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ORIGINAL PAPER

Low genetic differentiation and symmetric migration between urban and forest populations of great tits

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Abstract

Gene flow may be limited between urban and non-urban populations of wild animals that can influence their landscape-level genetic structure and potential to adapt to new ecological conditions. To test this idea, we genetically characterized great tit (*Parus major*) populations breeding in an urban and a forest area 3.5 km apart, differing in several phenotypic traits some of which may contribute to adaptation to urban living. We used 16 microsatellite markers to genotype 189 breeding adult individuals (119 urban and 70 forest birds) and (1) tested whether the two populations are genetically differentiated, and (2) estimated the rate and direction of migration between the sites. Heterozygosity tended to be lower in the urban than in the forest habitat. Genetic population structure analyses did not show a consistent clustering of breeding birds between the urban and forest sites, and this conclusion was not affected by the inclusion of phenotypic data in the analyses. The pairwise fixation index (F_{st}) was low (0.009) and only 1% of the total genetic variance was explained by variation between populations. Finally, there was detectable gene flow between the two areas, and its estimated values did not suggest asymmetry in the direction of migration. We conclude that great tits living in the city are genetically connected to the nearby forest population by reciprocal migration, which may explain the low level of genetic differentiation.

Keywords *Parus major* · Microsatellite · Urbanization · Population differentiation · Gene flow · Migration rate

Introduction

The extent of urbanized areas is constantly increasing globally due to the increasing human population. To avoid habitat loss and extinction, wildlife needs to adapt to rapidly changing conditions, and as a response, some species are finding their new ecological niche in urban areas (Szulkin et al. 2020). Urban and natural habitats differ in a wide range of environmental factors, however, that may exert new selection pressures on local wildlife, for example on their life history, physiology, and behaviour (Seress & Liker 2015; Johnson & Munshi-South, 2017; Liker 2020; Szulkin et al. 2020). Local adaptation by genetic changes requires both sufficient variation within population and selection pressures that are relatively strong compared to the effects of gene flow (Morjan & Rieseberg 2004; Keller et al. 2013). Theoretical studies suggest that many generations may be required to generate significant genetic divergence even between geographically distinct populations, especially in species with high mobility in which migrants can maintain some level of gene flow (Evans et al. 2010; Landguth et al. 2010). Hence, information on gene flow and genetic divergence (or the lack of it) between urban and non-urban populations are crucial for understanding the mechanisms of adaptive changes in urban wildlife (Johnson & Munshi-South, 2017; Liker 2020; Szulkin et al. 2020).

Urban populations may be genetically isolated from conspecifics living outside of the cities for several reasons. First, dispersal from and to urban habitats may be restricted by habitat fragmentation and physical barriers like roads that

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characterize these areas (Bhattacharya et al. 2002; Benítez-López et al. 2010). Cities are often surrounded by other kinds of highly transformed habitats (e.g. agricultural lands) that may be avoided by dispersing individuals (Haas 1995; Neuschulz et al., 2012). These features of the urbanized landscape can make movements costly by inducing stress, increasing mortality risk, and thus may select for reduced dispersal (Cheptou et al. 2008; Adalsteinsson et al. 2018). Some favourable characteristics of cities, like the milder winter microclimate in the temperate climate zone, the availability of water and nutrients (e.g. human waste, food provided for birds) or lower predator abundance (Fischer et al. 2012; Vincze et al. 2017; Stofberg et al. 2019) may discourage members of the local populations to leave their natal or breeding area. Thus, urbanization is predicted to strongly influence population isolation and gene flow which can induce significant changes in the genetic structure of urban and non-urban populations (Johnson & Munshi-South, 2017).

The effects of urbanization on gene flow and genetic differentiation between populations have been studied in an increasing number of wild species. Several studies found significantly reduced gene flow and a detectable genetic differentiation in urbanized habitats, and the results vary between studies and species with different ecological and dispersal characteristics (Johnson & Munshi-South, 2017; Munshi-South & Richardson 2020). For example, strong isolation and consequent genetic differentiation were demonstrated in small vertebrates with low spreading ability, such as the red-backed salamander (*Plethodon cinereus*), the European rabbit (*Oryctolagus cuniculus*), or the white-footed mouse (*Peromyscus leucopus*) (Noel et al. 2007; Munsi-South & Kharchenko, 2010; Ziege et al. 2020; Richardson et al. 2021). In contrast, particularly for birds and large mammals, such as wild boars (*Sus scrofa*), genetic differentiation between urban and non-urban populations is quite low due to high levels of gene flow (Evans et al. 2009; Minias et al. 2017; Stillfried et al., 2017).

The great tit (*Parus major*) is a common Palearctic passerine bird and is one of the most widely studied model species in urban ecological and genetic research (Marzluff 2017). It occupies both urban and forest habitats, has the potential to move relatively long distances in a short time, but usually has a small home range (del Hoyo et al., 2007). Although urban great tits typically have significantly lower breeding success compared to their forest conspecifics in the same geographical region (Seress et al. 2018; de Satgé et al., 2019; Corsini et al. 2020; Bukor et al., 2022), their urban populations often reach similar or higher density (Møller et al. 2012, and our unpublished data). These contrasting trends in reproductive success and density suggest that some urban sites may act as population sinks for great tits where immigrants from surrounding non-urban habitats can play a role in maintaining both high urban population densities (Bukor et al., 2022) and gene flow between habitats.

Studies on phenotypic variation and/or genetic signatures of selection demonstrated likely contemporary adaptations in great tits to anthropogenic effects including urbanization (e.g. Laine et al. 2016; Perrier et al. 2017; Caizergues et al. 2021; Salmón et al. 2021; Vincze et al., 2021). A few studies also tested if urban great tits are genetically differentiated from non-urban populations. For example, Björklund et al. (2010) found that genetic differentiation at neutral microsatellite markers was higher between urban parks in Barcelona (Spain) than between parks and a forest area close to the city. The research of Markowski et al. (2021), also using microsatellite markers, found a low but significant divergence between urban, suburban, and forest sites in and around Łódź (Poland). A genome-wide SNP (single nucleotide polymorphism) study comparing nine major cities in Europe with their adjacent woodland areas showed weak population structuring with small to moderate, but statistically significant genetic differentiation between habitats (Salmón et al. 2021, Supplementary Tables 1–2). Another study of SNPs also found that birds in urban areas have lower heterozygosity values than in less urbanized areas, and reported a low but significant neutral genetic differentiation along the urbanization gradient (Perrier et al. 2017). Overall, these studies suggest somewhat reduced gene flow between urban and forest populations of great tits. Furthermore, Björklund et al. (2010) also found that the direction of gene flow was the opposite of what would be expected by the idea of urban sink populations: they inferred a significantly higher migration rate from the city to the forest population than *vice versa*.

In this study, we tested population differentiation and the extent and direction of gene flow between two populations of great tits living in a city and a nearby forest area. Both populations have been extensively studied since 2013. Our earlier research demonstrated several phenotypic differences between these populations, including plumage morphology (Sándor et al. 2021, 2022), behaviour (Preiszner et al. 2017; Vincze et al., 2021), and reproductive success (Seress et al. 2018; Pipoly et al. 2019, 2022). Although our long-term ringing data indicate some exchanges of individuals between the two study sites (unpublished data), the detection of migrants is very rare and gene flow has not been studied so far in these populations by genetic markers. In general, all the previous studies of population structure in great tits were conducted in Western and Northern European populations, and we have no similar information from other parts of the species' range. Our specific aims in this study were to assess the genetic diversity of the two populations, the degree of their genetic differentiation, and the extent and direction of gene flow between them.

Material and methods

Sample collection and genotyping

For this study, we collected blood samples from 192 adult great tits breeding in artificial nest boxes between 2013 and 2018. We captured parent birds with a nest box trap 6–15 days after their first nestling had hatched. Upon capture, we determined the parents' sex based on their plumage characteristics and measured the body mass with a Pesola spring balance (± 0.1 g), the tarsus length of the left leg with a vernier calliper (± 0.1 mm; following Svensson's 'alternative' method (Svensson 1992), and the length of the longest primary feather of the right wing with a ruler (± 1 mm). We also ringed each bird with a unique combination of a numbered metal ring and three plastic colour rings that allows unique identification of individuals. Individuals were captured in two study sites, in the city Veszprém (47°05′17″N, 17°54′29″E) and in a close forest site Vilma-puszta (47°05′02″N, 17°52′01″E). Veszprém is a medium-sized city in Hungary (population size in 2022: 57 145 (HCSO 2023)), with its vegetation consisting of a mix of indigenous and introduced plant species. Nest boxes were placed in four areas of public green space, including public parks, university campuses, and a cemetery, that are surrounded by built-up areas and roads and experience frequent anthropogenic disturbance. These sites comprise one relatively large core site (23.3 ha), and three smaller sites (3.1, 7.3, and 4.4 ha) located approximately 60 m, 620 m, and 730 m from the core site, respectively. Individuals were sampled from all of these four plots to encompass our entire urban study site. Individually marked breeding great tits have been observed to move occasionally between these plots during our long-term study of the population.

In contrast, Vilma-puszta is a homogenous, Natura 2000 protected forest area, where the dominant tree species are the native downy oak (*Quercus pubescens*) and South European flowering ash (*Fraxinus ornus*). This site has low human disturbance, it has no paved roads, there is only one nearby farm, and there is no logging activity; though hikers and hunters occasionally visit the area. The study plot in this area covers 48.1 ha. The sampling of individuals covered the whole study site. The distance between the centres of the two study sites is 3.5 km. The forest site is separated from the city boundary by high-traffic roads, and open agricultural lands unsuitable for great tits, although there are small forest patches that can provide corridors for birds moving between the two areas (Fig. 1). We captured and ringed the sampled individuals either as breeding adults or as nestlings that hatched and were recruited later as breeding birds in the same study site. For individuals sampled as nestlings, we selected only one individual per brood for genotyping to avoid statistical biases resulting from the close genetic relatedness of siblings.

We collected blood samples with blood collection syringes and needles in 1.5 ml Eppendorf tubes filled with 1 ml ethanol and stored them at -20 °C until further processing. We extracted DNA with E.Z.N.A. Tissue DNA Kit (Omega Bio-Tek Inc, Norcross, USA) following the manufacturer's protocol, and stored them at -20 °C. We performed PCR amplification of 17 microsatellite markers (Table S1) designed by Saladin et al. (2003) and Saladin & Richner (unpublished manuscript), which we further optimized for the purpose of the study (Björklund et al. 2010; Bertrand et al. 2016; Delahaie et al. 2017). Each PCR was carried out in a 15.6-µl reaction volume containing 0.66 µl dNTP (2 mM, Fermentas, Massachusetts, USA), 0.66 µl MgCl2 (25 mM, Fermentas, Massachusetts, USA), 0.066 µl DreamTaq polymerase (Fermentas, Massachusetts, USA), 1.70 µl DreamTaq Buffer (Fermentas, Massachusetts, USA), 10.5 µl MilliQ water, and 2 µl of the respective primer pair (10 µM) mixed at a 1:1 ratio. To avoid nonspecific fragments and to increase the specificity of PCR we used Touchdown PCR method. The annealing temperature was progressively decreasing from 62 to 57 °C (PmaCAn2; PmaTAGAn71; PmaTAGAn88; PmaTAGAn96e; PmaTGAn42; PmaGAn27; PmaTGAn33; PmaTAGAn89; PmaTGAn54; PmaCAn1; PmaTAGAn73; PmaTAGAn78) and from 59 to 54 °C (PmaC25; PmaD105; PmaD22; PmaTAGAn84; PmaTGAn51) over the course of successive cycles. The final PCR profile was: initial denaturation at 95 °C for 2 min, followed by 10 cycles of 95 ºC for 30 s with the TD-PCR steps and 72 ºC for 45 s, after that 28 cycles with 57 or 54 °C annealing temperature, concluded by a final extension step at 72 °C for 7 min.

We amplified each marker separately by PCR, then mixed the fluorescently labelled PCR products into two pools (Table S1) for each individual. We separated the products in these two pools on an ABI 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). Allele sizes were checked using Peak Scanner v.1.0 software (Applied Biosystems, Foster City, CA, USA). To assess our genotype evaluation accuracy, 25% of the trace files were surveyed by two experts (BM and EN), resulting in 95.06% consistency. We re-checked the inconsistently evaluated genotypes to find the correct allele sizes. Allele determination remained ambiguous for three individuals, so these were excluded from the analyses resulting in 189 genotyped individuals (119 from Veszprém and 70 from Vilma-puszta). If the amplification of a locus failed, we performed two additional attempts before we recorded it as a failed amplification.

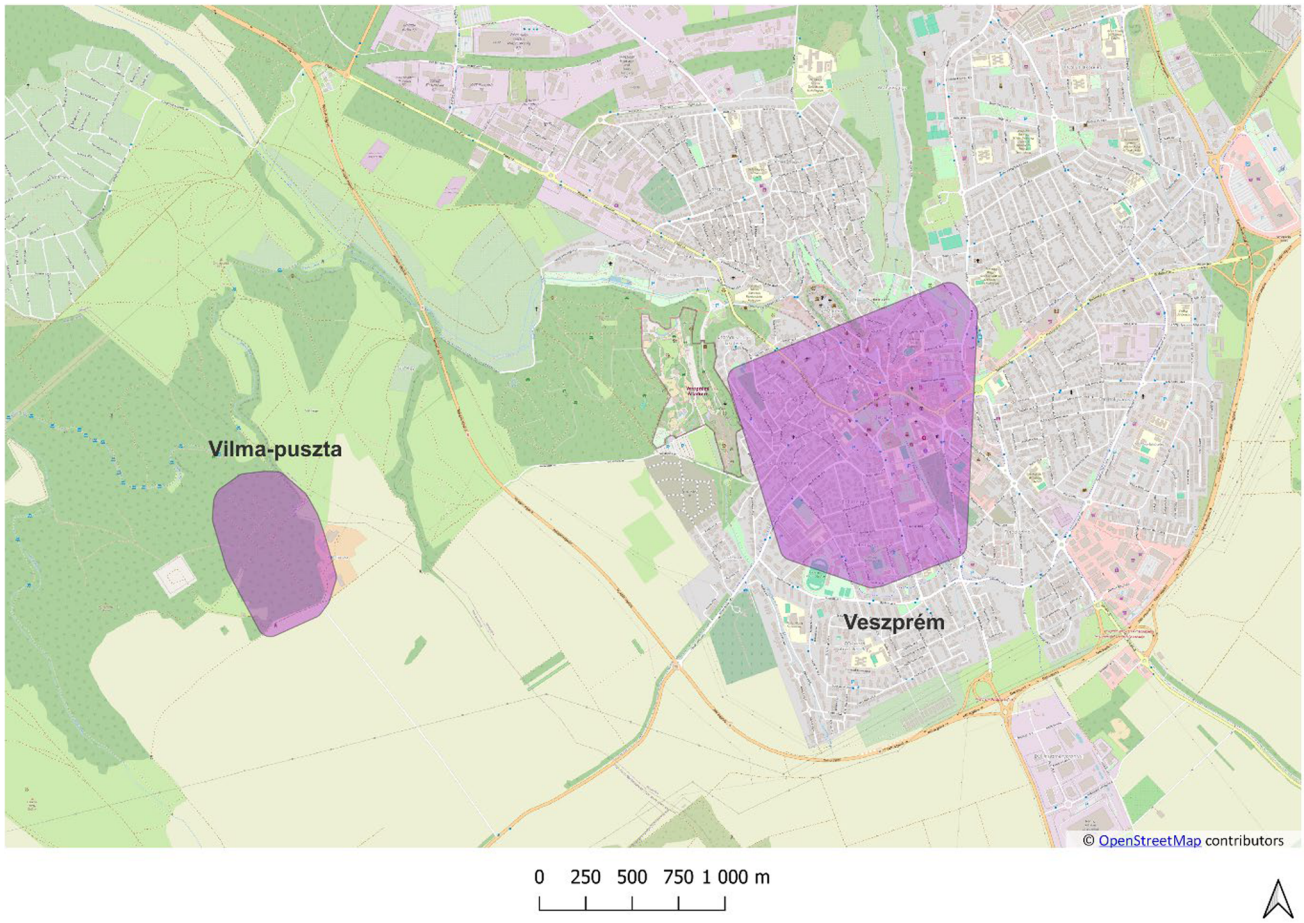

Fig. 1 Map of the sampling sites: Veszprém (urban), and Vilma-puszta (forest). The purple polygons show the areas of the artificial nest boxes used by breeding great tits. Dark green, light green, and yellow areas are forests, grassland, and agricultural fields, respectively (base map and data from OpenStreetMap and OpenStreetMap Foundation)

Statistical analyses

Preliminary tests of data quality and main genetic indices

We checked the presence of null alleles, large allele dropout, and stuttering using the Micro-Checker v.2.2.3 software (van Oosterhout et al. 2004). For finding null alleles, we used all four methods (Oosterhout, Chakraborty, Brookfield 1, and Brookfield 2) (Chakraborty et al. 1992; Brookfield 1996; van Oosterhout et al. 2004), and tested in the pooled samples (i.e. samples from the two sites together) and in the two sites separately. Microchecker analyses indicated the possible presence of null alleles in 10 loci when the entire dataset was analysed (i.e. the two study sites combined). Only a single microsatellite marker had a consistent result of null allele ratio > 0.2 (PmaTAGAn96; Table S1). Because of this high probability of null alleles according to all four calculation methods, this marker was excluded from the study (Table S1). The probability of null alleles was moderate (between 10 and 20%) in another four markers (PmaCAn1, PmaCAn2, PmaTGAn51, PmaTAGAn84), and another five showed low presence, under 10% (PmaC25, PmaGAn27, PmaTGAn54, PmaTAGAn78, PmaTAGAn89). To ensure that markers with potential null alleles did not cause bias in the results, we performed the population genetic analyses with the full set of 16 markers, as well as with two subsets consisting of markers with 1) only less than 20% null alleles (12 markers) or 2) no signs of null alleles (7 markers). Because the results of population genetics analyses were qualitatively similar regardless of using all 16, or the subsets of 12 or 7 microsatellite markers (and in line with the conclusions of Dakin & Avise 2004), in the main text, we present the results of analyses with 16 microsatellite markers. The analyses did not detect large allelic dropout in any locus, and it detected possible scoring errors due to stuttering in only one locus: PmaTAGAn84. This latter marker was also excluded from analyses that used the subsets of 12 and 7 markers. The possible presence of null alleles was also checked separately for the two sites (Table S2).

We measured the basic population genetic indices (number of alleles, number of effective alleles, heterozygosity values, and deviation from Hardy–Weinberg equilibrium) with GenAlEx v.6.5. (Peakall & Smouse 2012) and Genepop v4.2.1 (Rousset 2008). Differences in these indices between the two sites were tested by paired t-tests.

Population clusters

We calculated the most likely number of populations in which the genotyped individuals could be divided with Geneland v.3.4.2. (Guillot et al. 2012), testing the range from 1 to 10 populations, with 100 000 iterations and 100 thinning. To strengthen the results and visualizing them in a different way, a Bayesian clustering of genotype data was also performed using Structure 2.3.4 (Pritchard et al. 2000) software, with 1–10 populations, 3 repeats, burning period length of 750 000 and MCMC repeats after burning was set to 250 000. We accepted those birds clearly belonging to the same population where the probability exceeded 75%. We visualized the Structure results and calculated the most probable group numbers with Structure Harvester (Earl & von Holdt 2012). In this latter analysis, we estimated the most probable number of genetic clusters both by log probabilities L'(K) and the Evanno's method (ΔK). Furthermore, we performed an expanded cluster analysis that uses both genotypic and phenotypic data, as implemented in assignPOP and klaR R packages (Röver et al. 2004; Chen et al. 2018). This method has the advantage that it may separate groups by including new, easy-to-study variables, like morphological traits, in the analyses. We used three types of phenotypic data: tarsus length, wing length, and body mass (see above). AssignPOP performs Monte-Carlo and K-fold cross-validation procedures to estimate assignment accuracy and membership probabilities and allows the building of predictive models based on classification functions including linear discriminant analysis, support vector machine, naïve Bayes, decision tree, and random forest. We used the assign.MC function with Monte-Carlo procedure and random forest classification function in the analysis, and the accuracy.plot function to visualize the result.

Finally, we conducted an analysis of molecular variance (AMOVA) in GenAlEx v.6.5. software (Peakall & Smouse 2012) using default settings to partition total genetic variance into three components: among populations, among individuals within populations, and within individuals.

Level of genetic differentiation and migration rates

To estimate genetic differentiation between populations, we calculated pairwise F_{st} using the Geneland v 4.0.9 software (Peakall & Smouse, 2012). The migration rates between the two areas were calculated with the Migrate v5.0.4. software (Beerli 2006) using the default settings. This approach estimates migration rate as $M = m/\mu$, where m is the fraction of the new immigrants of the population per generation, and μ is the variability created by mutation. The software allowed us to specify the direction of migration, thus we could test whether the migration rate from the urban to forest population is different from the migration rate in the reverse direction.

Results

Main genetic indices

Successful amplification ranged from 170 to 188 samples per microsatellite marker, and allele numbers varied from 11 (PmaTAGAn73) to 33 (PmaTAGAn78), with an average of 20 alleles (Table S3). We estimated that the number of effective alleles for the entire sample set varies between 22.01% (5.7 alleles, PmaTGAn54) and 55.71% (9.4 alleles, PmaTAGAn84) of the observed allele numbers, with an average of 39.38% (Table S3). The expected heterozygosity values were high (≥ 0.8) in all cases, but the observed values were lower in most cases, ranging from 0.559 (PmaCAn1) to 0.881 (PmaTGAn33) (Table S3). Consequently, six of the 16 markers showed a significant deviation from the Hardy–Weinberg equilibrium (Table S3). The observed heterozygosity (Ho) was higher in the forest area than in the city (Table 1). On the other hand, no significant

Table 1 Summary statistics of the main genetic indices for the pooled samples (i.e. the two sites analysed together) and separately for the two spatial groups (urban: Veszprém, forest: Vilma-puszta)

	Two sites combined	Urban site	Forest site	T (df = 15)	p
Na	16.47 ± 1.834	17.12 ± 1.553	15.82 ± 1.384	1.835	0.086
Ne	7.578 ± 0.755	7.474 ± 0.73	7.683 ± 0.775	0.199	0.845
Ho	0.729 ± 0.022	0.703 ± 0.022	0.756 ± 0.027	2.171	0.046
He	0.853 ± 0.010	0.847 ± 0.01	0.859 ± 0.012	0.703	0.493

Na: number of alleles, Ne: number of effective alleles, Ho: observed heterozygosity, He: expected heterozygosity. The table shows the means ± SE of the 16 loci and the results of paired t-tests comparing the two sites (see Tables S3 and S4 for values of each locus)

differences were detected in allele numbers and expected heterozygosity (Table 1). The measures of allelic richness for each locus are shown separately for the two study areas in Table S4.

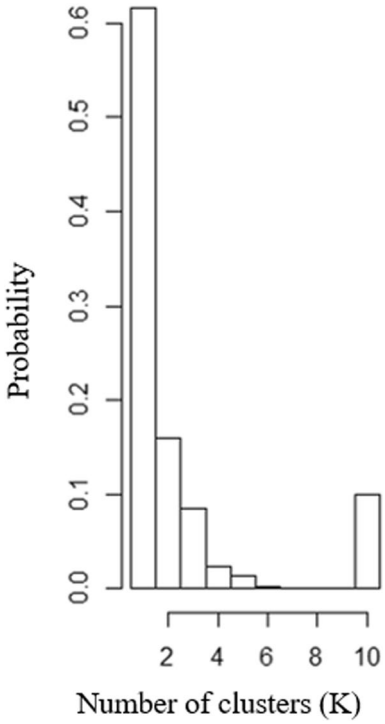

Fig. 2 The results of population structure analyses using Geneland. The histogram shows that there is an outlier probability (> 60%) for the model assuming that individuals belong to a single population (K = 1)

Genetic population clusters and genetic differentiation

The first set of population structure analysis by Geneland using all 16 markers showed that the genotyped individuals belong to a single population (K = 1) with the highest probability (> 60%, Fig. 2). The second most probable division that clusters the birds into two populations (K = 2) has a much lower probability (< 20%). We obtained similar results when we repeated the analyses with 12 and 7 markers (Table S5).

The Bayesian clustering of individuals by Structure largely confirmed these results. There were only 25 out of the 189 birds (13.23%) that could be assigned to one of the study sites (i.e. city or forest) with higher than 75% probability in the two cluster (K = 2) model, and Fig. 3 shows that the population assignments of individuals did not differ consistently between the two populations. We obtained the same results when the subset of 12 or 7 microsatellite markers was analysed (Fig S1 and S2). Structure Harvester analysis provided the highest support for 4 genetic clusters (K = 4; ΔK = 94.5; Table S6). However, the assignment of individuals to these 4 putative genetic clusters does not consistently differ between the two habitats (i.e. both study sites contains a mix of individuals from all clusters; Fig. 3). Please note that in this set of analyses it was not possible to compare the above scenarios (i.e. K = 2 and 4) with K = 1 because the ΔK value cannot be calculated for the latter model. Finally, the expanded clustering method that also included the three phenotypic variables (tarsus and wing length, and body mass) produced similar results: the probability of the individuals' assignments to the two groups was not consistently associated with the study sites (Fig S3).

In line with the above results of population structure, the AMOVA analysis found that the between populations (i.e. habitats) component of variance accounts only for 1% of the

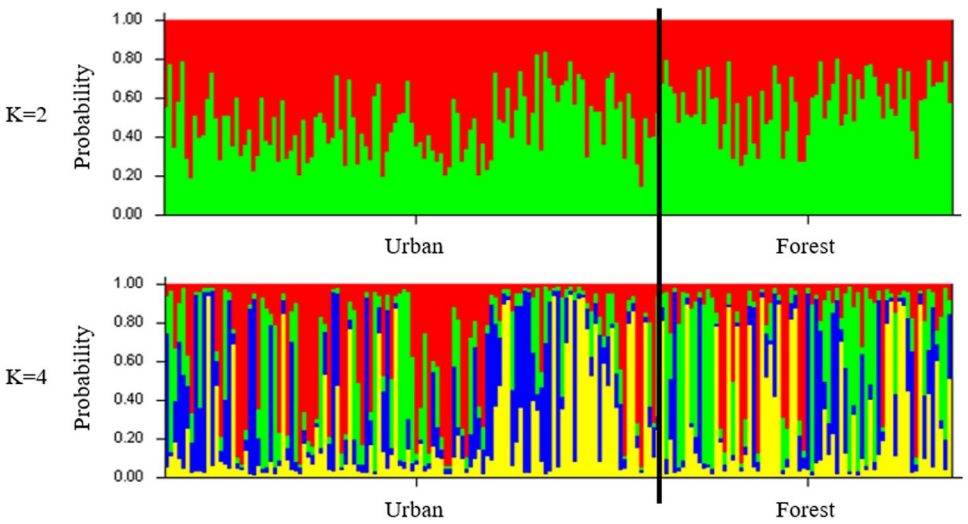

Fig. 3 Results of Bayesian population structure analyses by Structure with K = 2 (top panel) and K = 4 (bottom panel) groups. The two parts in each panel show the two study sites, whereas each vertical column shows the probability of assignment of an individual to putative genetic clusters based on 16 microsatellite genotypes. With K = 2 clustering the coloration shows the probability of belonging to the urban (red) or forest (green) groups

total genetic variance, while the variance within individuals explained 81% (Table S7). Similarly, we found a low albeit statistically significant genetic differentiation between the two study sites (pairwise $F_{st} = 0.009$, $p = 0.001$).

Migration rates

The mean estimate of migration rate (*M*) from forest site to urban site was 0.995 (95% CI: 0.78–1.2), whereas its estimate for the opposite direction was 0.934 (95% CI: 0.72–1.147). The almost completely overlapping credible intervals of these estimates indicate a negligible difference between the migration rates in the two directions (Fig. 4).

Discussion

Our study consistently indicated low genetic structuring between the two spatially separated groups of great tits living in an urban and a forest habitat close to each other. None of the clustering approaches separated the individuals according to their origin of habitat, even when we included phenotypic data in the analysis. The pairwise F_{st} (0.009) also indicated a low level of genetic differentiation, and between habitats variation explained only 1% of the total genetic variance. This lack of marked genetic differentiation between nearby urban and non-urban great tits is in line with the results of earlier studies of the species. For example, similarly low F_{st} values (< 0.05) were reported for most urban–rural comparisons in other geographic areas (using SNPs: Perrier et al. 2017; Salmón et al. 2021; using microsatellites: Markowski et al. 2021), although higher F_{st} was also found (Björklund et al. 2010, using microsatellites).

Nevertheless, some of our results suggest detectable genetic differences between urban and forest birds: one measure of genetic variation (observed heterozygosity) was significantly lower in the urban than in the forest population, and the F_{st} was statistically significant. Other studies of great tits also reported statistically significant divergence of urban populations (Björklund et al. 2010; Perrier et al. 2017; Markowski et al. 2021; Salmón et al. 2021), although not universally (e.g. Salmon et al. 2021 did not detect reduced heterozygosity in urban populations).

Several factors can contribute to this low but detectable level of genetic differences between urban and non-urban populations that appear to characterize the species over most of its studied geographic range. On the one hand, birds are likely to have smaller dispersal distances and/or home ranges (Vangestel et al. 2010, 2011; Luna et al. 2019; O'Donnell & delBarco-Trillo 2020) in urban compared to forested areas due to specific habitat characteristics of the cities (e.g. higher fragmentation, presence of food sources; see Introduction). This may lead to some level of genetic isolation and higher inbreeding rates in urban populations promoting genetic divergence. On the other hand, as shown by our results, there is gene flow between urban and adjacent non-urban populations of great tits. We estimated migration rates of about 0.78–1.20 from Vilma-puszta to Veszprém and 0.72–1.15 in the opposite direction; these results are broadly similar to those found in great tits between city populations of Barcelona (Björklund et al 2010). Indeed, our ringing data also confirm that individuals sometimes move between the two habitats (but such instances are very rare in our database: 12 out of 8087 ringed individuals between 2011 and 2024; most of them juvenile dispersers), and similar movements were reported by Björklund et al (2010). Thus, this level of gene flow may be sufficient to prevent strong genetic isolation and quick divergence of urban great tit populations. Additional factors that may act against genetic differentiation include large population sizes of great tits that may mitigate the effects of genetic drift on neutral markers, and the relatively recent urban history of the species (Perrier et al. 2017).

Gene flow from urban areas to their surrounding landscapes (and *vice versa*) may be reduced compared to that in natural habitats. For example, Salmón et al. (2021) found that genetic differentiation between non-urban sites across Europe was lower than between pairs of urban–rural sites or between different cities in the same geographic region. Collectively, these findings align with the expectations drawn from the ecological characteristics of cities (see Introduction) that urbanization can limit gene flow and contribute – albeit weakly – to genetic divergence. Similarly, Perrier

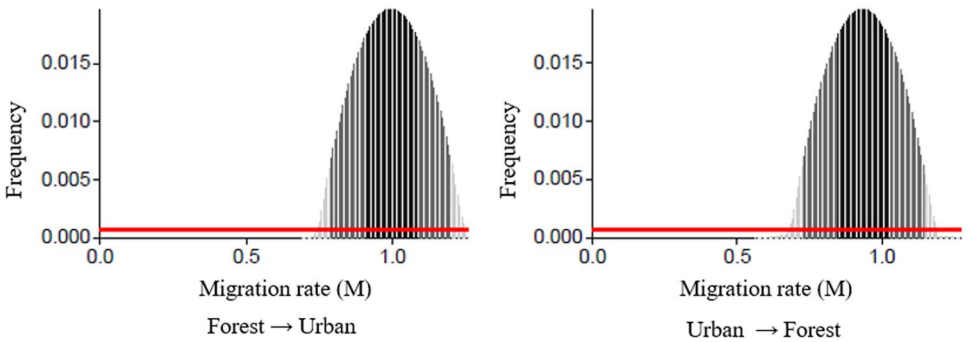

Fig. 4 Posterior distribution of migration rates (M) from the forest (Vilma-puszta) to the urban site (Veszprém; left panel), and vice versa (right panel). The calculation is based on data from all (n = 16) microsatellite loci. Red lines are the prior distribution used in the model

et al. (2017) concluded that genetic differentiation due to urbanization in great tits (and probably in other urbanized birds as well) may be on the rise, and that reduced gene flow, coupled with small genetic drift, is likely compatible with local adaptation processes.

However, reduced gene flow is not exclusive to urban areas but has also been documented in natural habitats. So far, several studies reported limited gene flow between populations of great and blue tits within mosaics of forest patches, where habitat connectivity is hindered, e.g. by intervening grasslands or agricultural landscapes. In such cases, even over distances of just a few kilometres, similar (e.g. Ortego et al. 2011; García-Navas et al. 2014) or even higher F_{st} values have been reported (Garrido-Bautista et al. 2024) than compared to urban populations (see above), suggesting that the effects of reproductive barriers on genetic differentiation may be similar or sometimes even more pronounced in non-urban fragmented landscapes than in urban environments. Studies also reported local adaptation in tits at small geographic scales in natural habitats (e.g. Garant et al. 2005; Postma & Noordwijk. 2005; Garrido-Bautista et al. 2024).

So far, the analyses of gene flow in great tits did not support the idea that urban populations are population sinks maintained by asymmetric immigration of individuals from non-urban areas. The estimates of migration rates in our study were close to identical from the city to the forest and *vice versa*. Björklund et al. (2010) found a higher migration rate from the city to the forest than in the opposite direction. These results suggest that the high density of great tits often observed in urban areas may be a consequence of local demographic processes, for example via better juvenile or adult survival in cities than in rural areas. Interestingly, the inferred direction of gene flow differs between our results and the study of Björklund et al (2010), although the distance between study populations was similar. One reason for the difference may be that these studies used distinct sampling methods (we used data only of confirmed, locally breeding individuals while this is not unambiguously the case in the other study). Study sites may also differ in several habitat characteristics (e.g. food abundance, predation risk, habitat connectivity) that influence the movement decisions of birds.

Despite the low level of genetic differentiation between rural and urban populations, we found several kinds of phenotypic differences between the individuals of the two sites involved in this study (for example in plumage and feather structure, reproductive effort, extra-pair behaviour, problem-solving, and risk-taking; Preiszner et al. 2017; Pipoly et al. 2019; Seress et al. 2018, 2021; Sándor et al. 2021, 2022; Vincze et al., 2021). These may be due to selection acting on specific genes in the cities, even if neutral markers remain undifferentiated (Laine et al. 2016; Salmon et al., 2021). It is likely that other mechanisms may also be involved in generating urban phenotypes, for example, phenotypic plasticity (Johnson & Munsi-South, 2017; Mercx et al., 2018), epigenetic mechanisms (Riyahi et al. 2015; Caizergues et al. 2021), or environmental constraints like reduced quality and quantity of available food (Seress et al. 2018, 2020) and climatic conditions like the heat island effect (Pipoly et al., 2022).

One limitation of our study is that we used microsatellites to test genetic differentiation instead of using SNPs, which have higher power due to the larger number of informative markers. However, microsatellites are still widely used due to the method's cost-effectiveness and its suitability to test genetic isolation between groups (e.g. Björklund et al. 2010; Bertrand et al. 2016; Delahaie et al. 2017), especially when the markers are highly variable as in our study (20 alleles per marker on average).

Conclusions for future biology

In conclusion, our study supports the general agreement of several other population genetic studies that, at neutral markers, the differentiation between urban and non-urban conspecific bird populations is low (Lemoine et al. 2016; Neale & Wheeler 2019; Szulkin et al. 2020). Importantly, our results suggest that the rate of movement between the two habitats is similar in both directions. This latter result does not support the hypothesis that urban populations are population sinks maintained by birds immigrating from forest areas. We suggest future studies to confirm this latter finding by other methods, for example by analysing ringing recoveries and by radio tracking of the movement of birds. Having a more complete knowledge on the degree of isolation of urban animal populations from non-urban ones can provide a better understanding of both their population dynamics and also their potential for genetic adaptations to local ecological conditions.

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Declarations

Conflict of interest The authors of the paper declare no conflict of interest.

Ethical approval All procedures used in the study were in accordance with the guidelines for animal care outlined by ASAB/ABS and Hungarian laws. Permits to study protected species and access to protected areas were provided by the Middle Transdanubian Inspectorate for Environmental Protection, Natural Protection and Water Management (permit numbers: 31559/2011, 24,861/2014 and VE-09Z/03454–8/2018). Permit for blood sampling was provided by the Pest County Government Office, Department of Food Chain Safety, Animal Health, and Plant and Soil Protection (permit nr. PE/EA/786–7/2018).

References

Adalsteinsson SA, Buler JJ, Bowman JL, D'Amico V, Ladin ZS, Shriver WG (2018) Post-independence mortality of juveniles is driven by anthropogenic hazards for two passerines in an urban landscape. J Avian Biol 49(8):e01555

Beerli P (2006) Comparison of Bayesian and maximum-lieklihood inference of population genetic parameters. Bioinformatics 22(3):341–345

Benítez-López A, Alkemade R, Verweij PA (2010) The impacts of roads and other infrastructure on mammal and bird populations: a meta-analysis. Biol Cons 143(6):1307–1316

Bertrand JAM, Delahaie B, Bourgeois YXC, Duval T, Garcia-Jimenez R, Cornuault J, Pujol B, Thébaud C, Milá B (2016) The role of selection and historical factors in driving population differentiation along an elevational gradient in an island bird. J Evol Biol 2(9):824–836

Bhattacharya M, Primack RB, Gerwein J (2002) Are roads and railroads barriers to bumblebee movement in a temperate suburban conservation area? Biol Cons 109(1):37–45

Björklund M, Ruiz I, Senar JC (2010) Genetic differentiation in the urban habitat: the great tits (*Parus major*) of the parks of Barcelona city. Biol J Lin Soc 99(1):9–19

Brookfield JFY (1996) A simple new method for estimating null allele frequency from heterozygote deficiency. Mol Ecol 5(3):453–455

Bukor B, Seress G, Pipoly I, Sándor K, Cs S, Vincze E, Liker A (2022) Double-brooding and annual breeding success of great tits in urban and forest habitats. Curr Zool. 68(5):517–525. https://doi.org/10.1093/cz/zoab096

Caizergues AE, Le Luyer J, Grégoire A, Szulkin M, Senar J-C, Charmantier A, Perrier C (2021) Epigenetics and the city: Non-parallel DNA methylation modifications across pairs of urban-forest Great tit populations. Evol Appl 15(1):149–165

Chakraborty R, de Andrade M, Daiger SP, Budlowe B (1992) Apparent heterozygote deficiencies observed in DNA typing data and their implications in forensic applications. Ann Hum Genet 56(1):45–57

Chen K-Y, Marschall EA, Sovic MG, Fries AC, Gibbs HL, Ludsin SA (2018) AssignPOP: an R package for population assignment using genetic, non-genetic, or integrated data in a machine-learning framework. Methods Ecol Evol 9(2):439–446

Cheptou P-O, Carrue O, Rouifed S, Cantarel A (2008) Rapid evolution of seed dispersal in an urban environment in the weed *Crepis sancta*. PNAS 105(10):3796–3799

Corsini M, Schöll EM, Di Lecce I, Chatelain M, Dubiec A, Szulkin M (2020) Growing in the city: urban evolutionary of avian growth rates. Wiley Evol Appl 14(1):69–84

del Hoyo J, Elliott A, Cristie DA. Handbook of the Birds of the World (2007) Volume 12, Picathares to Tits and Chickadees. Lynx Edicions, Barcelona

Dakin EE, Avise JC (2004) Microsatellite null alleles in parentage analysis. Heredity 93(5):504–509

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. https://doi.org/10.1111/jav.02108

Delahaie B, Cornuault J, Masson C, Bertrand JAM, Bourgeois YXC, Milá B, Thébaud C (2017) Narrow hybrid zones in spite of very low population differentiation in neutral markers in an island bird species complex. J Evol Biol 30(12):2132–2145

Earl DA, von Holdt BM (2012) Structure harvester: a website program for visualizing Structure output and implementing the Evanno method. Conserv Genet Resour 4(2):359–361

Evans KL, Gaston KJ, Frantz AC, Simeoni M, Sharp SP, McGowan A et al (2009) Independent colonization of multiple urban centres by a formerly forest specialist bird species. Proc Royal Soc B 276(1666):2403–2410

Evans KL, Hatchwell BJ, Parnell M, Gaston KJ (2010) A conceptual framework for the colonisation of urban areas: the blackbird Turdus merula as a case study. Biol Rev 85(3):643–667

Fischer JD, Cleeton SH, Lyons TP, Miller JR (2012) Urbanization and the predation paradox: the role of trophic dynamics in structuring vertebrate communities. Bioscience 62(9):809–818

Garant D, Kruuk LE, Wilkin TA, McCleery RH, Sheldon BC (2005) Evolution driven by differential dispersal within a wild bird population. Nature 433(7021):60–65

García-Navas V, Cáliz-Campal C, Ferrer ES, Sanz JJ, Ortego J (2014) Heterozygosity at a single locus explains a large proportion of variation in two fitness-related traits in great tits: a general or a local effect? J Evol Biol 27(12):2807–2819

Garrido-Bautista J, Comas M, Jowers MJ, Smith S, Penn DJ, Bakkali M, Moreno-Rueda G (2024) Fine-scale genetic structure and phenotypic divergence of a passerine bird population inhabiting a continuous Mediterranean woodland. Royal Soc Open Sci 11(6):240601

Guillot G, Renaud S, Ledevin R, Michaux J, Claude J (2012) A unifying model for the analysis of phenotypic, genetic and geographic data. Syst Biol 61(5):897–911

Haas CA (1995) Dispersal and use of corridors by birds in wooded patches on an agricultural landscape. Conserv Biol 9(4):845–854

HCSO, 2023: Hungarian Central Statistical Office data https://www.ksh.hu/stadat_files/fol/hu/fol0014.html, downloaded: 2023.05.06

Johnson MTJ, Munshi-South J (2017) Evolution of life in urban environments. Science 358(6363):eaam8327

Keller I, Alexander JM, Holderegger R, Edwards PJ (2013) Widespread phenotypic and genetic divergence along altitudinal gradients in animals. J Evol Biol 26(12):2527–2543

Laine VN, Gossmann TI, Schachtschneider KM, Garroway CJ, Madsen O, Verhoeven KJF et al (2016) Evolutionary signals of selection on cognition from the great tit genome and methylome. Nat Commun 7(1):10474

Landguth EL, Cushman SA, Schwartz MK, McKelvey KS, Murphy M, Luikart G (2010) Quantifying the lag time to detect barriers in landscape genetics. Mol Ecol 19(19):4179–4191

Lemoine M, Lucek K, Perrier C, Saladin V, Adriaensen F, Barba E et al (2016) Low but contrasting neutral genetic differentiation shaped by winter temperature in European great tits. Biol J Linnean Soc 118(3):668–685

Liker A (2020) Biologia Futura: adaptive changes in urban populations. Biologia Futura 71(1):1–8

Luna A, Palma A, Sanz-Aguilar A, Tella JL, Carrete M (2019) Personality-dependent breeding dispersal in rural but not urban burrowing owls. Sci Rep 9(1):2886

Markowski M, Minias P, Banbura M, Gladalski M, Kalinski A, Skwarska J, Wawrzyniak J, Zielinski P, Banbura J (2021) Genetic structure of urban and non-urban populations differs between two common parid species. Sci Rep 11(1):10428

Marzluff JM (2017) A decadal review of urban ornithology and a prospectus for the future. Ibis 159(1):1–13

Merckx T, Souffreau C, Kaiser A, Baardsen LF, Backeljau T, Bonte D et al (2018) Body-size shifts in aquatic and terrestrial urban communities. Nature 558(7708):113–116

Minias P, Wlodarczyk R, Minias A, Dziadek J (2017) How birds colonize cities: genetic evidence from a common waterbird, the Eurasian coot. J Avian Biol 48(8):1095–1103

Møller AP, Diaz M, Flensted-Jensen E, Grim T, Ibánez-Álamo JD, Jokimaki J et al (2012) High urban population density of birds reflects their timing of urbanization. Oecologia 170(3):867–875

Morjan CL, Rieseberg LH (2004) How species evolve collectively: implications of gene flow and selection for the spread of advantageous alleles. Mol Ecol 13(6):1341–1356

Munshi-South J, Kharchenko K (2010) Rapid, pervasive genetic differentiation of urban white-footed mouse (*Peromyscus leucopus*) populations in New York City. Mol Ecol 19(19):4242–4254

Munshi-South J, Richardson JL (2020) Landscape Genetic Approaches to Understanding Movement and Gene Flow in Cities. In: Szulkin M (ed) Urban Evolutionary Biology. Oxford University Press, Jason Munshi-South and Anne Charmantier, pp 54–73

Neale DB, Wheeler NC (2019) Neutral Genetic Variation. The Conifers: Genomes. Variation and Evolution. Springer, Cham, pp 181–224

Neuschulz EL, Brown M, Farwig N (2012) Frequent bird movements across a highly fragmented landscape: the role of species traits and forest matrix. Anim Conserv 16(2):170–179

Noel S, Ouellet M, Galois P, Lapointe F-J (2007) Impact of urban fragmentation on the genetic structure of the eastern red-backed salamander. Conserv Genet 8:599–606

O'Donnell K, del Barco-Trillo J (2020) Changes in the home range sizes of terrestrial vertebrates in response to urban disturbance: a meta-analysis. J Urban Ecol 6(1):juaa014

Ortego J, García-Navas V, Ferrer ES, Sanz JJ (2011) Genetic structure reflects natal dispersal movements at different spatial scales in the blue tit *Cyanistes caeruleus*. Animal Behav 82(1):131–137

Peakall R, Smouse PE (2012) GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research – an update. Bioinformatics 28:2537–2539

Perrier C, del Campo AL, Szulkin M, Demeyrier V, Gregoire A, Charmantier A (2017) Great tits and the city: distribution of genomic diversity and gene-environment associations along an urbanization gradient. Evol Appl 11(5):593–613

Pipoly I, Szabó K, Bókony V, Preiszner B, Seress G, Vincze E, Schroeder J, Liker A (2019) Higher frequency of extra-pair offspring in urban than forest broods of great tits (*Parus major*). Front Ecol Evol 7:229

Pipoly I, Preiszner B, Sándor K, Cs S, Seress G, Vincze E, Bókony V, Liker A (2022) Extreme hot weather has stronger impacts on avian reproduction in forests than in cities. Front Ecol Evol 10:825410

Postma E, van Noordwijk AJ (2005) Gene flow maintains a large genetic difference in clutch size at a small spatial scale. Nature 433(7021):65–68

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 Cogn 20:53–63

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. Genetics 155(2):945–959

Richardson JL, Michaelides S, Combs M, Djan M, Bisch L et al (2021) Dispersal ability predicts spatial genetic structure in native mammals persisting across an urbanization gradient. Evol Appl 14(1):163–177

Riyahi S, Sánchez-Delgado M, Calafell F, Monk D, Senar JC (2015) Combined epigenetic and intraspecific variation of the DRD4 and SERT genes influence novelty seeking behaviour in great tits *Parus major*. Epigenetics 10(6):516–525

Rousset F (2008) genepop'007: a complete re-implementation of the genepop software for Windows and Linux. Mol Ecol Resour 8(1):103–106

Röver C, Raabe N, Luebke K, Ligges U (2004) klaR: A package including various classification tools. University Dortmund, Dortmund, Germany

Saladin V, Bonfils D, Binz T, Richner H (2003) Isolation and characterization of 16 microsatellite loci in the Great Tit *Parus major*. Mol Ecol Notes 3(4):520–522

Saladin V, Richner H. A set of 48 microsatellite loci for the great tit including 15 novel markers. (unpublished manuscript; markers submitted to GenBank)

Salmón P, Jacobs A, Ahrén D, Biard C, Dingemanse NJ, Dominoni DM et al (2021) Continent-wide genomic signatures of adaptation to urbanisation in a songbird across Europe. Nat Commun 12:2983

Sándor K, Liker A, Sinkovics C, Péter Á, Seress G (2021) Urban nestlings have reduced number of feathers in Great Tits (*Parus major*). Ibis 163(4):1369–1378

Sándor K, Seress G, Sinkovics C, Péter Á, Liker A (2022) Differences in feather structure between urban and forest great tits – constraint or adaptation? J Avian Biol 2022:e02922

Seress G, Liker A (2015) Habitat urbanization and its effects on birds. Acta Zoologica Academiae Scientiarum Hungaricae 61(4):373–408

Seress G, Hammer T, Bókony V, Vincze E, Preiszner B, Pipoly I et al (2018) Impact of urbanization on abundance and phenology of caterpillars and consequences for breeding in an insectivorous bird. Ecol Appl 28(5):1143–1156

Seress G., Sándor K., Vincze E., Pipoly I., Bukor B., Ágh N., Liker A. Contrasting effects of the COVID-19 lockdown on urban birds' reproductive success in two cities. Scientific Reports 2021/11. 10 pp.

Stillfried M, Fickel J, Börner K, Wittstatt U, Heddergott M, OrtmannS K-SS, Frantz AC (2017) Do cities represent sources, sinks or isolated islands for urban wild boar population structure? J Appl Ecol 54(1):272–281

Stofberg M, Cunningham SJ, Sumasgutner P, Amar A (2019) Juggling a "junk-food" diet: responses of an urban bird to fluctuating anthropogenic-food availability. Urban Ecosystems 22:1019–1026

Svensson, L. Identification Guide to European Passerines. British Trust for Ornithology, Stockholm,1992

Szulkin M, Munshi-South J, Charmantier A (2020) Urban Evolutionary Biology. Oxford University Press, Oxford, UK, p 318

van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P (2004) Micro-checker: software for identifying and correcting genotyping errors in microsatellite data. Mol Ecol Notes 4(3):535–538

Vangestel C, Braeckman BP, Matheve H, Lens L (2010) Constraints on home range behaviour affect nutritional condition in urban house sparrows (*Passer domesticus*). Biol J Lin Soc 101(1):41–50

Vangestel C, Mergeay J, Dawson DA, Vandomme V, Lens L (2011) Spatial heterogeneity in genetic relatedness among house sparrows along an urban-rural gradient as revealed by individual-based analysis. Mol Ecol 20(22):4643–4653

Vincze E, Seress G, Lagisz M, Nakagawa S, Dingemanse NJ, Sprau P (2017) Does urbanization affect predation of bird nests? A meta-analysis. Front Ecol Evol 5:29. https://doi.org/10.3389/fevo.2017.00029

Vincze E, Bókony V, Lzs G, Seress G, Pipoly I, Cs S et al (2021) Consistency and plasticity of risk-taking behaviour towards humans at the nest in urban and forest great tits, *Parus major*. Animal Behav 179:161–172

Ziege M, Theodorou P, Jüngling H, Merker S, Plath M, Streit B, Lerp H (2020) Population genetics of the European rabbit along a rural-to urban gradient. Sci Rep 10(1):2448