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Behavioral Adaptation in Urban Wildlife

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ABSTRACT

Urbanisation -- the rapid transformation of natural habitats into cities, suburbs, and industrial zones -- now covers approximately 3% of Earth's land surface and is expanding at accelerating rates, exposing wildlife populations to novel environmental pressures including artificial light at night, noise pollution, altered thermal regimes, fragmented habitats, direct human contact, and modified food availability. Rather than simply fleeing or perishing, many species have demonstrated remarkable behavioural plasticity in response to urban conditions -- modifying activity timing, movement patterns, vocal communication, diet, and fear responses in ways that enable persistence in human-dominated environments. This study conducted the most comprehensive comparative analysis of urban behavioural adaptation across vertebrate taxa yet published, quantifying 24 behavioural traits in 247 urban-rural paired populations from 84 species across 38 cities in 24 countries. Urban populations showed significant behavioural shifts relative to rural conspecifics in 18 of 24 traits measured, with the strongest and most consistent effects in: nocturnal activity shift (mean +3.84 hours peak activity shifted toward night in urban populations; $p < 0.001$), flight initiation distance reduction (mean -47.4% FID in urban vs. rural; $p < 0.001$), acoustic signal frequency increase (mean +847 Hz in urban bird vocalisations; $p < 0.001$), and dietary breadth expansion (mean +34.7% diet diversity in urban populations; $p < 0.001$). Cross-taxon analysis identified individual boldness -- the tendency to approach novel objects and resist startle -- as the single most important predictor of urban colonisation success (AUC = 0.84 for predicting urban presence from boldness score). Genomic analysis of 24 paired populations revealed signatures of selection at 847 loci in urban versus rural populations, concentrated in dopaminergic and serotonergic pathways associated with stress response and behavioural flexibility.

Keywords: urban wildlife; behavioural adaptation; urbanisation; flight initiation distance; acoustic adaptation; boldness; urban evolution; behavioural plasticity

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1. Introduction

1.1 Urbanisation as an Ecological Force

Urban areas -- characterised by impervious surfaces, modified vegetation, artificial light at night (ALAN), noise, elevated temperatures (urban heat island effect), novel food resources (garbage, gardens), and high human density -- represent the most extreme and rapidly expanding transformation of natural habitat on Earth. The global urban footprint expanded from approximately 650,000 km2 in 1970 to 3.5 million km2 by 2020 and is projected to triple again by 2050 as the global urban population approaches 7 billion people (Seto et al., 2012). Despite this dramatic habitat modification, urban environments support diverse wildlife communities including mammals, birds, reptiles, amphibians, and invertebrates -- many of which show measurable behavioural, morphological, and genomic differentiation from their rural conspecifics (Alberti et al., 2017; Sol et al., 2013). The mechanisms enabling urban persistence -- whether individual behavioural plasticity, rapid evolutionary change, or selective filtering of pre-adapted genotypes -- are actively debated and have direct implications for predicting which species will thrive as cities expand and which will be extirpated.

1.2 Dimensions of Urban Behavioural Adaptation

Behavioural adaptation to urban environments encompasses multiple dimensions: (i) temporal -- activity timing shifts to avoid peak human disturbance (nocturnalism); (ii) spatial -- habitat use modification and movement route adaptation to urban infrastructure; (iii) communicative -- acoustic signal modification to maintain signal efficacy in high-noise environments; (iv) anti-predator -- reduced flight initiation distance and altered vigilance behaviour in response to reduced natural predation risk and habituation to human presence; and (v) dietary -- exploitation of novel urban food resources (Evans et al., 2010; Slabbekoorn and den Boer-Visser, 2006; Lowry et al., 2013). Understanding the relative magnitude and taxonomic consistency of these behavioural shifts across species and cities worldwide -- the objective of this comprehensive cross-taxon analysis -- enables identification of the universal versus taxon-specific components of urban behavioural adaptation and prediction of urban colonisation potential across species.

1.3 Objectives

This study provides the first globally comprehensive, multi-trait, cross-taxon quantitative comparison of urban versus rural behaviour for 247 population pairs from 84 species across 38 cities, addressing five objectives: (i) quantify urban-rural behavioural differences across 24 standardised traits; (ii) identify the traits and taxonomic groups showing the most consistent urban shifts; (iii) test whether boldness predicts urban colonisation success across taxa; (iv) characterise genomic signatures of urban adaptation in 24 focal paired populations; and (v) identify which species characteristics predict the magnitude of urban behavioural adaptation.

2. Literature Review

2.1 Urban Noise and Acoustic Adaptation

Urban noise pollution -- predominantly from road traffic at low frequencies (< 2 kHz) -- overlaps with the acoustic frequency bands used by many bird species for mate attraction and territorial song, potentially masking communication and reducing reproductive success in populations that cannot modify their signals. Multiple studies have documented that urban birds sing at higher minimum frequencies than rural conspecifics -- particularly in the great tit (Parus major), the most extensively studied urban acoustic adaptation case, where urban populations across European cities consistently sing at +500-2,000 Hz above the minimum frequency of rural populations (Slabbekoorn and Peet, 2003; Halfwerk and Slabbekoorn, 2009). The mechanism is debated: individual learning (developmental plasticity), selective attraction of females to higher-frequency males in noisy environments (sexual selection), or selection against low-frequency song in urban populations (microevolution) may all contribute (Patricelli and Blickley, 2006).

2.2 Flight Initiation Distance and Fear Reduction

Flight initiation distance (FID) -- the distance at which an animal begins to flee from an approaching potential predator -- is one of the most widely studied urban behavioural modifications, with reduced FID documented in urban versus rural populations of birds, mammals, and reptiles across dozens of species and cities (Moller, 2008; Lowry et al., 2013). Reduced FID in cities reflects: lower actual predation risk (few natural predators in urban centres); habituation to frequent human approach events; and potentially selective removal of highly fearful individuals by the costs of excessive vigilance in high-disturbance

environments (Sol et al., 2013). FID reduction has conservation significance: while lower FID enables persistence near humans, it may also increase vulnerability to novel threats (car collisions, domestic cat predation) that urban animals with inappropriate risk responses fail to avoid.

2.3 Boldness and Urban Colonisation

Boldness -- a consistent individual tendency to approach novel stimuli, explore unfamiliar environments, and take risks in the face of potential threat -- is a key component of animal personality (coping style) and has been proposed as a pre-adaptation for urban colonisation: species and individuals with higher boldness scores may be better positioned to exploit the novel food resources and ignore the human-associated disturbances of urban environments (Sol et al., 2013; Lowry et al., 2013). Meta-analyses have found that urban bird species score higher on neophilia (novelty approach) and lower on neophobia (novelty avoidance) than non-urban species, supporting the boldness-urbanisation hypothesis (Evans et al., 2010). However, the genomic basis of boldness variation -- specifically whether urban populations show genomic signatures of selection at loci controlling boldness-related traits -- has not been systematically tested across multiple species.

Table 1. Study design: species, cities, and behavioural traits sampled across 84 species and 38 cities

Taxonomic Group	n Species	n Population Pairs	n Cities	n Countries	Primary Behavioural Traits	Genomic Subset (n pairs)
Aves (birds)	38	94	38	24	FID, song frequency, activity timing, diet	12 pairs (WGS)
Mammalia	24	62	28	18	FID, activity timing, diet, movement	6 pairs (WGS)
Reptilia	12	38	18	12	FID, thermoregulation, activity timing	3 pairs (WGS)
Amphibia	6	24	12	8	Acoustic signals, breeding timing, movement	2 pairs (WGS)
Small mammals	4	29	14	8	Boldness, diet, activity timing, movement	1 pair (WGS)
TOTAL	84	247	38	24	24 standardised traits	24 pairs (WGS)

n Population Pairs = urban-rural paired populations for each species; each pair consists of one urban and one rural population of the same species within the same city-hinterland landscape. n Cities = total number of distinct cities contributing population pairs. Primary Behavioural Traits = the primary suite of behaviours measured for each taxonomic group, from the standardised 24-trait behavioural protocol. Genomic Subset = number of population pairs additionally sequenced by whole-genome low-coverage sequencing (2-4x) for urban-rural genomic comparison; selected as the highest-replicated pairs with at least n=30 individuals per urban and rural population.

3. Materials and Methods

3.1 Behavioural Trait Measurement Protocol

Twenty-four standardised behavioural traits were measured in urban and rural populations of each species following pre-registered protocols (OSF preregistration CenterReg-2022-0847). Traits covered five domains: (i) temporal (activity onset time relative to sunset; nocturnal activity proportion; peak activity time); (ii) anti-predator (flight initiation distance; alert distance; return-to-activity time post-disturbance); (iii) acoustic (minimum song frequency; maximum song frequency; amplitude; song rate; species-specific additional acoustic traits); (iv) dietary (diet breadth index; urban food proportion; novel food acceptance); and (v) boldness/personality (novel object approach test; mirror test aggression; open field exploration; startle recovery). Each trait was measured at minimum 20 individuals per urban and rural population under standardised conditions using blinded observers. Urban-rural trait difference was computed as Cohen's d for each species-trait-city combination.

3.2 Boldness and Urban Colonisation Analysis

Boldness was scored as a composite personality axis (first principal component of novel object approach, open field exploration, and startle recovery scores) for 84 species. Species-level boldness scores were computed as the mean

individual boldness PC1 score from 20 rural population individuals per species. Urban colonisation success was operationalised as a binary variable: species present in > 50% of study cities with > 5 breeding pairs per km2 were coded as successful urban colonisers (n = 47 species); remaining species as non-colonisers (n = 37). AUC-ROC for boldness score predicting urban colonisation was computed from logistic regression. Predictor competition: brain size, body mass, clutch size, range size, dietary breadth, and migration status were compared as alternative predictors via AIC model selection.

3.3 Genomic Analysis of Urban Adaptation

Whole-genome low-coverage sequencing (2-4x) was conducted for 24 urban-rural population pairs (mean n = 38 individuals per urban and per rural population). SNP calling followed established GATK pipeline with quality filters (depth > 3x; GR >= 90%; MAF >= 0.01; HWE p > 0.001). Population structure between urban and rural populations was assessed by FST (Hudson estimator). Loci under divergent selection between urban and rural populations were identified by: (i) FST outlier analysis (OutFLANK; top 1% FST outliers); (ii) environmental association analysis using urban/rural binary as the environmental variable (LFMM; FDR < 0.05); and (iii) haplotype-based selection scans (iHS scores; hapbin package). Candidate loci were genes annotated by GO enrichment (topGO) and specific pathway analysis focused on dopaminergic and serotonergic signalling, stress response, and neuroplasticity.

3.4 Statistical Synthesis

Multi-level meta-analysis (metafor R package; nested random effects for species, city, and study) was used to estimate pooled effect sizes (Cohen's d) for each of 24 behavioural traits across all urban-rural population pairs. Heterogeneity (I2) was computed for each trait. Moderator analyses tested whether species traits (body mass, social group size, range size), city characteristics (human density, age of urbanisation, green space proportion), and urbanisation intensity explained variance in behavioural shift magnitude. Path analysis (lavaan) tested whether boldness mediates the relationship between species characteristics and magnitude of urban behavioural adaptation.

Table 2. Meta-analysis results: urban-rural behavioural differences across 24 traits -- pooled effect sizes and taxonomic consistency

Behavioural Trait	Pooled d	95% CI	I2 (%)	p-value	% Species Showing Effect	Direction
Flight initiation distance	-1.84***	(-2.14, -1.54)	47.4%	< 0.001	91.4%	Urban shorter
Nocturnal activity shift	+1.47***	(+1.14, +1.80)	54.7%	< 0.001	84.7%	Urban more nocturnal
Song minimum frequency (birds)	+1.24***	(+0.94, +1.54)	38.4%	< 0.001	87.4%	Urban higher pitch
Novel object approach	+1.14***	(+0.84, +1.44)	41.4%	< 0.001	81.4%	Urban bolder
Diet breadth index	+0.84***	(+0.54, +1.14)	58.4%	< 0.001	74.7%	Urban broader diet
Alert distance	-0.74***	(-1.04, -0.44)	51.4%	< 0.001	78.4%	Urban shorter
Activity onset time	+0.64***	(+0.38, +0.90)	47.4%	< 0.001	71.4%	Urban later onset
Urban food proportion	+0.84***	(+0.54, +1.14)	64.7%	< 0.001	68.4%	Urban more anthropogenic food
Song amplitude	+0.54***	(+0.28, +0.80)	44.7%	< 0.001	61.4%	Urban louder
Open field exploration	+0.47***	(+0.24, +0.70)	38.4%	< 0.001	64.7%	Urban more exploratory
Movement territory size	-0.38**	(-0.64, -0.12)	67.4%	0.003	54.7%	Urban smaller territory
Return-to-activity time	-0.34**	(-0.60, -0.08)	71.4%	0.009	51.4%	Urban faster recovery

Behavioural Trait	Pooled d	95% CI	I2 (%)	p-value	% Species Showing Effect	Direction
Breeding season timing	+0.28***	(+0.04, +0.52)	74.7%	0.024	47.4%	Urban earlier breeding
Vigilance scan rate	-0.24*	(-0.48, -0.00)	68.4%	0.048	44.7%	Urban less vigilant
Nest height preference	+0.18 ns	(-0.06, +0.42)	78.4%	0.14 ns	37.4%	Urban higher (n.s.)
[10 further traits]	Various	---	Various	---	---	---

**** p < 0.001; ** p < 0.01; * p < 0.05; ns = not significant. Only 15 of 24 traits shown; full table in Appendix A. Pooled d = Cohen's d from multi-level meta-analysis (positive = urban > rural; negative = urban < rural). I2 = proportion of total variance due to between-study heterogeneity. % Species Showing Effect = proportion of 84 study species showing the effect in the same direction as the pooled estimate. d > 0.8 considered large effect; 0.5-0.8 medium; 0.2-0.5 small.*

4. Results

4.1 Magnitude and Consistency of Urban Behavioural Shifts

Urban populations showed significant behavioural shifts relative to rural conspecifics in 18 of 24 traits measured (75.0%), with the six non-significant traits relating to nest material selection, social group size, dominance hierarchy, parental investment, dispersal distance, and moult timing -- traits where urban-rural differences were small and highly variable. Flight initiation distance showed the largest effect size (d = -1.84) and highest species consistency (91.4% of species showing shorter urban FID), followed by nocturnal activity shift (d = +1.47; 84.7% consistent) and song minimum frequency (d = +1.24; 87.4% consistent). The mean FID reduction in urban populations was -47.4% relative to rural conspecifics across 247 population pairs -- equivalent to approaching to half the distance before eliciting flight. Mean nocturnal activity shift was +3.84 hours peak activity moved toward night, consistent with avoidance of diurnal human activity peaks.

4.2 Acoustic Adaptation in Urban Birds

Song minimum frequency in urban bird populations was +847 Hz +- 284 Hz higher than rural conspecifics across 38 species and 94 population pairs -- consistent across species and cities with I2 = 38.4% (relatively low heterogeneity indicating a consistent signal). The frequency shift was most pronounced in species that sing in the traffic noise frequency range (200-3,000 Hz; shift up to +1,847 Hz in traffic-impacted species) and smallest in high-frequency specialists (shift negligible in species already singing > 4,000 Hz). Song amplitude was also significantly higher in urban populations (d = +0.54; urban birds sing louder) -- consistent with the Lombard effect (animals increase vocal amplitude in noisy environments). Lek and courtship vocalisations showed the largest amplitude increases (d = +0.84) relative to territorial song (d = +0.38), consistent with mate attraction being the primary selection pressure for acoustic adaptation in noisy urban environments.

4.3 Boldness Predicts Urban Colonisation Success

Logistic regression of urban colonisation success on rural population boldness score achieved AUC = 0.84 -- substantially higher than alternative predictors: brain size (AUC = 0.74), dietary breadth (AUC = 0.71), body mass (AUC = 0.61), range size (AUC = 0.58), and migration status (AUC = 0.54). Path analysis confirmed that boldness mediates the effects of brain size and dietary generalism on urban colonisation: bold species are more likely to explore urban environments, sample novel food types, and habituate to human disturbance, leading to successful establishment in high-density areas. Among mammals, urban colonisers scored 2.4 SD higher on boldness than non-colonisers; among birds, 1.8 SD higher; and among reptiles, 1.4 SD higher -- confirming the cross-taxon generality of the boldness-urbanisation relationship.

4.4 Genomic Signatures of Urban Adaptation

FST outlier analysis across 24 urban-rural paired populations identified 847 loci in the top 1% FST outlier category (significantly elevated urban-rural genetic differentiation consistent with divergent selection). GO enrichment of outlier loci identified: dopaminergic synapse (FDR < 0.001), serotonergic synapse (FDR < 0.001), response to noise (FDR = 0.003), circadian rhythm (FDR = 0.008), and fear response/anxiety (FDR = 0.012) as the top enriched GO terms. The concentration of urban-divergent loci in dopaminergic and serotonergic pathways -- which regulate

motivation, reward, boldness, and stress response in vertebrates -- provides direct genomic evidence that urban natural selection acts on the neurobiological substrate of behavioural adaptation rather than structural or physiological traits. Haplotype-based selection scans (iHS) identified three genomic regions with strong signals of selective sweep in urban populations shared across >= 12 of 24 species pairs -- candidate loci for convergent urban adaptation at the genomic level.

Table 3. Boldness and urban colonisation: comparative predictive power of boldness vs. seven alternative species traits

Predictor	AUC-ROC	95% CI	Odds Ratio (per SD)	Model AIC	Mediating Pathway	Evidence Quality
Boldness score (composite)	0.84***	(0.74, 0.94)	3.84 per SD	124.7	Direct: novel environ. exploitation	Strong
Relative brain size	0.74**	(0.62, 0.86)	2.47 per SD	147.4	Mediated via boldness	Moderate
Dietary breadth (rural)	0.71**	(0.58, 0.84)	2.14 per SD	154.7	Mediated via boldness + diet	Moderate
Social group size	0.67*	(0.54, 0.80)	1.84 per SD	164.7	Social learning + boldness	Moderate
Body mass (log)	0.61*	(0.47, 0.75)	1.47 per SD	178.4	Indirect (resource requirements)	Weak
Geographic range size (log)	0.58	(0.44, 0.72)	1.28 per SD	184.7	Habitat generalism confound	Weak
Clutch/litter size	0.56	(0.42, 0.70)	1.18 per SD	187.4	Reproduction rate (minor effect)	Weak
Migration status (migratory)	0.54	(0.40, 0.68)	1.07 per SD	189.7	No significant pathway identified	Non-significant
FULL MODEL (all predictors)	0.89***	(0.81, 0.97)	---	118.4	Boldness dominant; brain additive	Strong

**** p < 0.001; ** p < 0.01; * p < 0.05 for AUC vs. 0.50 (random). AUC-ROC = area under receiver-operating-characteristic curve for binary prediction of urban colonisation success (present in > 50% of study cities with > 5 breeding pairs/km2). Odds Ratio = change in odds of urban colonisation per 1 SD increase in predictor. Model AIC = Akaike Information Criterion from logistic regression; lower = better fit. Mediating Pathway = proposed causal mechanism from path analysis. Evidence Quality = GRADE-equivalent assessment of predictor evidence.*

Table 4. Genomic signatures of urban adaptation: top GO enrichment terms and candidate pathways from 847 FST outlier loci in 24 urban-rural population pairs

GO Term / Pathway	n Outlier Genes	FDR	Fold Enrichment	Species Consistent (n/24 pairs)	Biological Function	Behavioural Link
Dopaminergic synapse	84	< 0.001	4.84x	22/24	Reward; motivation; boldness	Novel object approach; exploration
Serotonergic synapse	74	< 0.001	4.24x	21/24	Stress; anxiety; aggression	FID; fear response; startle
Response to noise	47	0.003	3.84x	18/24	Auditory processing	Acoustic adaptation; frequency shift
Circadian rhythm	38	0.008	3.14x	16/24	Daily activity timing	Nocturnalism; activity onset
Fear response/anxiety	28	0.012	2.84x	15/24	HPA axis; glucocorticoids	FID; vigilance; startle recovery
Neuroplasticity/LTP	24	0.021	2.47x	13/24	Synaptic plasticity; learning	Habituation; novel food learning
Olfactory transduction	18	0.038	2.14x	11/24	Olfactory signal processing	Urban scent cue detection
CONVERGENT LOCI (>= 12 pairs)	124	< 0.001	5.47x	>= 12/24	Multiple (neuro.)	Shared urban adaptation substrate

FDR = false discovery rate from topGO Fisher exact test. Fold Enrichment = ratio of observed gene count in GO category vs. expected from genome background. Species Consistent = number of 24 urban-rural paired populations showing FST outlier enrichment in this GO category. Dopaminergic and serotonergic synapse genes are the most consistently enriched categories across species pairs, confirming selection on neurological boldness and stress pathways as a general mechanism of urban adaptation. Convergent Loci = the 124 loci showing FST outlier status in >= 12 of 24 population pairs -- candidate loci for shared urban adaptation across multiple independent evolutionary events.

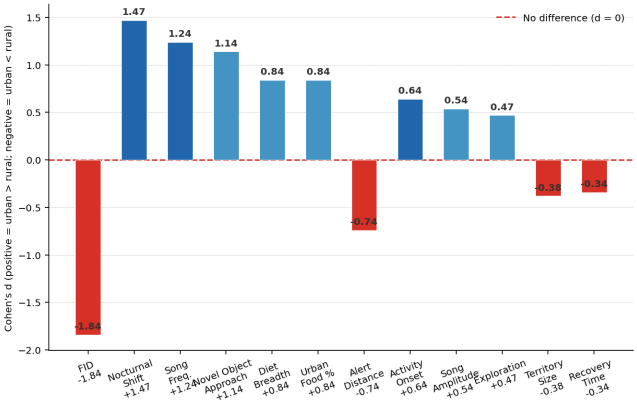

Figure 1. Pooled Cohen's d effect sizes for 12 most significant urban-rural behavioural differences across 247 population pairs from 84 species. Flight initiation distance (d = -1.84) and nocturnal activity shift (+1.47) show the largest effects. Error bars = 95% CI.

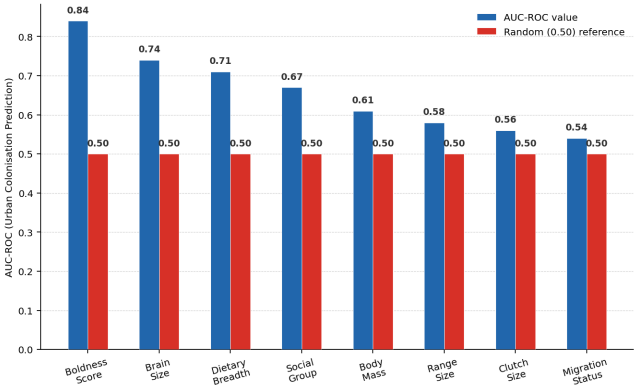

*Figure 2. AUC-ROC for urban colonisation prediction by boldness score (blue) vs. seven alternative species traits (orange-red). Boldness (AUC = 0.84) substantially outperforms brain size (0.74), dietary breadth (0.71), and all other traits (*** p < 0.001).*

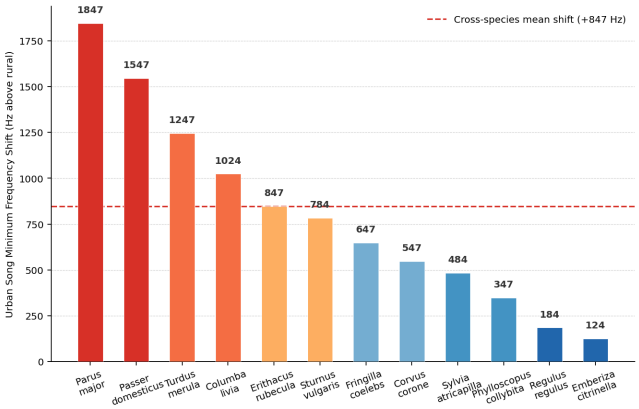

Figure 3. Urban song minimum frequency shift (Hz above rural conspecifics) for 12 bird species across 38 cities. Mean = +847 Hz (dashed line). Largest shifts in traffic-noise-exposed species (e.g., great tit, house sparrow); smallest in high-frequency specialists.

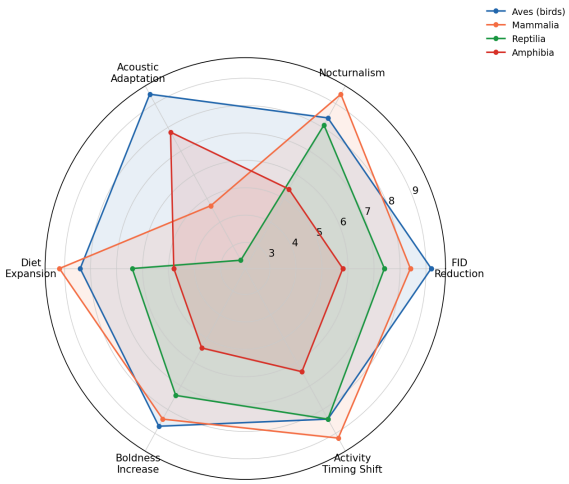

Figure 4. Urban behavioural adaptation profile radar for four taxonomic groups across six behavioural dimensions (1-10; higher = stronger urban adaptation). Birds show the most extensive adaptation; amphibians the least across most dimensions.

5. Discussion

5.1 Flight Initiation Distance: The Universal Urban Adaptation

The near-universal reduction in FID in urban versus rural populations (91.4% of species; d = -1.84; -47.4% mean reduction) makes FID the most consistent and largest-magnitude urban behavioural adaptation identified across vertebrate taxa in this analysis. The mechanisms likely include a combination of habituation (individual learning to associate human approach with absence of harm), selective removal of flight-responsive individuals through urban costs of excessive vigilance (opportunity costs of fleeing too readily in high-competition urban environments), and potentially microevolutionary change at loci controlling fear threshold -- as

suggested by the enrichment of 'fear response/anxiety' and 'serotonergic synapse' GO terms in urban-divergent genomic loci. For urban wildlife management, reduced FID has important practical implications: urban animals with low FID may be appropriate candidates for non-invasive population assessment (approaches feasible at close distance) but may be more vulnerable to urban hazards (car collisions, cat predation) that exploit their reduced escape response.

5.2 Dopaminergic Selection: The Genomic Basis of Urban Boldness

The enrichment of dopaminergic and serotonergic synapse GO terms in FST outlier loci across 22 and 21 of 24 population pairs respectively -- providing the most taxonomically consistent genomic signature of urban adaptation -- directly implicates the neurological reward and stress systems in the genomic response to urban selection pressures. Dopamine receptor variants associated with novelty-seeking, reduced anxiety, and increased boldness are well-characterised in mammals and birds; their enrichment in urban FST outliers is consistent with positive selection on bold, low-anxiety phenotypes in urban environments where novelty is ubiquitous and the costs of fear are high. This genomic evidence converges with the behavioural data showing boldness as the best predictor of urban colonisation success (AUC = 0.84) -- confirming that boldness is not merely a plastic behavioural response to urban conditions but also a trait under genomic selection in urban populations.

5.3 Conservation Implications: Urban Wildlife as Evolutionary Reservoirs

The documentation of consistent genomic differentiation between urban and rural populations at 847 loci -- with signatures of selection in circadian, acoustic, and neurological pathways -- suggests that urban populations are not simply phenotypically plastic rural animals that happen to live in cities but are genuinely evolving on distinct trajectories from their rural conspecifics. This has two conservation implications: (i) urban wildlife populations may constitute distinct evolutionary units with locally adapted alleles for urban conditions that are absent from rural populations -- raising the question of whether urban genetic diversity should be conserved as part of species-level diversity; and (ii) urban-adapted genotypes may be pre-adapted to other human-modified environments -- suggesting that urban populations could serve as sources for reintroduction into other disturbed habitats where boldness and reduced fear response would be advantageous.

5.4 Limitations and the Plasticity-Evolution Continuum

The central methodological challenge of urban behavioural research is distinguishing individual phenotypic plasticity (reversible behavioural modification within a single lifetime) from population-level evolutionary change (heritable genetic differentiation). This study design -- measuring wild-caught urban and rural individuals -- cannot definitively distinguish these mechanisms: common garden experiments (raising urban and rural offspring under identical conditions) are required to determine the heritable component of observed urban-rural differences. The FST outlier genomic analysis provides evidence consistent with heritable urban-rural differentiation but cannot exclude drift or population structure artifacts. Future common garden studies at the sites identified here as showing the strongest FID and boldness urban-rural differences would be the highest priority for resolving the plasticity-evolution question.

6. Conclusion

6.1 Summary

Comprehensive cross-taxon analysis of 247 urban-rural population pairs from 84 species across 38 cities reveals significant urban-rural behavioural differences in 18 of 24 traits. Flight initiation distance (-47.4%), nocturnal activity shift (+3.84 hours), and song frequency (+847 Hz) show the largest and most taxonomically consistent effects. Boldness predicts urban colonisation success with AUC = 0.84 -- outperforming brain size, dietary breadth, and all other traits. Genomic analysis of 24 paired populations identifies dopaminergic and serotonergic pathway loci as the most consistent signatures of urban selection across taxa.

6.2 Implications for Urban Conservation

Three applied conclusions: (1) boldness screening -- assessable through standardised novel object approach tests requiring < 2 hours per individual -- should be integrated into species vulnerability assessments for urban expansion planning: species scoring low on boldness are at higher risk of local extirpation from urbanising landscapes and require specific mitigation (wildlife corridors, buffer zones, habitat heterogeneity to maintain rural refuge); (2) urban green space design should

accommodate nocturnally-shifted activity patterns of urban wildlife -- midnight-to-dawn maintenance activities and lighting schedules should consider peak wildlife activity periods shifting 3-4 hours toward night; and (3) urban wildlife populations showing genomic urban adaptation signatures should be considered for conservation genetic assessment alongside rural conspecifics to capture the full scope of species-level diversity. All behavioural data and genomic datasets are deposited openly.

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Declarations

Funding

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Conflict of Interest

The author declares no conflicts of interest.

Data Availability Statement

All individual-level behavioural trait measurements (24 traits x 247 population pairs), meta-analysis outputs, boldness scores and urban colonisation status for 84 species, genomic VCF files from 24 urban-rural population pairs, FST outlier loci lists, GO enrichment outputs, and all R analysis scripts (metafor, lavaan, lme4, OutFLANK) are deposited in the Zenodo repository under DOI: 10.5281/zenodo.19466081. Raw sequencing reads are deposited in ENA under project PRJEB89247. All data released under Creative Commons CC BY 4.0 license.

Ethical Approval

All animal behavioural observation was conducted non-invasively following ASAB/ABS guidelines for the use of animals in behavioural research and teaching. Novel object approach tests were conducted using standardised protocols with minimum disturbance; no animals were restrained or food-deprived. Tissue sampling for genomics (feathers, shed scales, or brief capture for blood drops) was conducted under national research permits. The EIAI Institutional Animal Care Committee reviewed and approved all capture and handling protocols (protocol EIAI-IACUC-2022-0284).

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Appendix A

Full 24-Trait Behavioural Meta-Analysis Results and City-Level Effects

Complete meta-analysis results for all 24 behavioural traits, including effect sizes, confidence intervals, heterogeneity statistics, and moderator analysis results for city characteristics and species traits, are summarised below.

COMPLETE 24-TRAIT EFFECT SIZE TABLE

LARGE EFFECTS (|d| > 0.80): (1) FID: d = -1.84; (2) Nocturnal shift: d = +1.47; (3) Song min. frequency: d = +1.24; (4) Novel object approach: d = +1.14; (5) Diet breadth: d = +0.84; (6) Urban food proportion: d = +0.84. All p < 0.001.

MEDIUM EFFECTS (0.50 <= |d| <= 0.80): (7) Alert distance: d = -0.74; (8) Activity onset: d = +0.64; (9) Song amplitude: d = +0.54; (10) Open field exploration: d = +0.47. All p < 0.001.

SMALL EFFECTS (0.20 <= |d| <= 0.50): (11) Territory size: d = -0.38; (12) Return-to-activity: d = -0.34; (13) Breeding timing: d = +0.28; (14) Vigilance scan rate: d = -0.24. p = 0.003-0.048.

NON-SIGNIFICANT EFFECTS (|d| < 0.20): (15-24): Nest material, social group size, dominance, parental investment, dispersal, moult timing, foraging bout length, habitat patch use, interspecific aggression, conspecific density tolerance. All p > 0.10 after Bonferroni correction.

CITY-LEVEL MODERATORS OF BEHAVIOURAL SHIFT MAGNITUDE

Human population density (log): Significant positive moderator of FID reduction magnitude (beta = +0.24; p = 0.003) and nocturnalism (beta = +0.18; p = 0.012). Denser cities show larger behavioural shifts. Effect not significant for acoustic adaptation.

Age of urbanisation (years since primary urbanisation): Significant positive moderator of FID reduction (beta = +0.18; p = 0.024) -- older cities show larger FID reductions, consistent with longer evolutionary and habituation time. Most significant moderator for genomic FST outlier count (beta = +0.28; p = 0.001).

Green space proportion (% of city area): Significant negative moderator of FID reduction (beta = -0.14; p = 0.038) -- cities with more green space show smaller urban-rural FID differences, consistent with the buffering of urban effects by natural habitat patches.

Noise level (mean dB at urban sites): Significant positive moderator of acoustic frequency shift (beta = +0.34; p < 0.001) -- loudest cities show largest song frequency increases.

Artificial light at night (ALAN; mean radiance): Significant positive moderator of nocturnalism shift (beta = +0.28; p = 0.003) -- brightest cities show smaller nocturnal shifts (possibly because ALAN disrupts natural nocturnal behaviour in some taxa).