRD50	urban
1	6100001	6200000	75	LOC121096262	urban
5	76300001	76700000	90	RELN	urban
5	76600001	76700000	54	ORC5	urban
1	16900001	17000000	6	CDS1	non-urban
1	16900001	17000000	6	NKX6-1	non-urban
1	53600001	53700000	5	BOD1L1	non-urban
1	53600001	53700000	5	LOC121088338	non-urban
1	53600001	53700000	5	LOC121088347	non-urban
1	53600001	53700000	5	NKX3-2	non-urban
2	62600001	62700000	9	FAM155A	non-urban
5	76200001	76300000	8	DNAJC2	non-urban
5	76200001	76300000	8	NAPEPLD	non-urban
5	76200001	76300000	8	PMPCB	non-urban
5	76200001	76300000	8	PSMC2	non-urban
5	76200001	76300000	8	SLC26A5	non-urban
5	76300001	76400000	6	RELN	non-urban
13	6800001	6900000	6	CAB39	non-urban
13	6800001	6900000	6	ITM2C	non-urban
13	7000001	7100000	13	LOC121096813	non-urban
13	7000001	7100000	13	LOC121097025	non-urban
13	7000001	7100000	13	PER2	non-urban
13	7000001	7200000	21	PIK3CB	non-urban

Discussion

Our work shows that kestrels maintain substantial gene flow between urban and non-urban habitats, resulting in a largely panmictic population. This pattern is consistent with dispersal of juveniles after fledging over long distances, which counterbalances the sedentary habits of adults, preventing local inbreeding (Damiani et al., 2022). Although PCA revealed weak differentiation between habitats, the low proportion of explained variance and the overlap among individuals indicate the absence of clear population subdivision, a pattern further supported by admixture results. Consistently, heterozygosity did not differ between habitats and inbreeding coefficients were slightly negative, providing no evidence of inbreeding. These results indicate that demographic subdivision is negligible, and suggest that the observed genetic differences might be driven by habitat-specific selective pressures in our study area.

Signature of selection

In line with findings on urban great tits (Salmón et al., 2021), where selective sweeps were associated with functional genes rather than shared haplotypes across urban populations, our haplotype-based (iHS) analysis revealed no evidence of recent haplotypic sweeps within the urban kestrels, although the power of this statistics may be limited by sample size. In contrast, the composite selection signal analysis identified signals of selection in proximity to functional genes, suggesting that selective processes may contribute to local adaptation in both urban and non-urban kestrels. No significant relationship was detected between recombination rate and the distribution of candidate SNPs, suggesting that recombination might not be a major factor shaping the observed selection signals.

In urban kestrels, the CSS approach identified a strong candidate region on chromosome 1 containing the gene *ANKRD50*, which is involved in endosome-to-plasma membrane transport (Kvainickas et al., 2017). Previous studies associated this gene with Newcastle Disease Virus (NDV) infection (Deist et al., 2017; Rowland et al., 2018). NDV, caused by avian *avulavirus 1* (formerly *avian paramyxovirus type 1*), is a highly contagious avian pathogen with mortality rates of up to 80% (Kitalyi, 1998). Therefore, the signal of selection near this gene in urban kestrels could suggest a pathogen-mediated selection associated with denser populations and, possibly, contact with urban pigeons, which are particularly exposed to this virus (Biancifiori and Fioroni, 1983). This result is consistent with prior work that found transcriptomic evidence of altered immune pathways in urban kestrels (Damiani et al. 2024). Similarly, Mascarenhas et al. (2023) identified outlier loci potentially associated with urban adaptation in the bananaquit (*Coereba flaveola*), including a gene involved in the immunological response (Endogenous retrovirus group K member 113 Pol protein-like, *ERVVK113*). Overall, these findings point to immune-related processes as possible important mechanisms driving adaptation of animals to city life.

A gene involved in neuronal functions, *RELN*, was identified as a candidate in both habitats, with a stronger signal (i.e., a higher number of associated SNPs) in urban kestrels (see Table 1) (Minor et al., 2018; Bentz et al., 2021). Selection on this gene in urban kestrels may reflect neurobiological adjustments to the cognitive and sensory challenges imposed by cities. Urban birds often exhibit enhanced problem-solving abilities and behavioural flexibility, traits that can improve foraging efficiency and reproductive success in anthropogenic habitats (e.g., Preiszner et al., 2017). Although evidence for

genetically based cognitive divergence in urban birds remains mixed, work on great tits shows that inhibitory control has a heritable component and is associated with variation in neurotransmitter-related genes (Thompson et al., 2025). Further work will be needed to assess whether these genetic changes facilitate behavioural flexibility and cognitive performance in urban landscapes. Non-urban kestrels experienced selective pressures on genes involved in lipid metabolism, specifically *CDS1* and *PIK3CB*. Both genes play central roles in the regulation of membrane lipid composition and intracellular metabolic signalling (Margarita et al., 2019; Xu et al., 2022). The identification of these genes under selection in non-urban kestrels is consistent with previous transcriptomic evidence from the same population, where non-urban nestlings showed differential expression of genes associated with lipid metabolism, insulin signalling, and carbon and pyruvate pathways (Damiani et al., 2024). Thus, urban environments might impose metabolic constraints or energetic demands that differ from those in non-urban habitats, potentially linked to altered diet composition, prey availability, or energetic costs associated with city life. In the same context, we identified *NAPEPLD* as a candidate gene in non-urban kestrels. This gene is involved in endocannabinoid signaling, a pathway derived from membrane lipids that regulates neuronal processes, metabolism, and responses to environmental stress. This pathway plays a role in the regulation of energy balance and food intake in chickens, where NAPE-PLD is upregulated in the hypothalamus in response to low-energy diets, supporting its involvement in metabolic adaptation to nutritional challenges (Jehl et al., 2019). Addressing differences in diet among habitats would be important to assess the role of nutrition in driving selection on *NAPEPLD*.

In addition, the gene *FAM155A*, also identified as a candidate in non-urban kestrels, was associated with reproductive and immune performance in sheep and partridge chicks (Pasandideh et al., 2020; Zhang et al., 2024), and body mass increase in pigs (Cai et al., 2022). It might be that non-urban kestrels nesting in Mediterranean natural habitats experience selective pressures that impinge on the regulation of growth, metabolism, and reproduction, consistent with previous findings on gene expression (Damiani et al., 2024).

In prior work, we observed habitat-related differences in blood concentration of DNA damage (Giovanetti et al., 2024) and expression of genes associated with DNA damage (Damiani et al., 2024). Thus, urban and non-urban kestrels might differ in both amount of DNA damage generated but also in the capacity to repair it. The gene *BOD1L1*, identified as a candidate in our analysis, is involved in the maintenance of genome stability and DNA damage response including double-stranded break repair (Kucharski et al., 2025). Thus, these results might support a potential role of DNA repair processes in shaping habitat-specific selective pressures.

Finally, urbanisation can profoundly influence circadian and photoperiodic regulation in birds (e.g., Dominoni, 2015). Light pollution represents one of the most pervasive anthropogenic disturbances in cities, and may contribute to the divergent selective pressures observed between urban and non-urban habitats. *PER2* is a well-established regulator of circadian rhythms in birds (Yoshimura et al., 2000), and its selection in non-urban individuals may reflect contrasting light environments and associated physiological demands across habitats. Further research is needed to elucidate how altered light-dark

cycles in urban areas modulate circadian pathways and potentially drive evolutionary responses in urban-dwelling bird populations.

In conclusion, our study provides novel evidence for habitat-driven selection on specific genes in a panmictic bird population. Immune-related genes were consistently highlighted, supporting the idea that pathogen exposure might shape adaptive responses in both urban and non-urban habitats. These findings align with recent genomic studies in urban birds showing selection on immune, metabolic, and oxidative stress pathways (Mascarenhas et al., 2023). Future studies linking genotype to phenotypic are crucial to understand the evolutionary mechanisms underlying bird adaptation to urban environments. The lack of replication across multiple urban and non-urban populations limits the generality of our conclusions, and leaves open the possibility that some differentiated loci reflect demographic processes rather than selection. Expanding this approach to additional areas will be essential to identify genomic signatures of urban adaptation in birds of prey and other vertebrates.

Declarations

Funding statement

Data collection and analyses were funded by Ornis italica (Italy). G. Damiani was supported by a PhD scholarship D.M. 117/2023 funded by 'NextGenerationEU, National Recovery and Resilience Plan (NRRP) of the Italian Ministry for University and Research (MUR)', and by Technosmart Europe srl. S. Messina was funded by the NRRP of the MUR, through the Young Researchers Program (ID: SOE_0000189).

Acknowledgements

We thank Terna s.p.a. and ACEA s.p.a. for granting access to the power lines and the nest boxes. The work was authorised by the Institute for Environmental Protection and Research ISPRA (protocol n. 12495/2020 and 11640/2022) and the Regione Lazio (protocol n. G03417/2020 and G04115/2022).

References

1. Alberti M (2015) Eco-evolutionary dynamics in an urbanizing planet. *Trends Ecol Evol* 30:114–126
2. Alberti M, Correa C, Marzluff JM, Hendry AP, Palkovacs EP, Gotanda KM, Hunt VM, Apgar TM, Zhou Y 2017 Global urban signatures of phenotypic change in animal and plant populations. *Proc. Natl. Acad. Sci. USA* 114, 8951–8956
3. Alexander DH, Novembre J, Lange K (2009) Fast model-based estimation of ancestry in unrelated individuals. *Genome Res* 19:1655–1664
4. Aronson MF, La Sorte FA, Nilon CH, Katti M, Goddard MA, Lepczyk CA, Warren PS, Williams NSG, Cilliers S, Clarkson B, Dobbs C, Dolan R, Hedblom M, Klotz S, Kooijmans JL, Kühn I, MacGregor-Fors I, McDonnell M, Mörtberg U, Pyšek P, Siebert S, Sushinsky J, Werner P, Winter M 2014 A global

- analysis of the impacts of urbanization on bird and plant diversity reveals key anthropogenic drivers. *Proc.R. Soc. Lond., B* – 281, 20133330
5. Audet J-N, Ducatez S, Lefebvre L (2016) The town bird and the country bird: problem solving and immunocompetence vary with urbanization. *Behav Ecol* 27:637–644
 6. Bentz AB, George EM, Wolf SE, Rusch DB, Podicheti R, Buechlein A, Rosvall KA (2021) Experimental competition induces immediate and lasting effects on the neurogenome in free-living female birds. *Proc. Nat. Acad. Sci.* 118, e2016154118
 7. Biancifiori F, Fioroni A (1983) An occurrence of Newcastle disease in pigeons: virological and serological studies on the isolates. *Comp Immunol Microbiol Infec Dis* 6:247–252
 8. Bolger AM, Lohse M, Usadel B (2014) Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* 30:2114–2120
 9. Browning BL, Zhou Y, Browning SR 2018 A one-penny imputed genome from next-generation reference panels. *Am J Hum Genet* 103, 338–348
 10. Cai Z, Christensen OF, Lund MS, Ostersen T, Sahana G (2022) Large-scale association study on daily weight gain in pigs reveals overlap of genetic factors for growth in humans. *BMC Genomics* 23:133
 11. Camacho C, Coulouris G, Avagyan V (2009) BLAST+: architecture and applications. *BMC Bioinformatics* 10:421
 12. Casagrande S, Dell’Omo G, Costantini D, Tagliavini J (2006) Genetic differences between early- and late-breeding Eurasian kestrels. *Evol Ecol Res* 8:1029–1038
 13. Corsini M, Schöll EM, Di Lecce I, Chatelain M, Dubiec A, Szulkin M 2021 Growing in the city: urban evolutionary ecology of avian growth rates. *Evol Appl* 14, 69–84
 14. Costantini D, Dell’Omo G (2020) The kestrel: ecology, behaviour and conservation of an open-land predator. Cambridge University Press
 15. Costantini D, Messina S, Sebastiano M, Marasco V (2025) Life at new extremes: integrating stress physiology and the bio-exposome in the Anthropocene. *Ann NY Acad Sci* 1548:66–79
 16. Crooks KR, Sanjayan M (eds) (2006) Connectivity conservation, vol 14. Cambridge University Press
 17. Damiani G, Jennings V, Dell’Omo G, Costantini D (2022) GPS-tracking reveals annual variation in home-range and sedentary behaviour in Common Kestrels breeding in central Italy. *Avocetta* 46:87–96
 18. Damiani G, Sebastiano M, Dell’Omo G, Costantini D (2024) Blood transcriptome analysis of common kestrel nestlings living in urban and non-urban environments. *Sci Tot Environ* 928:172585
 19. Damiani G, Dell’Omo G, Costantini D (2025) City life anticipates the breeding of a bird of prey without affecting its reproductive success. *Environ Res* 273:121235
 20. Danecek P, Auton A, Abecasis G, Albers CA, Banks E, De Pisto MA, Handsaker RE, Lunte G, Marth GT, Sherry ST, McVean G, Durbin R, Group GPA 2011 The variant call format and VCFtools. *Bioinformatics* 27, 2156–2158

21. Danecek P, Bonfield JK, Liddl J, Marshall J, Ohan V, Pollard MO, Whitwham A, Keane T, McCarthy SA, Davies RM, Li H 2021 Twelve years of SAMtools and BCFtools. *GigaScience* 102), giab008
22. de Satgé J, Strubbe D, Elst J, De Laet J, Adriaensen F, Matthysen E 2019 Urbanisation lowers great tit *Parus major* breeding success at multiple spatial scales. *J Avian Biol.* 50, 1–15
23. Deist MS, Gallardo RA, Bunn DA, Dekkers JC, Zhou H, Lamont SJ 2017 Resistant and susceptible chicken lines show distinctive responses to Newcastle disease virus infection in the lung transcriptome. *BMC Genomics* 18, 989
24. Dell'Omo G, Costantini D, Wright J, Casagrande S, Shore RF 2008 PCBs in the eggs of eurasian kestrels indicate exposure to local pollution. *Ambio* 37, 452–456
25. Dominoni DM (2015) The effects of light pollution on biological rhythms of birds: an integrated, mechanistic perspective. *J Ornithol* 156:409–418
26. Fugère V, Hendry AP (2018) Human influences on the strength of phenotypic selection. *Proc. Natl. Acad. Sci. USA* 115, 10070–10075
27. Garrison E, Marth G (2012) Haplotype-based variant detection from short-read sequencing. arXiv preprint arXiv :12073907
28. Gautier M, Vitalis R 2012 rehh: an R package to detect footprints of selection in genome-wide SNP data from haplotype structure. *Bioinformatics* 28, 1176–1177
29. Giovanetti L, Caliani I, Damiani G, Dell'Omo G, Costantini D, Casini S 2024 A blood-based multi-biomarker approach reveals different physiological responses of common kestrels to contrasting environments. *Environ Res* 251, 118674
30. Grossman SR, Shylakhter I, Karlsson EK, Byrne EH, Morales S, Frieden G, Hostetter E, Angelino E, Garber M, Zu O, Lander ES, Schaffner SF, Sabeti PC 2010 A composite of multiple signals distinguishes causal variants in regions of positive selection. *Science* 327, 883–886
31. Jehl F, Désert C, Klopp C, Brenet M, Rau A, Leroux S, Boutin M, Lagoutte L, Muret K, BlumY, Esquerré D, Gourichon D, Burlot T, Collin A, Pitel F, Benani A, Zerjal T, Lagarrigue S (2019) Chicken adaptive response to low energy diet: main role of the hypothalamic lipid metabolism revealed by a phenotypic and multi-tissue transcriptomic approach. *BMC Genomics* 20:1033
32. Johnson MTJ, Munshi-South J (2017) Evolution of life in urban environments. *Science* 358:eaam8327
33. Kettel EF, Gentle LK, Quinn JL, Yarnell RW (2018) The breeding performance of raptors in urban landscapes: a review and meta-analysis. *J Ornithol* 159:1–18
34. Kitalyi AJ (1998) Village chicken production systems in rural Africa: household food security and gender issues. Food & Agriculture Organization, Rome
35. Kolberg L, Raudvere U, Kuzmin I, Adler P, Vilo J, Peterson H 2023 g: Profiler—interoperable web service for functional enrichment analysis and gene identifier mapping 2023 update. *Nucleic Acids Res* 51, W207–W212

36. Korneliussen TS, Albrechtsen A, Nielsen R (2014 ANGSD) Analysis of Next Generation Sequencing Data. *BMC Bioinformatics* 15:356
37. Kucharski TJ, Higgs MR, Compton DA, Bechstedt S (2025) A busy BOD1-y: the diverse functions of an intracellular signaling regulatory protein family. *Biochem Societ Transact* 53:1479–1491
38. Kvainickas A, Jimenez Orgaz A, Nägele H, Diedrich B, Heesom KJ, Dengjel J, Cullen PJ, Steinberg F (2017) Retromer-and WASH-dependent sorting of nutrient transporters requires a multivalent interaction network with ANKRD50. *J Cell Sci* 130(2), 382–395
39. Hendry AP, Farrugia TJ, Kinnison MT (2008) Human influences on rates of phenotypic change in wild animal populations. *Mol Ecol* 17:20–29
40. Hendry AP, Gotanda KM, Svensson EI (2017) Human influences on evolution, and the ecological and societal consequences. *Philos Trans R Soc Lond B* 372:20160028
41. Hille SM, Nesje M, Segelbacher G (2003) Genetic structure of kestrel populations and colonization of the Cape Verde archipelago. *Mol Ecol* 12:2145–2151
42. Huang W, Sun X, Zhang X, Xu R, Qian Y, Zhu J (2024) Neural correlates of early-life urbanization and their spatial relationships with gene expression, neurotransmitter, and behavioral domain atlases. *Mol Neurobiol* 61:6407–6422
43. Lawrence M, Huber W, Pagès H, Aboyoun P, Carlson M, Gentleman R, Morgan MT, Carey VJ (2013) Software for computing and annotating genomic ranges. *PLoS Comput Biol* 9, e1003118
44. Li H, Durbin R (2009) Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics* 25:1754–1760
45. Low GW, Chattopadhyay B, Garg KM, Irestedt M, Ericson PG, Yap G, Tang Q, Wu S, Rheindt FE (2018) Urban landscape genomics identifies fine-scale gene flow patterns in an avian invasive. *Heredity* 120, 138–153
46. Margaria JP, Ratto E, Gozzelino L, Li H, Hirsch E (2019) Class II PI3Ks at the intersection between signal transduction and membrane trafficking. *Biomolecules* 9:104
47. Mascarenhas R, Meirelles PM, Batalha-Filho H (2023) Urbanization drives adaptive evolution in a Neotropical bird. *Curr Zool* 69:607–619
48. Michaiel AM, Bernard A (2022) Neurobiology and changing ecosystems: Toward understanding the impact of anthropogenic influences on neurons and circuits. *Front Neural Circuits* 16:995354
49. Minor KM, Letko A, Becker D, Drögemüller M, Mandigers PJJ, Bellekom SR, Drögemüller C (2018) Canine NAPEPLD-associated models of human myelin disorders. *Sci Rep* 8:5818
50. Munshi-South J, Zoln CP, Harris SE (2016) Population genomics of the Anthropocene: Urbanization is negatively associated with genome-wide variation in white-footed mouse populations. *Evol Appl* 9:546–564
51. Negro JJ, Prenda J, Ferrero JJ, Rodríguez A, Reig-Ferrer A (2020) A timeline for the urbanization of wild birds: the case of the lesser kestrel. *Quat Sci Rev* 249, 106638

52. Owen JC (2011) Collecting, processing, and storing avian blood: a review. *J Field Ornithol* 82:339–354
53. Pasandideh M, Gholizadeh M, Rahimi-Mianji G (2020) Identification of two novel SNPs affecting lambing traits in sheep by using a 50K SNP-Chip. *Small Ruminant Res* 191:106193
54. Perrier C, Lozano del Campo A, Szulkin M, Demeyrier V, Gregoire A, Charmantier A 2018 Great tits and the city: distribution of genomic diversity and gene–environment associations along an urbanization gradient. *Evol Appl* 11, 593–613
55. Preiszner B, Papp S, Pipoly I, Seress G, Vincze E, Liker A, Bókony V (2017) Problem-solving performance and reproductive success of great tits in urban and forest habitats. *Anim Cognit* 20:53–63
56. Purcell S et al (2007) PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am J - Hum Genet* 81:559–575
57. Randhawa IAS, Khatkar MS, Thomson PC, Raadsma HW (2014) Composite selection signals can localize the trait specific genomic regions in multi-breed populations of cattle and sheep. *BMC Genet* 15:34
58. Riegert J, Fainová D, Bystřická D 2010 Genetic variability, body characteristics and reproductive parameters of neighbouring rural and urban common kestrel (*Falco tinnuculus*) populations. *Popul Ecol* 52, 73–79
59. Rowland K, Wolc A, Gallardo RA, Kelly T, Zhou H, Dekkers JC, Lamont SJ (2018) Genetic analysis of a commercial egg laying line challenged with Newcastle disease virus. *Front Genet* 9:326
60. Ruegg KCal et al (2021) The American Kestrel (*Falco sparverius*) genoscape: implications for monitoring, management, and subspecies boundaries. *Auk* 138:ukaa051
61. Rutkowski R, Rejt L, Szczuka A (2006) Analysis of microsatellite polymorphism and genetic differentiation in urban and rural kestrels *Falco tinnunculus* (L. *Pol J Ecol* 54:473–480
62. Rutkowski R, Rejt Ł, Tereba A, Gryczyńska-Sięmiątkowska A, Janic B (2010) Population genetic structure of the European kestrel *Falco tinnunculus* in Central Poland. *Eur J Wild Res* 56:297–305
63. Sabeti PC al.2002 Detecting recent positive selection in the human genome from haplotype structure. *Nature* 419, 832–837
64. Sabeti PC et al 2007 Genome-wide detection and characterization of positive selection in human populations. *Nature* 449, 913–918
65. Salmón P, Jacobs A, Ahrén D, Biard C, Dingemanse NJ, Dominoni DM, Helm B, Lundberg M, Senar JC, Sprau P, Visser ME, Isaksson C 2021 Continent-wide genomic signatures of adaptation to urbanisation in a songbird across Europe. *Nat Comm* 12, 2983
66. Schell CJ (2018) Urban evolutionary ecology and the potential benefits of implementing genomics. *J Hered* 1092:138–151
67. Smeds L, Qvarnström A, Ellegren H (2016) Direct estimate of the rate of germline mutation in a bird. *Genom Res* 26:1211–1218

68. Storey JD (2002) A direct approach to false discovery rates. *J R Stat Soc* 64:479–498
69. Strimmer K 2008 fdrtool: a versatile R package for estimating local and tail area-based false discovery rates. *Bioinformatics* 24, 1461–1462
70. Sumasgutner P, Nemeth E, Tebb G, Krenn HW, Gamauf A (2014) Hard times in the city—attractive nest sites but insufficient food supply lead to low reproduction rates in a bird of prey. *Front Zool* 11:48
71. Tan A, Abecasis GR, Kang HM (2015) Unified representation of genetic variants. *Bioinformatics* 31:2202–2204
72. Thompson MJ, Gervais L, Bharath D, Caro SP, Chainé AS, Perrier C, Réale D, Charmantier A (2025) Tit wit: environmental and genetic drivers of cognitive variation along an urbanization gradient. *Anim Cognit* 28:56
73. United Nations, Department of Economics (2024) World Population Prospects 2024: Summary of Results. Stylus Publishing, LLC, NY
74. Village A (2010) The kestrel. A&C Black
75. Voight BF, Kudaravalli S, Wen X, Pritchard JK (2006) A map of recent positive selection in the human genome. *PLoS Biol* 4:e72
76. Wang H, Zhao X, Wen J, Wang C, Zhang X, Ren X, Zhang J, Li H, Muhatai G, Qu L 2023 Comparative population genomics analysis uncovers genomic footprints and genes influencing body weight trait in Chinese indigenous chicken. *Poult Sci* 102, 103031
77. Weir BS, Cockerham CC (1984) Estimating F-statistics for the analysis of population structure. *Evol* 38:1358–1370
78. Winchell KM, Reynolds RG, Prado-Irwi SR, Puente-Rolón AR, Revell LJ (2016) Phenotypic shifts in urban areas in the tropical lizard *Anolis cristatellus*. *Evolution* 70:1009–1022
79. Xu Y, Zhang S, Guo Y, Gao L, Zhang H, Chen W, Huang Y (2022) Chicken CDS2 isoforms presented distinct spatio-temporal expression pattern and regulated by insulin in a breed-specific manner. *Poult Sci* 101:101893
80. Yasumura M et al (2014) IL1RAPL1 knockout mice show spine density decrease, learning deficiency, hyperactivity and reduced anxiety-like behaviours. *Sci Rep* 4:6613
81. Yoshimura T, Suzuki Y, Makino E, Suzuki T, Kuroiwa A, Matsuda Y, Ebihara S (2000) Molecular analysis of avian circadian clock genes. *Molec Br Res* 78:207–215
82. Zhang X, Yang L, Xie Z, Gan J, Zh P, Song J, Kang H, Zhang Z, Liu L, Xiang H, Li H 2024 Genetic assessment and candidate genes identification for breed-specific characteristics of Qingyuan partridge chicken based on runs of homozygosity. *BMC Genomics* 25, 577

Figures

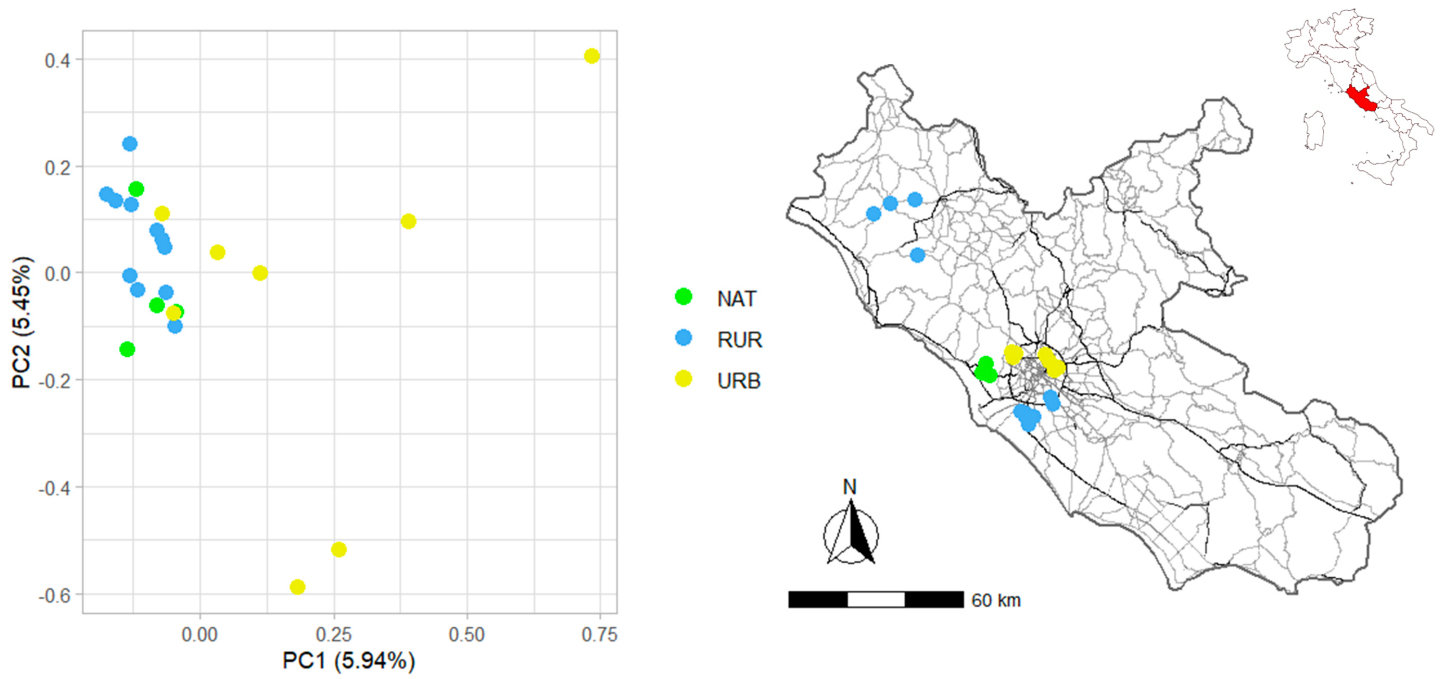


Figure 1

(Left panel) Principal component analysis (PCA) of urban (URB), rural (RUR) and natural (NAT) common kestrels performed on 469,867 SNPs filtered per linkage disequilibrium. The first principal component (PC1 = 5.94%) shows a partial genetic segregation between urban and non-urban individuals, while the second principal component (PC2 = 5.4%) suggests a further differentiation within the urban population. (Right panel) Geographic distribution of sampled individuals.

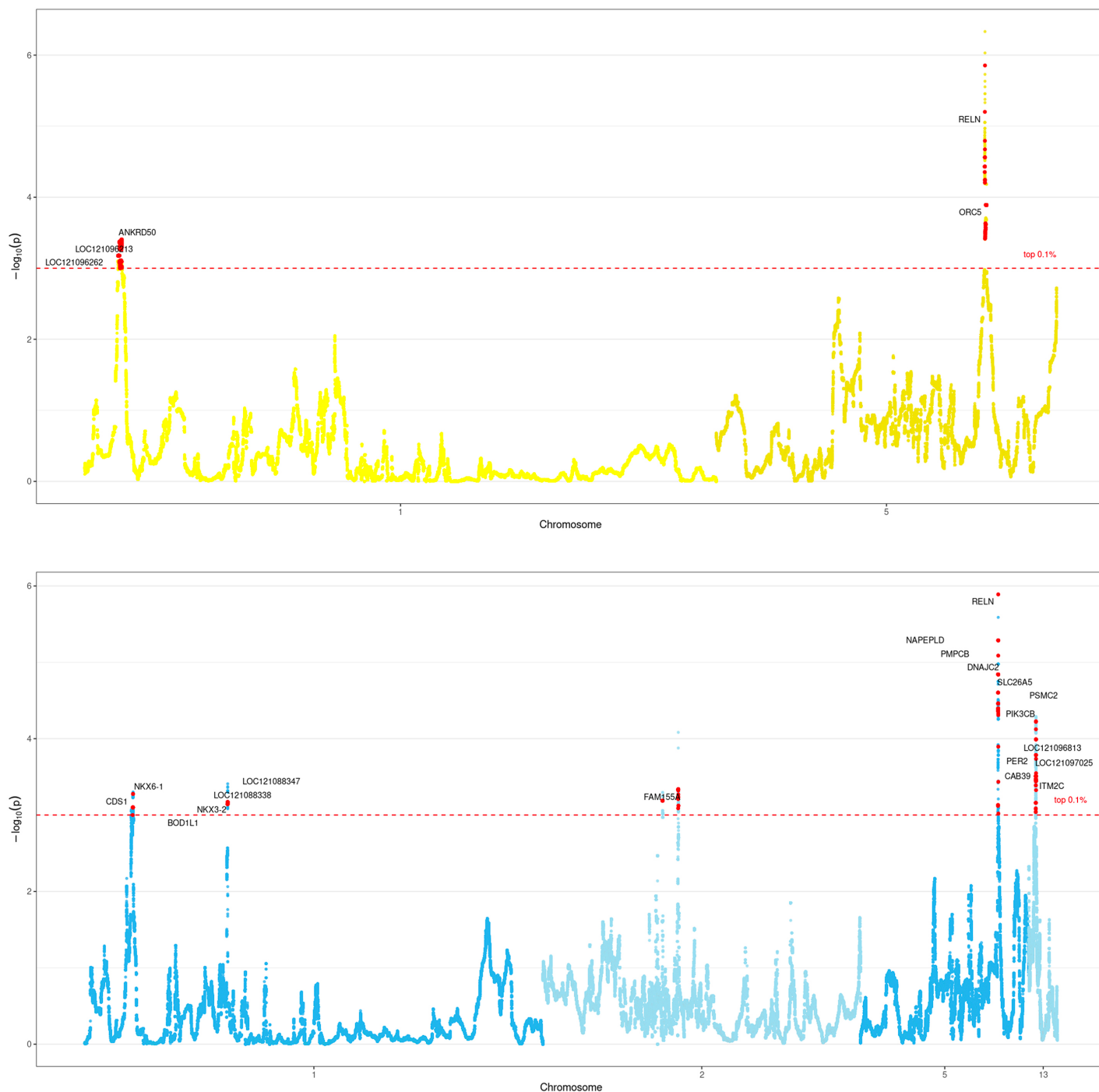


Figure 2

Manhattan plot of $-\log_{10}(p)$ values of smoothed CSS in urban (top, yellow) and non-urban (bottom, blue) population. The dotted red line indicates the genome wide 0.1% (upper cut-off) thresholds of the empirical smoothed scores. Loci exceeding this threshold and remaining significant after False Discovery Rate (FDR) correction are shown as red points. Positions of candidate genes putatively under selection are labelled with their gene names.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [graphicalabstract.jpg](#)
- [Supplementarynew.docx](#)