Comparative Urban Evolution in Two Closely Related Desert Songbirds, Northern Cardinals and Pyrrhuloxia

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**Abstract**

Urbanization has reshaped ecosystems globally and has created new selective regimes to which wild animals must adapt if they are to persist in the new urban ecosystem. Urbanization presents a variety of additional novel challenges (e.g. artificial light at night, noise pollution, novel resource distributions, etc.) that may differently affect native taxa. Selection by urbanization on genes involved in behavior, cognition, and immune function has been demonstrated in several avian species. Studies of closely related sympatric species may shed new light on how organisms experience genetic change in urban settings. Here we analyzed whole genome sequences of northern cardinals (*Cardinalis cardinalis*) and pyrrhuloxia (*C. sinuatus*) from urban and rural areas of Arizona, USA to test for the presence of urban-related parallel evolution and introgression. We identified genes involved in neural function, muscle development, blood physiology, and immune function across both species that appear to have undergone urban selection. We demonstrate evidence of parallel urban selection on the same genes in both cardinal species and between our focal species and two other avian taxa in the literature. Our findings emphasize the novel dynamics of behavior, energetics, and immune challenges faced by native species in a rapidly urbanizing world.

**Introduction**

Urbanization can reshape natural selective environments and create novel challenges for wild species. Genetic variation underlying phenotypic changes associated with urbanization has been documented in several animal species, including genes that affect boldness behavior (Mueller et al., 2013; van Dongen et al., 2015), cognitive ability (Mueller et al., 2020), and immune function (Minias, 2023; Pikus et al., 2021).

The effects of urbanization can be consistent within a species across different urban environments (e.g. Mueller et al., 2013; Winchell et al., 2023). For example, great tits (*Parus major*) display parallel evolution in neural and developmental genes in urban environments across the European continent (Salmón et al., 2021). Likewise, burrowing owls (*Athene cunicularia*) also show parallel evolution in genes related to cognition across many South American cities (Mueller et al., 2020). Yet despite these lines of evidence for parallelism in urban evolutionary responses, recent epigenetic research has found differences in even the fine-scale mechanisms that underlie species responses to urbanization (Caizergues et al., 2022; McNew et al., 2017). Whether selection resulting from urbanization acts on the same genes in closely related species remains an under-explored area of research.

Species that share a similar genetic background and a similar ecological niche yet differ in their persistence in an urban environment present a unique opportunity to identify potential genetic mechanisms underlying species responses to urbanization. Northern cardinals (*Cardinalis cardinalis*) and pyrrhuloxia (*Cardinalis sinuatus*) both occupy the metropolitan area of Tucson, Arizona and largely share an ecological niche, but northern cardinals occupy more highly urbanized areas than pyrrhuloxia (Jackson and McGraw unpublished data). Although northern cardinals and pyrrhuloxia are 5.1 million years divergent (CI: 4.2 - 6.0 MY; Barker et al., 2015; Hooper & Price, 2017; Kumar et al., 2017) they also infrequently hybridize, although no wild hybrids have been genetically confirmed, and genomic data demonstrate no evidence of introgression (Kaiya Provost pers. comm.). Slight differences arising from the largely similar genetic backgrounds of these two species may therefore underlie the differences in urban persistence, and a comparison of the two may illuminate mechanisms associated with urban adaptation.

We analyzed whole genomes of urban and rural northern cardinals and pyrrhuloxia to identify regions that may have undergone positive selection in the urban environment. We then tested for overrepresented Gene Ontology terms and for evidence of parallel selection across taxa at the gene level. We predicted that pyrrhuloxia would show a fainter signal of selection, but that some similar genes would be under selection in both species.

**Methods**

Tissue collection, DNA sequencing, and SNP filtering:

Between 2021-2022, we collected blood samples from a total of 12 birds at residences around Tucson, Arizona spanning approximately 22 miles of the city: 6 northern cardinals and 6 pyrrhuloxia (Table 1, Figure 1). *Cardinalis* species in rural areas were difficult if not impossible to capture in part due to fieldwork limitations during the COVID-19 pandemic, so we also accessed muscle tissue samples from 12 birds (6 northern cardinals and 6 pyrrhuloxia) from the University of Washington Burke Museum and the Museum of Southwestern Biology. This produced a sample size of 6 per species per population (urban vs. rural), which is sufficient for identifying outlier regions between populations (Hahn, 2018). We extracted DNA from each sample at the University of Colorado using a Qiagen DNeasy Blood & Tissue kit, and we measured DNA concentrations on a Thermofisher Qubit 3.0. Whole genome paired-end 150 base pair sequencing libraries were prepared and sequenced using the Illumina NovaSeq 6000 platform by Novogene, Sacramento, CA at approximately 5x coverage.

All code used to analyze these genomes can be found on GitHub (<https://github.com/dannyjackson/CardinalisGenomics>; will publish a release with a DOI before submitting). We trimmed raw sequence fasta files using Trimmomatic (Bolger et al., 2014) and analyzed for quality using FastQC (Andrews, 2010). The publicly available annotated northern cardinal genome is not chromosome-level and each scaffold could not reliably be assigned as either an autosome or sex chromosome; therefore, we used bwa mem (Li & Durbin, 2009) to align the trimmed sequence reads to the reference small tree finch (Camarhynchus parvulus) genome STF-HiC (1.28 GB; Genbank accession number: GCF\_901933205.1). The small tree finch is the closest relative with an available chromosome-level genome. Then we sorted the bam files using samtools (H. Li et al., 2009) and picard-tools (Picard, 2019), clipped overlapping read pairs using BamUtil (Jun et al., 2015), realigned around indels using GATK (3.7, McKenna et al., 2010) and indexed the resulting using samtools and picard-tools. We dropped all scaffolds and sex chromosomes from downstream analyses. We used angsd (Korneliussen et al., 2014) to generate a list of varying sites with a minimum SNP p-value of 1e-6, minimum individual depth of 4, minimum of 20 individuals, minimum minor allele frequency of 0.05, and minimum mapping quality of 30. This left 2,618,696 SNPs. We additionally tested for and did not find evidence of batch effects between museum and field collected samples (Lou & Therkildsen, 2022). After filtering, we had an average depth of coverage of 5.08 across all samples.

Population Structure Analyses

We analyzed relationships between our samples using Principal Component Analyses (PCA) and ADMIXTURE. We first used angsd to generate a bcf file which we input into plink to filter for unlinked SNPs (--indep-pairwise 50kb 1 0.5). We used pcangsd to output a covariance matrix from which we computed eigenvectors that we visualized in R and to output an ADMIXTURE plot. These revealed that two of our rural pyrrhuloxia samples from eastern Arizona may be close relatives, so we removed the individual with lower depth of coverage from all downstream analyses (UWBM77718). We show results from the population structure analyses without that individual, but the full results are in the supplement (Figure S1).

Tests for positive selection and introgression

To identify regions of the genome that have either recently undergone positive selection due to urbanization or that are associated with urban versus rural areas, we used two statistical approaches. First, we employed FST scans to compare urban populations with rural populations of the same species using 50 kb windows with a step size of 12.5 kb (Da Silva Ribeiro et al., 2022). Then, we employed RAiSD to compute μ statistics across genomes from the urban population and separately across genomes from the rural population. We considered regions that were significant within urban but not within rural populations of the same species to be regions of relevance to selection within an urban environment. RAiSD determines window sizes by number of informative sites rather than total base pair numbers, so we used windows containing 50 SNPs. For both analyses, we retained any region in the top 1% of statistics (either FST or μ) for GO term analysis.

To identify general selective trends, we tested for significant overrepresentation of Gene Ontology (GO) terms among gene lists within the top 1% of these analyses using the PANTHER classification system. We compared the lists of genes of interest to a filtered list of all genes in the reference genome. To curate our list of background genes, we filtered out any site with low mappability (< 0.95 SNP calling rate in SNPable http://lh3lh3.users.sourceforge.net/snpable.shtml) and with less than 3x average depth from our unfiltered bams or greater than 2 standard deviations above the mean depth of the sample (28.15). We also excluded any significant genes that may have been identified in these regions, to reduce errors from poor alignment. Genes excluded from the background gene list were also removed from any ultimate lists of candidate genes, as we inferred that they were in regions prone to error. We corrected for multiple testing by using a false discovery rate using the Benjamini-Hochberg FDR test with a significance level of *q* < 0.2 (Z.-H. Li et al., 2013; Osborn et al., 2013; Wagner et al., 2020). Because FST and RAiSD are sensitive to different evolutionary processes, we tested for overrepresented GO terms for each species in a combined list of genes identified by both programs as well as independent lists from each statistic. We visualized the overrepresented GO terms in rrvgo (Sayols, 2023), which produces a scatter plot from the PCoA of the dissimilarity matrix of the semantic similarity of overrepresented GO terms. Terms in these plots cluster by meaning, but the units of the axes are meaningless and are therefore not included.

Finally, to test for significant parallel evolution in response to urbanization across avian taxa, we compared our lists of candidate genes between cardinal species and with lists curated in two major investigations of avian urban genomic responses. One of these studies investigated urban adaptation of burrowing owls (*Athene cunicularia*) across multiple cities in Argentina and the other investigated urban adaptation of great tits (*Parus major*) across multiple European cities (Mueller et al., 2020; Salmón et al., 2021). We first tested for significant overlap between taxa and then highlight genes that show signatures of selection across multiple taxa.

**Results**

Population Structure Analyses (PCA, admixture)

All individuals first separated out species along PC1 (28.84% variation). PC2 (3.78% variation) separated northern cardinals by ecotype, with clusters associated with the Sonoran basin, montane conifer forests, and the Madrean archipelago, while pyrrhuloxia did not segregate distinctly by ecotype; neither species clustered by urbanization category (Figure 1). The ADMIXTURE analysis recovered no clusters below the species level but showed more variation within northern cardinals that suggests a genomic cline from the Madrean archipelago to the montane conifer forests to the Sonoran basin.

Overrepresented GO Terms

In northern cardinals, GO terms related to blood physiology (e.g. GO:0070527 platelet aggregation, *q* = 0.016), tissue integrity (e.g. GO:0098609 cell-cell adhesion, *q* = 0.022), muscle development (e.g. GO:0007519 skeletal muscle tissue development, *q* = 0.058), and response to external stimuli (GO: 0032102 negative regulation of response to external stimulus, *q* = 0.022) emerged as overrepresented categories in analyses of genes identified by the combination of FST and RAiSD (Figure 2). Two genes drove the signal associated with blood physiology, FGA (fibrinogen alpha chain) and FGB (fibrinogen beta chain), both of which are located in an ~15kb long segment of chromosome 4. Analyses of genes identified in just FST or RAiSD revealed similar patterns, with the addition of two GO terms that were overrepresented in the genes identified by FST (GO:1901564 organonitrogen compound metabolic process, *q* = 0.124) and in RAiSD (GO:0050878, regulation of body fluid levels, *q* = 0.013).

In pyrrhuloxia, no genes passed the FDR threshold from the combined list of FST and RAiSD outliers. In FST outliers, organic cyclic compound metabolic processes, nervous system development, tissue regeneration, and insulin-like growth factor receptor signaling pathway each emerged (Figure 3). Tissue regeneration was overrepresented in the outliers identified by RAiSD (Figure 3). Two GO terms – GO:0031099 regeneration and GO:0042246 tissue regeneration – were identified as overrepresented in both FST and RAiSD gene lists. Two candidate genes are involved in the insulin-like growth factor (IGF) receptor signaling pathway, GIGYF2 (GRB10 Interacting GYF Protein) and IGF1 (Insulin Like Growth Factor 1). We identified 15 candidate genes associated with nervous system development.

Across GO categories identified as associated with urbanization, more genes associated with GO terms related to blood physiology and muscle development showed a signal of selection in northern cardinals compared to pyrrhuloxia (Figure S2). Additionally, northern cardinals showed more representation of genes associated with negative regulation of response to external stimulus (GO:0032102). Pyrrhuloxia showed greater representations of genes in various disjunct categories, including insulin-like growth factor receptor signaling pathway (GO:0048009), tissue regeneration (GO:0042246), and regulation of phosphatase activity (GO:004666).

Parallel Evolution

Pairwise comparisons of species found significant evidence of overlapping genes in five of the six pairs (northern cardinals and pyrrhuloxia q = 1.32x10-15, northern cardinals and burrowing owls q = 2.81x10-05, northern cardinals and great tits q = 0.012, pyrrhuloxia and burrowing owls q = 0.008, burrowing owls and great tits q = 0.012; FDR corrected Fisher’s exact test), with only pyrrhuloxia and great tits not demonstrating significant overlap (q = 0.601).

Between cardinal species, 132 genes were identified by at least one analysis in both species but no GO terms were overrepresented in the list of overlapping genes. The three genes identified by both programs in both species are CDKAL1 (CDK5 Regulatory Subunit Associated Protein 1 Like 1), COL15A1 (Collagen Type XV Alpha 1 Chain), and TGFBR1 (Transforming Growth Factor Beta Receptor 1), which are all present within the same 3.4 Mb block of chromosome 2 and which shows distinct evidence of a selective sweep in urban pyrrhuloxia (Figure 4, Figure S3).

No genes were identified as a candidate gene associated with urbanization in all four species, but one was identified in three of the four species (NRXN3; found in northern cardinals, burrowing owls, and great tits; Figure 5). 45 genes were identified in one of the two cardinal species and in one additional non-cardinal taxa. In this list of 45 genes, GO terms associated with muscle development (e.g. GO:0090257 regulation of muscle system process *q* = 0.041) and neuronal processes (e.g. GO:0007399 nervous system development, *q* < 0.001) were overrepresented.

**Discussion**

We demonstrate a polygenic response to urbanization by two cardinal species with important differences that may underlie the increase in urban occupation by northern cardinals compared to pyrrhuloxia. The urban environment appears to have selected for changes to the blood physiology, muscle development, and neural development of both species. Pyrrhuloxia displayed less evidence of a selective response to urbanization, with no significant GO-terms emerging from analyses of the combined list of genes identified across both FST and RAiSD. Northern cardinals are larger than pyrrhuloxia and therefore may have had a more favorable pre-existing muscular physiology that has allowed them to better adapt to the urban environment. The behavioral and hematological differences between these species are understudied but our results suggest that traits in these realms may predict a species’ ability to persist in developed areas, particularly in ecologies similar to the Sonoran Desert.

GO terms illustrate potential functions that selection acted upon for our candidate genes, but these are not always definitive. For example, the two genes that drove the signal associated with blood physiology in northern cardinals, FGA (fibrinogen alpha chain) and FGB (fibrinogen beta chain) influence both clotting and immune system responses. FGA is associated with immunosuppressive activities and has been shown to be downregulated in chickens resistant to Marek’s disease virus (Dong et al., 2017), and both are shown to be downregulated in response to *Riemerella anatipestifer* infection in ducks (Zhou et al., 2013). Clotting may be essential for adaptation to an urban environment, where species may interact with or even consume novel sharp objects including plastics, may be more at risk of harm from an introduced predator like a house cat (*Felis catus*), or may experience intraspecific conflicts at higher rates or with greater intensity over more concentrated resources. The signals of selection on genes involved in tissue regeneration in Pyrrhuloxia support this interpretation. Additionally, changes to blood physiology may improve the species’ responses to extended periods of dehydration. Two additionally identified GO terms support this interpretation: organonitrogen compound metabolic process and regulation of body fluid levels both suggest an adaptation to deal with low-water availability. Alternatively, avian diseases can spread more rapidly through bird feeders or interactions with poultry and released avian pets, and selection may instead act on these genes due to their immune functions. These are not mutually exclusive interpretations, as the same genotype may improve clotting, fluid retention, and immune function.

However, the evidence for selection on muscle function in northern cardinals is more straightforward. Four genes identified in northern cardinals were involved in muscle development. DMD (Dystrophin) and SGCZ (Sarcoglycan Zeta) both produce parts of the dystrophin-associated glycoprotein complex (DGC), which is a fundamental structural protein in skeletal and cardiac muscle cells and regulates insulin receptor activity in these cells with major effects on muscle development (Eid Mutlak et al., 2020). MYF5 and MYF6 (Myogenic Factor 5 and 6), essential factors in muscle tissue development, were also outlier genes. 33 additional genes were identified in various aspects of tissue integrity: cell-adhesion, junction, morphogenesis, and signaling (Table S1). Pyrrhuloxia too showed some evidence of selection on muscle development, particularly with respect to the insulin-like growth factor (IGF) receptor signaling pathway. IGF receptors are associated with muscle development in response to acute exercise or in preparation for migration (Price et al., 2011), rapid nestling growth (Lodjak et al., 2017), more rapid moult (Lendvai et al., 2021), ovarian function (Onagbesan et al., 1999), and increased oxidative stress (Lendvai et al., 2024). IGF receptors also play a role in diabetes and this signal could emerge due to increased carbohydrates in the diets of urban birds (Fall & Ingelsson, 2014).

Three genes were identified by both RAiSD and FST in both northern cardinals and pyrrhuloxia: CDKAL1, COL15A1, and TGFBR1. These three genes are associated with muscle development in different ways. CDKAL1 is associated with obesity in multiple studies (Fall & Ingelsson, 2014) and type 2 diabetes (Ragvin et al., 2010) and is overexpressed in skeletal muscle tissues in women with polycystic ovarian syndrome (Manti et al., 2020), with variants leading to misfolded insulin (Ghosh et al., 2022). COL15A1 is associated with chicken pectoral muscle disorders (Bordini et al., 2022). TGFBR1 expression is associated with corticosterone exposure in tree swallows (Tachycineta bicolor) and with cardovascular disease (Yagyu et al., 2023), asthma (Stikker et al., 2023), and various cancers in humans (Ramer et al., 2016). These diverse pathologies likely arise from TGFBR1 because it mediates inflammation and endothelial cell response to tissue injury (He et al., 2022).

Across multiple forms of analysis, northern cardinals demonstrate stronger signals of selection associated with urbanization. No GO terms were significantly overrepresented in analyses of the full gene list identified in pyrrhuloxia. In comparisons of positively selected gene lists across all urban-associated GO terms, genes were overrepresented in northern cardinals compared to pyrrhuloxia a majority of the time, especially with respect to biological functions that could facilitate adaptation to urbanization (Figure S1). Among other GO terms, northern cardinals demonstrated more genes than pyrrhuloxia related to negative regulation of response to external stimulus. Of note among these genes, SUSD4 (Sushi domain-containing protein 4) can impact behavior, with SUSD4 knockout mice exhibiting anxiety behaviors (Zhu et al., 2020).

The nine genes that were associated with a significant GO term in both taxa – DMD, CDKAL1, NLGN3, SLIT3, SDK1, EPHA10, CHEK1, STT3A, and CACTIN – had a diverse array of functions (Figure 4, Table S1). Not only did we see independent evidence of selection, but our candidate genes also overlapped with findings from burrowing owls and great tits with overrepresentation of both muscle development and neural genes. Several candidate genes of note –PRKN for example – were not associated with any overrepresented GO terms related to neural function in our analyses of either cardinal species. This function only emerged in GO term analyses of overlapping genes identified in cardinals and either burrowing owls or great tits. This highlights the utility of comparative studies across multiple organisms for uncovering patterns in genomic data.

Our analyses uncovered evidence of both divergent selective responses and some parallel selection between northern cardinals and pyrrhuloxia in response to urbanization. Importantly, the genes that we identified are largely associated with muscle development and neural function, both of which have emerged from studies of other avian taxa as core processes that are selected upon by urban environments. We highlight immune functions as an additional axis of important urban adaptation. Despite showing different levels of persistence in an urban environment, both cardinal taxa demonstrate selective responses on similar functional categories, although pyrrhuloxia showed a less consistent signal of selection. Comparative morphological, behavioral, and physiological studies of these taxa informed by our findings could elucidate the phenotypic effects emerging from these genetic differences. This work contributes to the growing body of literature showing the parallel responses of avian taxa to urbanization, and novelly contributes evidence of species-specific genetic nuance in evolution to human disturbance.

**References**

Andrews, S. (2010). *FastQC: A quality control tool for high throughput sequence data.* https://cir.nii.ac.jp/crid/1370584340724053142

Barker, F. K., Burns, K. J., Klicka, J., Lanyon, S. M., & Lovette, I. J. (2015). New insights into New World biogeography: An integrated view from the phylogeny of blackbirds, cardinals, sparrows, tanagers, warblers, and allies. *The Auk*, *132*(2), 333–348. https://doi.org/10.1642/auk-14-110.1

Bolger, A. M., Lohse, M., & Usadel, B. (2014). Trimmomatic: A flexible trimmer for Illumina sequence data. *Bioinformatics*, *30*(15), 2114–2120. https://doi.org/10.1093/bioinformatics/btu170

Bordini, M., Soglia, F., Davoli, R., Zappaterra, M., Petracci, M., & Meluzzi, A. (2022). Molecular Pathways and Key Genes Associated With Breast Width and Protein Content in White Striping and Wooden Breast Chicken Pectoral Muscle. *Frontiers in Physiology*, *13*. https://doi.org/10.3389/fphys.2022.936768

Caizergues, A. E., Le Luyer, J., Grégoire, A., Szulkin, M., Senar, J., Charmantier, A., & Perrier, C. (2022). Epigenetics and the city: Non‐parallel DNA methylation modifications across pairs of urban‐forest Great tit populations. *Evolutionary Applications*, *15*(1), 149–165. https://doi.org/10.1111/eva.13334

Da Silva Ribeiro, T., Galván, J. A., & Pool, J. E. (2022). Maximum SNP *FST* Outperforms Full-Window Statistics for Detecting Soft Sweeps in Local Adaptation. *Genome Biology and Evolution*, *14*(10), evac143. https://doi.org/10.1093/gbe/evac143

Dong, K., Chang, S., Xie, Q., Black-Pyrkosz, A., & Zhang, H. (2017). Comparative transcriptomics of genetically divergent lines of chickens in response to Marek’s disease virus challenge at cytolytic phase. *PLOS ONE*, *12*(6), e0178923. https://doi.org/10.1371/journal.pone.0178923

Eid Mutlak, Y., Aweida, D., Volodin, A., Ayalon, B., Dahan, N., Parnis, A., & Cohen, S. (2020). A signaling hub of insulin receptor, dystrophin glycoprotein complex and plakoglobin regulates muscle size. *Nature Communications*, *11*(1), 1381. https://doi.org/10.1038/s41467-020-14895-9

Fall, T., & Ingelsson, E. (2014). Genome-wide association studies of obesity and metabolic syndrome. *Molecular and Cellular Endocrinology*, *382*(1), 740–757. https://doi.org/10.1016/j.mce.2012.08.018

Ghosh, C., Das, N., Saha, S., Kundu, T., Sircar, D., & Roy, P. (2022). Involvement of Cdkal1 in the etiology of type 2 diabetes mellitus and microvascular diabetic complications: A review. *Journal of Diabetes & Metabolic Disorders*, *21*(1), 991–1001. https://doi.org/10.1007/s40200-021-00953-6

Hahn, M. W. (2018). *Molecular Population Genetics*. Oxford University Press.

He, W., Chen, P., Chen, Q., Cai, Z., & Zhang, P. (2022). Cytokine storm: Behind the scenes of the collateral circulation after acute myocardial infarction. *Inflammation Research*, *71*(10), 1143–1158. https://doi.org/10.1007/s00011-022-01611-0

Hooper, D. M., & Price, T. D. (2017). Chromosomal inversion differences correlate with range overlap in passerine birds. *Nature Ecology &amp; Evolution*, *1*(10), 1526–1534. https://doi.org/10.1038/s41559-017-0284-6

Jun, G., Wing, M. K., Abecasis, G. R., & Kang, H. M. (2015). An efficient and scalable analysis framework for variant extraction and refinement from population scale DNA sequence data. *Genome Research*, gr.176552.114. https://doi.org/10.1101/gr.176552.114

Korneliussen, T. S., Albrechtsen, A., & Nielsen, R. (2014). ANGSD: Analysis of Next Generation Sequencing Data. *BMC Bioinformatics*, *15*(1), 356. https://doi.org/10.1186/s12859-014-0356-4

Kumar, S., Stecher, G., Suleski, M., & Hedges, S. B. (2017). TimeTree: A Resource for Timelines, Timetrees, and Divergence Times. *Molecular Biology and Evolution*, *34*(7), 1812–1819. https://doi.org/10.1093/molbev/msx116

Lendvai, Á. Z., Tóth, Z., Mahr, K., Osváth, G., Vogel-Kindgen, S., & Gander, B. A. (2021). Effects of experimental increase in insulin-like growth factor 1 on feather growth rate, moult intensity and feather quality in a passerine bird. *Journal of Experimental Biology*, *224*(14), jeb242481. https://doi.org/10.1242/jeb.242481

Lendvai, Á. Z., Tóth, Z., Mahr, K., Pénzes, J., Vogel-Kindgen, S., Gander, B. A., & Vágási, C. I. (2024). IGF-1 induces sex-specific oxidative damage and mortality in a songbird. *Oecologia*, *205*(3), 561–570. https://doi.org/10.1007/s00442-024-05587-y

Li, H., & Durbin, R. (2009). Fast and accurate short read alignment with Burrows–Wheeler transform. *Bioinformatics*, *25*(14), 1754–1760. https://doi.org/10.1093/bioinformatics/btp324

Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., Marth, G., Abecasis, G., & Durbin, R. (2009). The Sequence Alignment/Map format and SAMtools. *Bioinformatics*, *25*(16), 2078–2079. https://doi.org/10.1093/bioinformatics/btp352

Li, Z.-H., Xu, H., Zheng, W., Lam, S. H., & Gong, Z. (2013). RNA-Sequencing Analysis of TCDD-Induced Responses in Zebrafish Liver Reveals High Relatedness to In Vivo Mammalian Models and Conserved Biological Pathways. *PLOS ONE*, *8*(10), e77292. https://doi.org/10.1371/journal.pone.0077292

Lodjak, J., Mägi, M., Sild, E., & Mänd, R. (2017). Causal link between insulin-like growth factor 1 and growth in nestlings of a wild passerine bird. *Functional Ecology*, *31*(1), 184–191. https://doi.org/10.1111/1365-2435.12679

Lou, R. N., & Therkildsen, N. O. (2022). Batch effects in population genomic studies with low-coverage whole genome sequencing data: Causes, detection and mitigation. *Molecular Ecology Resources*, *22*(5), 1678–1692. https://doi.org/10.1111/1755-0998.13559

Manti, M., Stener-Victorin, E., & Benrick, A. (2020). Skeletal Muscle Immunometabolism in Women With Polycystic Ovary Syndrome: A Meta-Analysis. *Frontiers in Physiology*, *11*. https://doi.org/10.3389/fphys.2020.573505

McKenna, A., Hanna, M., Banks, E., Sivachenko, A., Cibulskis, K., Kernytsky, A., Garimella, K., Altshuler, D., Gabriel, S., Daly, M., & DePristo, M. A. (2010). The Genome Analysis Toolkit: A MapReduce framework for analyzing next-generation DNA sequencing data. *Genome Research*, *20*(9), 1297–1303. https://doi.org/10.1101/gr.107524.110

McNew, S. M., Beck, D., Sadler-Riggleman, I., Knutie, S. A., Koop, J. A. H., Clayton, D. H., & Skinner, M. K. (2017). Epigenetic variation between urban and rural populations of Darwin’s finches. *BMC Evolutionary Biology*, *17*(1). https://doi.org/10.1186/s12862-017-1025-9

Minias, P. (2023). The effects of urban life on animal immunity: Adaptations and constraints. *Science of The Total Environment*, *895*, 165085. https://doi.org/10.1016/j.scitotenv.2023.165085

Mueller, J. C., Carrete, M., Boerno, S., Kuhl, H., Tella, J. L., & Kempenaers, B. (2020). Genes acting in synapses and neuron projections are early targets of selection during urban colonization. *Molecular Ecology*, *29*(18), 3403–3412. https://doi.org/10.1111/mec.15451

Mueller, J. C., Partecke, J., Hatchwell, B. J., Gaston, K. J., & Evans, K. L. (2013). Candidate gene polymorphisms for behavioural adaptations during urbanization in blackbirds. *Molecular Ecology*, *22*(13), 3629–3637. https://doi.org/10.1111/mec.12288

Onagbesan, O. M., Vleugels, B., Buys, N., Bruggeman, V., Safi, M., & Decuypere, E. (1999). Insulin-like growth factors in the regulation of avian ovarian functions. *Domestic Animal Endocrinology*, *17*(2), 299–313. https://doi.org/10.1016/S0739-7240(99)00046-6

Osborn, M. P., Park, Y., Parks, M. B., Burgess, L. G., Uppal, K., Lee, K., Jones, D. P., & Jr, M. A. B. (2013). Metabolome-Wide Association Study of Neovascular Age-Related Macular Degeneration. *PLOS ONE*, *8*(8), e72737. https://doi.org/10.1371/journal.pone.0072737

Picard, T. (2019). *Broad Institute*. https://cir.nii.ac.jp/crid/1370013168858971926

Pikus, E., Włodarczyk, R., Jedlikowski, J., & Minias, P. (2021). Urbanization processes drive divergence at the major histocompatibility complex in a common waterbird. *PeerJ*, *9*, e12264. https://doi.org/10.7717/peerj.12264

Price, E. R., Bauchinger, U., Zajac, D. M., Cerasale, D. J., McFarlan, J. T., Gerson, A. R., McWilliams, S. R., & Guglielmo, C. G. (2011). Migration- and exercise-induced changes to flight muscle size in migratory birds and association with IGF1 and myostatin mRNA expression. *Journal of Experimental Biology*, *214*(17), 2823–2831. https://doi.org/10.1242/jeb.057620

Ragvin, A., Moro, E., Fredman, D., Navratilova, P., Drivenes, Ø., Engström, P. G., Alonso, M. E., Mustienes, E. de la C., Skarmeta, J. L. G., Tavares, M. J., Casares, F., Manzanares, M., van Heyningen, V., Molven, A., Njølstad, P. R., Argenton, F., Lenhard, B., & Becker, T. S. (2010). Long-range gene regulation links genomic type 2 diabetes and obesity risk regions to HHEX, SOX4, and IRX3. *Proceedings of the National Academy of Sciences*, *107*(2), 775–780. https://doi.org/10.1073/pnas.0911591107

Ramer, I., Varier, I., Zhang, D., Demicco, E. G., Posner, M. R., Misiukiewicz, K., Genden, E. M., Miles, B. A., Teng, M. S., & Sikora, A. G. (2016). Racial disparities in incidence of human papillomavirus-associated oropharyngeal cancer in an urban population. *Cancer Epidemiology*, *44*, 91–95. https://doi.org/10.1016/j.canep.2016.07.004

Salmón, P., Jacobs, A., Ahrén, D., Biard, C., Dingemanse, N. J., Dominoni, D. M., Helm, B., Lundberg, M., Senar, J. C., Sprau, P., Visser, M. E., & Isaksson, C. (2021). Continent-wide genomic signatures of adaptation to urbanisation in a songbird across Europe. *Nature Communications*, *12*(1). https://doi.org/10.1038/s41467-021-23027-w

Sayols, S. (2023). rrvgo: A Bioconductor package for interpreting lists of Gene Ontology terms. *microPublication Biology*, *2023*, 10.17912/micropub.biology.000811. https://doi.org/10.17912/micropub.biology.000811

Stikker, B. S., Hendriks, R. W., & Stadhouders, R. (2023). Decoding the genetic and epigenetic basis of asthma. *Allergy*, *78*(4), 940–956. https://doi.org/10.1111/all.15666

van Dongen, W. F. D., Robinson, R. W., Weston, M. A., Mulder, R. A., & Guay, P.-J. (2015). Variation at the DRD4 locus is associated with wariness and local site selection in urban black swans. *BMC Evolutionary Biology*, *15*(1), 253. https://doi.org/10.1186/s12862-015-0533-8

Wagner, D. N., Curry, R. L., Chen, N., Lovette, I. J., & Taylor, S. A. (2020). Genomic regions underlying metabolic and neuronal signaling pathways are temporally consistent in a moving avian hybrid zone. *Evolution*, *74*(7), 1498–1513. https://doi.org/10.1111/evo.13970

Winchell, K. M., Campbell-Staton, S. C., Losos, J. B., Revell, L. J., Verrelli, B. C., & Geneva, A. J. (2023). Genome-wide parallelism underlies contemporary adaptation in urban lizards. *Proceedings of the National Academy of Sciences*, *120*(3), e2216789120. https://doi.org/10.1073/pnas.2216789120

Yagyu, T., Noguchi, T., Asano, Y., Ida, K., Ogata, S., Nishimura, K., & Matsuda, H. (2023). Association Between Genetic Diagnosis and Clinical Outcomes in Patients With Heritable Thoracic Aortic Disease. *Journal of the American Heart Association*, *12*(8), e028625. https://doi.org/10.1161/JAHA.122.028625

Zhou, Z., Li, X., Xiao, Y., Wang, X., Tian, W., Peng, X., Bi, D., Sun, M., & Li, Z. (2013). Gene expression responses to Riemerella anatipestifer infection in the liver of ducks. *Avian Pathology*, *42*(2), 129–136. https://doi.org/10.1080/03079457.2013.770127

Zhu, H., Meissner, L. E., Byrnes, C., Tuymetova, G., Tifft, C. J., & Proia, R. L. (2020). The Complement Regulator Susd4 Influences Nervous-System Function and Neuronal Morphology in Mice. *iScience*, *23*(3). https://doi.org/10.1016/j.isci.2020.100957