

ORIGINAL ARTICLE

Genetic diversity and population structure in contemporary house sparrow populations along an urbanization gradient

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House sparrow (*Passer domesticus*) populations have suffered major declines in urban as well as rural areas, while remaining relatively stable in suburban ones. Yet, to date no exhaustive attempt has been made to examine how, and to what extent, spatial variation in population demography is reflected in genetic population structuring along contemporary urbanization gradients. Here we use putatively neutral microsatellite loci to study if and how genetic variation can be partitioned in a hierarchical way among different urbanization classes. Principal coordinate analyses did not support the hypothesis that urban/suburban and rural populations comprise two distinct genetic clusters. Comparison of F_{ST} values at different hierarchical scales revealed drift as an important force of population differentiation. Redundancy analyses revealed that genetic structure was strongly affected by both spatial variation and level of urbanization. The results shown here can be used as baseline information for future genetic monitoring programmes and provide additional insights into contemporary house sparrow dynamics along urbanization gradients.

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INTRODUCTION

Population connectivity, mediated by dispersal, affects various ecological and evolutionary key features, such as population growth rates, spatial distribution of genetic diversity, local adaptation and global dynamics (Ronce, 2007). Because population connectivity shapes the resilience of populations against demographic, genetic and environmental disturbance (Hanski, 1999), it also constitutes a key determinant of their long-term viability. Assessing dispersal rates using capture–mark–recapture is often cumbersome and time-consuming, and low probabilities of resighting or retrapping due to the inaccessibility of open areas (for example, private gardens) and strong human disturbance in urban centers, may additionally hamper its use. Nowadays, the wealth of genetic markers, which are readily available for most species and the advent of new statistical analytical methods have provided researchers with a powerful alternative to indirectly measure dispersal using genotype data (Neigel, 1997; Prugnolle and de Meeus, 2002). In addition, a body of literature has presented numerous correlations between genetic variation and fitness traits, such as growth, survival and disease resistance (Falconer and MacKay, 1996; Reed and Frankham, 2003), and many conservation biologists are therefore concerned in preserving the remnant genetic variation within a species. Knowledge on the geographical distribution of existing genetic variation has thus become an invaluable source of information when aiming to delineate appropriate biological conservation units (Taylor and Dizon, 1996; Gebremedhin *et al.*, 2009). A solid understanding of genetic population substructure and exchange of migrants between populations may complement demographic

studies and has become a major goal of many ecological studies as it can inform both population ecologists and conservation managers.

One of the species that has recently drawn the attention of conservation biologists is the house sparrow (*Passer domesticus*). Once a thriving ubiquitous species (Anderson, 2006), house sparrows have suffered a dramatic decline in abundance and distribution during the last decades (Hole *et al.*, 2002; Chamberlain *et al.*, 2007; De Laet and Summers-Smith, 2007) and evidence has mounted that these reductions vary considerably in space and time (De Laet and Summers-Smith, 2007; Shaw *et al.*, 2008). Within strongly built-up areas, urban populations, which have suffered massive declines, can generally be differentiated from suburban ones, which have remained rather stable. Apart from the extent of the population decline, the onset in rural areas also preceded that in the urban one, and although rural population numbers seem to have stabilized (albeit at lower population equilibria), urban populations continue to plummet (De Laet and Summers-Smith, 2007). Yet, despite this demographic heterogeneity, post-decline data on small-scale genetic structuring along urbanization gradients are still lacking for this species. This paucity of information may hamper future conservation efforts and advocates for an urgent need to bridge this lacuna.

House sparrows are among the most sedentary of all passerine birds, being characterized by short postnatal dispersal distance (1–1.7 km; Anderson, 2006) and strong levels of site tenacity after their first breeding attempt (Summers-Smith, 1988). Based on this sedentary behavior and the difference in timing, magnitude and progress of the population decline, urban/suburban and rural

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populations are thought to comprise two distinct and independent units that may call for different conservation strategies (Anderson, 2006; De Laet and Summers-Smith, 2007; Shaw *et al.*, 2008). Such dichotomy implicitly assumes that both habitats are characterized by a high degree of isolation and are to a large extent self-sustainable (Wilson, 2004). Likewise, the small-scale heterogeneity in demographic features within the built-up environment at least casts doubt that urban and suburban areas constitute a single independent unit. This view, however, was challenged by Heij (1985) who concluded that both urban and rural study populations were highly dependent on suburban immigrants for their long-term viability. Several other studies also failed to reveal (large) genetic dissimilarities between house sparrow populations in the absence of strong geographical barriers (Fleisher, 1983; Parkin and Cole, 1984; Kekkonen *et al.*, 2010). Yet, all these studies had in common that they described dispersal and/or genetic structure well before the onset of the population decline.

In contrast, Hole (2001) found low but significant genetic structuring and loss of connectivity between neighboring farms in a rural area after the decline had commenced. Local extinction without recolonization may transform contiguous populations into patchy ones, a pattern currently (mainly) observed in urban areas (Shaw *et al.*, 2008; Vangestel, 2011). As house sparrows most likely (used to) disperse according to a stepwise pattern (Kekkonen *et al.*, 2010), loss of intermediate 'stepping-stones' combined with the intrinsic sedentary nature of house sparrows (Anderson, 2006) may have reduced or even inhibited contemporary dispersal between adjacent colonies (Hole, 2001). Hole (2001) was among the first to suggest that such loss of (intermediate) populations may gradually increase distances between remaining source–sink populations up to a point at which house sparrow metapopulation dynamics become constrained and populations genetically diverge, ultimately resulting in an extinction vortex that spreads through the landscape. Less vagile species (such as the house sparrow), in particular, are expected to be highly vulnerable to such landscape alterations (Hole, 2001; Sekercioglu *et al.*, 2002) and to show a tendency towards strong local population structuring (Stangel, 1990). Given the observed large-scale reductions in urban house sparrow numbers (De Laet and Summers-Smith, 2007), we could hence expect that contemporary urban house sparrow populations are particularly susceptible to genetic drift, while exchange of migrants between contemporary populations may become problematic.

Here we quantify genetic variation across microsatellite loci to test whether house sparrows show small-scale genetic differentiation and changes in genetic diversity along an urban–rural gradient, and to relate this to putative demographic changes. Besides its relevance for future conservation strategies, these results may further contribute to our general understanding of population dynamics of species in contemporary anthropogenic landscapes.

MATERIALS AND METHODS

Study site and species

House sparrows were sampled in the greater area of Ghent (northern Belgium) and in an adjacent rural area near the village of Zomergem, ca. 12 km northwest of Ghent. The degree of urbanization, measured as the ratio of built-up to total grid cell area (each cell measuring 90 000 m² on the ground), ranged between 0–0.10 (henceforth referred to as 'rural' area), 0.11–0.30 ('suburban' area) and >0.30 ('urban' area), respectively (Arcgis version 9.2, ESRI, Redlands, CA, USA) (Figure 1). Although we currently lack long-term data on house sparrow densities within our study area, we believe it is reasonable to expect substantial demographic heterogeneity along the urbanization gradient

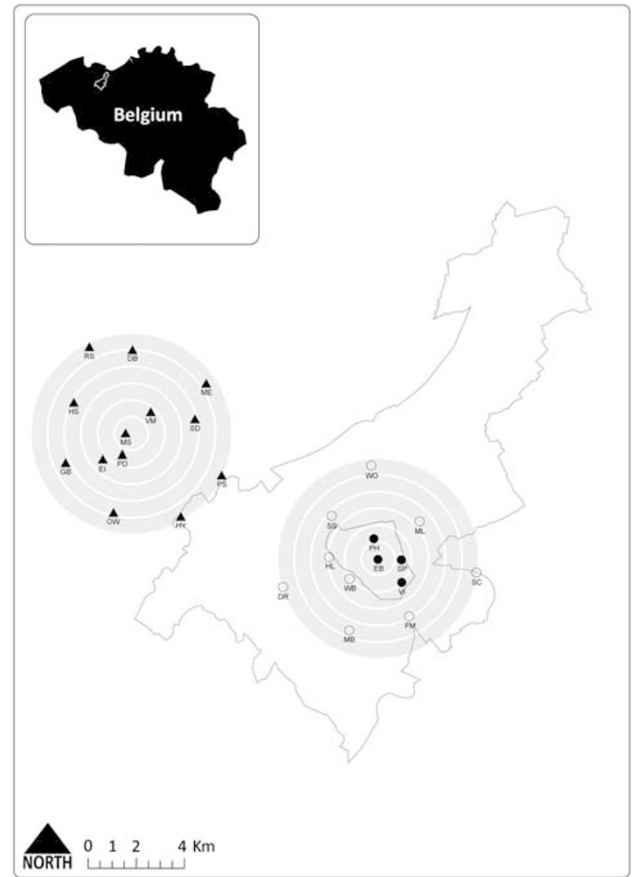


Figure 1 Map showing the location of the 26 urban (filled circles), suburban (open circles) and rural (filled triangles) populations under study. The inner contour encompasses the city center of Ghent, the outer contour encompasses the surrounding municipalities.

for the following reasons. First, earlier studies (Vangestel *et al.*, 2010; Vangestel, 2011) within the same study area suggested that house sparrows experience the urban environment as the most stressful one, based on the slower feather growth rates measured in urban compared with suburban and rural populations. This phenotypic trait was earlier shown to be positively related to a range of fitness components and to comprise a reliable proxy for nutritional condition and energetic stress (Grubb, 2006 and references therein). Secondly, census counts using a mapping-based survey technique revealed equally low urban house sparrow density (0.57 birds ha⁻¹) as in other large cities, such as London and Hamburg, which have well-documented house sparrow declines. In contrast, suburban and rural population sizes were several magnitudes larger (Vangestel, 2011). Between the years 2003 and 2009, a total of 690 adult house sparrows were captured by standard mist netting in 26 study plots in rural, suburban and urban areas (roughly equal sex ratios obtained per plot). As there was no evidence that allele frequencies substantially shifted during the course of the sampling period, samples were pooled across years. Upon capture, standard morphological measurements were taken (details in Vangestel *et al.*, 2010) and a small sample of body feathers was collected for DNA analysis.

Morphological variation

A general linear mixed model was applied to study variation in average body weight between urbanization classes. Sex, month and hour were added as fixed covariates, whereas year and study plot were modeled as random factors to account for a possible clustered data structure. Measures for body condition were obtained by adding tarsus size as a fixed covariate to the previous model (Jakob *et al.*, 1996). All statistical analyses were performed in SAS v9.2 (SAS Institute 2008, Cary, NC, USA).

DNA extraction, PCR and genotyping

We applied a Chelex resin-based method (InstaGene Matrix, Bio-Rad, Hercules, CA, USA) (Walsh *et al.*, 1991) to extract genomic DNA from a total of ten plucked body feathers per individual. In all, 16 microsatellite markers (both traditional 'anonymous' microsatellites as well as those developed based on expressed sequence tags; Table 1) were selected based on their polymorphism and stutter profile. Polymerase chain reactions were organized in four multiplex sets and compatibility between primer pairs was checked using AutoDimer (Vallone and Butler, 2004). The first multiplex reaction contained *Pdoμ1* (Neumann and Wetton, 1996), *Pdo32*, *Pdo47* (Dawson *et al.*, 2012) and *TG04-012* (Dawson *et al.*, 2010); the second one contained *Pdoμ3* (Neumann and Wetton, 1996), *Pdoμ5* (Griffith *et al.*, 1999), *TG13-017* and *TG07-022* (Dawson *et al.*, 2010); the third multiplex reaction contained *Pdo10* (Griffith *et al.*, 2007), *Pdo16*, *Pdo19*, *Pdo22* (Dawson *et al.*, 2012) and *TG01-040* (Dawson *et al.*, 2010); the last set consisted of *Pdo9* (Griffith *et al.*, 2007), *TG01-148* and *TG22-001* (Dawson *et al.*, 2010). PCR reactions were performed on a 2720 Thermal Cycler (Applied Biosystems, Foster City, CA, USA) in 9 μl volumes and contained approximately 3 μl (30 ng) of genomic DNA, 3 μl QIAGEN Multiplex PCR Mastermix (Qiagen, Venlo, The Netherlands) and 3 μl primer mix (concentrations were 0.1 μM (*Pdoμ1*), 0.12 μM (*TG01-148*), 0.16 μM (*Pdo10*, *Pdo19*, *Pdo22*, *Pdo32*, *TG04-012*) and 0.2 μM (*Pdoμ3*, *Pdoμ5*, *Pdo9*, *Pdo16*, *Pdo47*, *TG01-040*, *TG07-022*, *TG13-017*, *TG22-001*). The PCR profile contained an initial denaturation step of 15 min at 95 °C, followed by 35 cycles of 30 s at 94 °C, 90 s at 57 °C and 60 s at 72 °C. Finally, an additional elongation step of 30 min at 60 °C and an indefinite hold at 4 °C was included. Prior to genotyping, the DNA concentration of each sample was quantified using a ND1000 spectrometer (Nanodrop Technologies, Wilmington, DE, USA) and adjusted to a standard concentration of 10 ng μl⁻¹. To rule out contamination of reagents, negative controls were applied during extraction and PCR. PCR products were visualized on an ABI3730 Genetic Analyzer (Applied Biosystems), an internal LIZ-600 size standard was applied to determine allele size, standard samples were included in each run and fragments were scored using the software package GENEMAPPER 4.0 (Applied Biosystems). All loci under study were autosomal as inferred from the chromosomal location of their homologs on the genome of the zebra finch, *Taeniopygia guttata* (Vangestel *et al.* unpublished data) and confirmed autosomal in house sparrow by the successful

amplification of the loci in both sexes and by the presence of heterozygotes in female individuals for each locus.

We used MICRO-CHECKER (Van Oosterhout *et al.*, 2004) to identify scoring errors that could be attributed to stuttering, differential amplification of size-variant alleles causing large allele drop-out or the presence of null alleles. All microsatellite loci were checked for Hardy–Weinberg and linkage equilibrium with GENEPOP 4.0 (Raymond and Rousset, 1995; Rousset, 2008) and a family-wise error rate of 0.05 was obtained by applying a Bonferroni correction (Weir, 1990).

Genetic diversity and population structure

For each locus-by-population combination, genetic diversity was quantified by allelic richness ($A_{[g]}$), observed heterozygosity (H_o) and unbiased expected heterozygosity (H_e) (Nei, 1978). Measures of heterozygosity were computed in FSTAT 2.9.3.2 (Goudet, 1995), whereas allelic richness was calculated with ARES 1.2.2 (Van Loon *et al.*, 2007). As estimates of allelic richness vary with sample size (Kalinowski, 2004) and patterns can be obscured or even reversed when samples of different sizes are compared (Van Loon *et al.*, 2007), a rarefaction procedure was applied (*sensu* Hurlbert, 1971). Estimates were extrapolated to the average number of gene copies per population ($g = 40$), rather than to the smallest observed sample size, which might result in a loss of information or decrease in accuracy (Van Loon *et al.*, 2007). Heterogeneity in genetic diversity between urban, suburban and rural populations was tested by running 1000 permutations. We calculated F_{ST} indices (Weir and Cockerham, 1984) using Genetix 4.05.2 (Belkhir *et al.*, 2004) to assess pairwise genetic differentiation among all populations and overall genetic differentiation within each urbanization class. The significance of pairwise F_{ST} values was evaluated using 1000 permutations.

We conducted an individual-based Bayesian analysis implemented with STRUCTURE 2.2 (Pritchard *et al.*, 2000) to delineate clusters (K) of individuals based on their multilocus genotypes. Without a *priori* knowledge of source populations, this algorithm groups individuals by minimizing deviation from Hardy–Weinberg and linkage disequilibrium (Pritchard *et al.*, 2000). We applied an admixture model with ten independent runs of $K = 1–26$, 200 000 Markov Chain Monte Carlo repetitions, a burn-in period of 100 000, correlated allele frequencies and no prior information on the population of origin. The modal value of the *ad hoc* quantity ΔK , based on the second order rate of change of the likelihood function ($\ln \Pr(X|K)$), was used as criterion to detect the true K (Evanno *et al.*, 2005). This approach overcomes difficulties in estimating the true K in case likelihood values increase with stepwise values of K (Pritchard *et al.*, 2007). When ΔK failed to reveal an unambiguous signal, we selected the model with the highest mean value of $\ln \Pr(X|K)$ as the most parsimonious one (Pritchard *et al.*, 2007).

Next, we applied an alternative clustering algorithm with INSTRUCT (Gao *et al.*, 2007). This extension of STRUCTURE does not assume Hardy–Weinberg equilibrium within clusters and jointly estimates levels of population inbreeding and population structure. Apart from estimating a population inbreeding coefficient, the settings used were identical to those applied in STRUCTURE. Replicate cluster analyses were aligned with CLUMPP 1.1.2 (Jakobsson and Rosenberg, 2007) and visualized with DISTRICT 1.0 (Rosenberg, 2004). Subsequently, we conducted a Principal Coordinate Analysis (PCoA) to summarize pairwise F_{ST} values obtained from an Analysis of Molecular Variance (AMOVA) as implemented in GENALEX 6.4 (Peakall and Smouse, 2006). This multivariate dimension-reducing method is exploratory in nature and graphical displays of the most important principal axes were used to visualize genetic similarity among populations within urbanization classes (Jombart *et al.*, 2009). Finally, we conducted an AMOVA in ARLEQUIN 3.5.1.2 (Excoffier *et al.*, 2005) to examine how genetic variation was partitioned over three hierarchical scales: within populations, between populations within urbanization classes and among urbanization classes. As the statistical power of a nested AMOVA depends on the number of populations that can be permuted among higher hierarchical levels (rather than on the number of individuals or loci), we first applied the multinomial theorem to our sampling scheme to ascertain that the minimum P -value was adequately small (Fitzpatrick, 2009).

A hierarchical analysis of genetic variance across spatial scales provides a first estimate of a deviation from migration-drift equilibrium, and of the extent of

Table 1 Locus-specific summary statistics for all 16 microsatellite markers used

Locus	EMBL	N	N_A	H_o	H_e	F_{ST}	Estimated f_{null}
	accession						
<i>TG01-040</i> ^a	DV576233	537	6	0.40	0.44	0.016	0.028 (0.01)
<i>TG01-148</i> ^a	CK301512	486	3	0.42	0.38	0.020	-0.023 (0.02)
<i>TG04-012</i> ^a	CK306810	549	5	0.53	0.59	-0.001	0.039 (0.016)
<i>TG07-022</i> ^a	DV948210	493	5	0.37	0.41	0.023	0.026 (0.012)
<i>TG13-017</i> ^a	CK313422	547	8	0.52	0.64	0.007	0.074 (0.015)
<i>TG22-001</i> ^a	CK317333	478	11	0.34	0.41	0.019	0.054 (0.013)
<i>Pdoμ1</i>	AM287188	550	20	0.80	0.85	0.008	0.024 (0.009)
<i>Pdoμ3</i>	AM287190	515	19	0.83	0.85	0.015	0.015 (0.007)
<i>Pdoμ5</i>	Y15126	523	22	0.76	0.82	0.040	0.033 (0.011)
<i>Pdo9</i>	AF354423	442	31	0.65	0.75	0.059	0.052 (0.013)
<i>Pdo10</i>	AF354424	596	18	0.78	0.82	0.014	0.021 (0.011)
<i>Pdo16</i>	AM158995	549	17	0.81	0.84	0.008	0.016 (0.01)
<i>Pdo19</i>	AM158998	573	9	0.60	0.62	0.025	0.008 (0.011)
<i>Pdo22</i>	AM159001	578	16	0.73	0.72	0.013	-0.005 (0.011)
<i>Pdo32</i>	AM159011	491	20	0.59	0.75	0.010	0.093 (0.015)
<i>Pdo47</i>	AM159027	562	17	0.68	0.83	0.021	0.078 (0.013)

Abbreviations: f_{null} , estimated null allele frequency; H_e , expected heterozygosity; H_o , observed heterozygosity; N , number of individuals genotyped; N_A , number of distinct alleles per locus; F_{ST} , genetic differentiation.

^aEST-based microsatellites.

dispersal limitation. Here, three spatial clusters are present: rural, suburban and urban samples. If a metapopulation is at or near migration-drift equilibrium, genetic differentiation at a lower hierarchical level (within clusters) cannot exceed the genetic differentiation at a higher hierarchical level (among clusters). If on the other hand, genetic differentiation among populations within a spatial cluster is larger than among clusters, this provides proof that the genetic structure is more determined by genetic drift than by dispersal. This follows from the principle that drift is stronger in small populations (local scale) than in large populations (cluster scale). Hence, such patterns represent a deviation from migration-drift equilibrium, reflecting a future loss of genetic diversity (genetic extinction debt).

To investigate in more detail the extent to which the genetic structure (genetic distance) and genetic diversity was affected by spatial structure (dispersal limitation) on the one hand, and the degree of urbanization on the other hand, we performed a redundancy analysis (RDA) in Canoco 4.5 (ter Braak and Šmilauer, 2002). As dependent variables, we used genetic structure (PCoA loadings of the first six PCoA axes for each population), and in a separate analysis measures of genetic variation (H_e per locus). As explanatory variables, we used two sets of variables. The first is the spatial variable set (S), consisting of the geographic locations of the populations in a two-dimensional grid (x, y) and transformations thereof (Borcard *et al.*, 1992): $xy, x^2, y^2, x^2y, xy^2, x^3, y^3, \ln(x)$ and $\ln(y)$. The second set represents the urbanization degree (U) in a radius of 50, 250 and 1250 m around the population centers (hence labeled U50, U250 and U1250, respectively). Using these two sets of explanatory variables, we performed variance partitioning (Borcard *et al.*, 1992) to decompose the total explained variance (in genetic structure and genetic diversity, respectively) into the unique contribution of spatial variables (S|U), of urbanization variables (U|S) and the intersection of both ($S \cap U$). We used an automatic forward selection procedure implemented in Canoco to maximally improve the fit of each model. In addition, we estimated the unique contribution of each variable to the model and the significance thereof using 999 monte carlo permutations. Finally, we determined the unique contribution of spatial variables and of urbanization variables in reduced models, using only variables that contributed significantly to the earlier full models. This approach is much more powerful than (partial) Mantel tests as these use only a fraction of the available information (all variation is compressed into a single variable), whereas multivariate ordination approaches such as RDA use more information and are much more sensitive to subtle patterns (Fortin and Dale, 2005).

Population bottlenecks and dispersal

To detect genetic signatures of recent population bottlenecks, we visually inspected allele frequency distributions for mode shifts from low to intermediate allele frequency with BOTTLENECK 1.2.02 (Cornuet and Luikart, 1996; Piry *et al.*, 1999). Strong reductions in effective population size result in contrasting rates at which allelic diversity and heterozygosity at Hardy–Weinberg equilibrium (H_e) are lost (Cornuet and Luikart, 1996), with particularly strong losses of rare alleles. Whereas the latter has a small impact on H_e only (Hedrick *et al.*, 1986), it strongly affects the expected heterozygosity at mutation–drift equilibrium (H_{eq}) as these estimates depend on the absolute number of alleles (Ewens, 1972), and the transient disruption of mutation–drift equilibrium in bottlenecked populations generates an excess in heterozygosity ($H_e > H_{eq}$) (Luikart and Cornuet, 1998). As H_{eq} distributions obtained through coalescence-based simulations strongly hinge on the (unknown) model of microsatellite evolution (Hawley *et al.*, 2006), the occurrence of a bottleneck was assessed by visual inspection (mode shift) rather than formal testing.

To quantify recent gene flow between urban, suburban and rural areas, we pooled all populations within a particular urbanization class into a single population cluster and identified the cluster of origin for each individual by computing posterior probability densities of unknown population allele frequencies and subsequent individual multilocus genotype probabilities (partial assignment technique *sensu* Rannala and Mountain, 1997). Next, we generated a likelihood distribution of simulated genotypes and excluded a particular population cluster as possible origin if the likelihood of a genotype fell in the tail-end of this distribution (Cornuet *et al.*, 1999). By excluding two

of three clusters the putative source of an individual could be identified, and this individual was considered a ‘disperser’ if the population in which it was captured differed from the presumed cluster of origin (Cornuet *et al.*, 1999). Although the statistical power of assignment tests ultimately depends on the level of population differentiation (Paetkau *et al.*, 2004), these tests are believed to be robust against deviations from migration–drift equilibrium as assignment is performed at an ecological (not evolutionary) timescale (Peery *et al.*, 2008). Analyses were performed in GENECLASS 2.0 (Piry *et al.*, 2004) and focal individuals were removed from the sample when allele frequencies were estimated (‘leave-one-out’ procedure).

RESULTS

Neither weight nor body condition varied significantly between urbanization classes (respectively $F_{2,17.1} = 0.65$; $P = 0.54$ and $F_{2,17.4} = 0.66$; $P = 0.53$). All locus-by-population combination were highly polymorphic, with numbers of alleles per locus ranging between 3 (*TG01-148*) and 31 (*Pdo9*). Apart from *Pdo47* (three populations), *Pdoμ5* (two populations) and *Pdo9, Pdo32, TG01-148, TG07-022* and *TG13-017* (one population each), all locus-by-population combinations were in Hardy–Weinberg equilibrium after Bonferroni correction. There was no evidence that disequilibria in the loci mentioned above reflected scoring errors due to large allele drop-out or stutter, and removing them did not alter any of the observed patterns, hence results based on the analysis of all loci are presented. There was also no evidence for linkage disequilibrium between any pair of loci (Table 1).

Genetic diversity and population structure

Mean numbers of alleles per locus ranged between 4.8 and 7.9, allelic richness ranged between 5.86 and 9.62 and expected heterozygosity ranged between 0.60 and 0.71. Based on permutation tests, none of these indices differed significantly between urbanization classes (all $P > 0.05$). Levels of pairwise population differentiation ranged between -0.028 and 0.120 (mean $F_{ST} \pm$ s.e.m.: 0.018 ± 0.001) (Table 2). When pooling all populations per urbanization class, pairwise values of the differentiation index F_{ST} equaled 0.0019 (urban-rural), 0.0013 (suburban-rural) and 0.0007 (urban-suburban), whereas overall levels of differentiation within each cluster equaled 0.0138 (urban), 0.0119 (suburban) and 0.0267 (rural), respectively. Thus, genetic differentiation within each cluster is an order of magnitude higher than among clusters, which is evidence for strong deviation from migration–drift equilibrium.

Details of all RDA analyses are presented in the Supplementary Material (Supplementary Appendix S1). Redundancy analyses on genetic diversity with both spatial and urbanization variables explained 36.2% of the variance in genetic diversity found in populations (expected heterozygosity per locus), although no single variable contributed significantly to the model ($P > 0.07$). The variance partitioning showed that spatial variables alone explained 6.7%, though no single variable had a significant contribution. Urbanization uniquely explained 11.5% of the variance, but again no variable contributed significantly.

When considering genetic structure based on the PCoA loadings of each population, spatial and urbanization variables jointly explained 34.6% of the variance in genetic structure. Two urbanization and one spatial variable contributed significantly, yielding a reduced model that explained 31.4% of the total variance ($P = 0.001$). Given that urbanization variables were included in the model, spatial variables uniquely explained 34.0% of the total variance, with a single significant variable (y^2) explaining 20% of the variance in genetic structure ($P = 0.001$). The unique contribution of urbanization (U|S)

was 18.4%, of which U250 was retained in a reduced model ($R^2 = 11.4\%$, $P = 0.02$).

When the RDA on the genetic structure was limited to rural populations or (sub)urban populations, a (marginally) significant spatial pattern emerged after forward selection of the variable y^3 (rural $R^2 = 0.19$, $P = 0.036$) or the variable y ((sub)urban $R^2 = 0.17$, $P = 0.046$). When this was repeated for the degree of urbanization per cluster while correcting for spatial covariation, patterns persisted in (sub)urban areas (variable U250, $R^2 = 0.23$, $P = 0.03$), but not in the rural one.

We failed to identify distinct population clusters along the urbanization gradient, which is consistent with the low level of genetic differentiation among populations. Whereas clustering in STRUCTURE did not show a clear mode for ΔK , posterior probabilities gradually declined from $K > 7$ onwards, with $K = 1$ selected as the most parsimonious model (Figures 2a–d). When taking variation in inbreeding into account, posterior probabilities incremented with increasing values of K and reached a plateau near $K = 13$. To overcome this problem, we used ΔK to delineate the most appropriate model. This, however, did not reveal a clear mode either, although lower numbers of clusters were associated with the largest ΔK values ($K = 1$ and $K = 2$, respectively) (Figures 2e–h).

Lack of support for strong genetic population structuring was confirmed by the fact that very few individuals showed a unique affinity to a specific urbanization class. Overall, PCoA analysis revealed weak clustering of individuals from adjacent populations but did not support strong clustering of the different urbanization classes. Both main PCoA axes together explained 83.74% of the total genetic variation (Figure 3). Based on a hierarchical AMOVA model, 0% genetic variation was assigned to differences between urbanization classes, 2.2% variation to between population variation within urbanization classes and 97.8% variation to variation within populations. As the minimum P -value for three groups of 13, 9 and 4 populations was much smaller than 0.001, lack of support for genetic population structure at higher hierarchical levels was not due to low statistical power.

Population bottlenecks and gene flow

Of the 26 populations assessed, only the most central urban population (EB-U) showed genetic evidence of a recent population bottleneck. Here, allelic richness was low whereas heterozygosity was retained at a level equivalent to that of other populations, and a clear mode was present at intermediate (rather than low) frequency alleles. In all other populations, allele frequency distributions were typically L-shaped (see Supplementary Appendix S2).

Because of the weak level of genetic population structuring, statistical power to quantify gene flow among urbanization classes was low as well. Only 10.1% of all individuals (rural 31 individuals; suburban 32 individuals; urban 7 individuals) were unambiguously assigned to a single source cluster. Among the rural individuals, 61% were assigned as resident, whereas 39% were assigned as immigrant from the suburban population cluster. Among the suburban individuals, 78% were assigned as resident and 22% were assigned as immigrant from the rural cluster. Finally, among the urban individuals, all individuals were assigned as immigrant, 43% of which originated from the suburban cluster and 57% from the rural cluster. Most of the other individuals showed a putative mixed ancestry (73.9%), whereas a smaller fraction (15.9%) was assigned as immigrant from an unknown population source (Table 3).

DISCUSSION

Despite the rapid and steep decline of house sparrow populations in many metropolitan areas across Europe (De Laet and Summers-Smith, 2007 and references therein), genetic population studies remain remarkably scant. Spatial analysis of putatively neutral microsatellite genotypes in 26 house sparrow populations along an urbanization gradient in Flanders revealed evidence for high historical connectivity or recent common ancestry. Redundancy analyses showed that local genetic diversity *per se* was not significantly affected by dispersal limitation (spatial variables) or by the degree of urbanization, but the genetic structure, that is, how the regional genetic diversity was distributed across the landscape, was significantly affected by both spatial variation (dispersal limitation) and the degree of urbanization. The most central urban population showed a genetic signature of a recent bottleneck, however, no such signal was apparent in more peripheral urban populations. Small census sizes in the urban periphery (Vangestel, 2011) and the suggested unidirectional gene flow from suburban and rural populations into urban peripheral ones, further support the hypothesis that urban populations act as demographic sinks in which genetic drift is (at least partly) countered by gene flow.

The spatially hierarchical analysis of genetic differentiation, with much higher differentiation among nearby populations than among more distant clusters, showed evidence for strong deviations from migration-drift equilibrium. This indicates that genetic drift is currently a much more important process than gene flow. Consequently, we can expect a future reduction in genetic diversity, especially among clusters. Contrary to our expectation, we found no evidence of stronger isolation and decrease in numbers in urban populations compared with suburban or rural ones. This may either indicate that urban populations remained stable in the recent past—possibly at a lower carrying capacity—or that we failed to pick up the genetic signal associated with a demographic population bottleneck. However, the bottleneck test assumes a closed population, whereas this is likely not the case, compromising our potential to detect demographic bottleneck events (Keller *et al.*, 2001). Unfortunately, lack of historical data series prevents us from testing this hypothesis directly. Dispersal rates estimated from individual-based assignment tests indicated moderate site tenacity in suburban and rural populations, supporting the presumed sedentary status of house sparrows (Anderson, 2006). A complete lack of immigrants from urban populations in either of these populations, however, was unexpected, especially in view of the fact that the urban population cluster consisted exclusively of immigrants from suburban and rural populations. Such unidirectional gene flow provides additional evidence for an urban ‘dispersal sink’ (*sensu* Dias, 1996) that may have masked bottleneck signatures in all urban populations apart from the most central one. Whereas weak genetic structuring across the urbanization gradient did not allow us to formally test whether rates of immigration decreased relative to drift, towards more central urban populations, such a scenario may be especially apparent in large metropolitan cities where distances between core populations and potential demographic sources can be several orders of magnitude larger.

Notwithstanding the weak genetic differentiation, the RDA provided evidence of spatial genetic structure (and hence, dispersal limitation), even within the rural and within the (sub)urban cluster. Furthermore, the hierarchical spatial analysis clearly provided evidence that genetic drift is currently a more important driver of genetic structure than gene flow. This may reflect a reduction in gene flow compared with historical levels, a reduction in average local

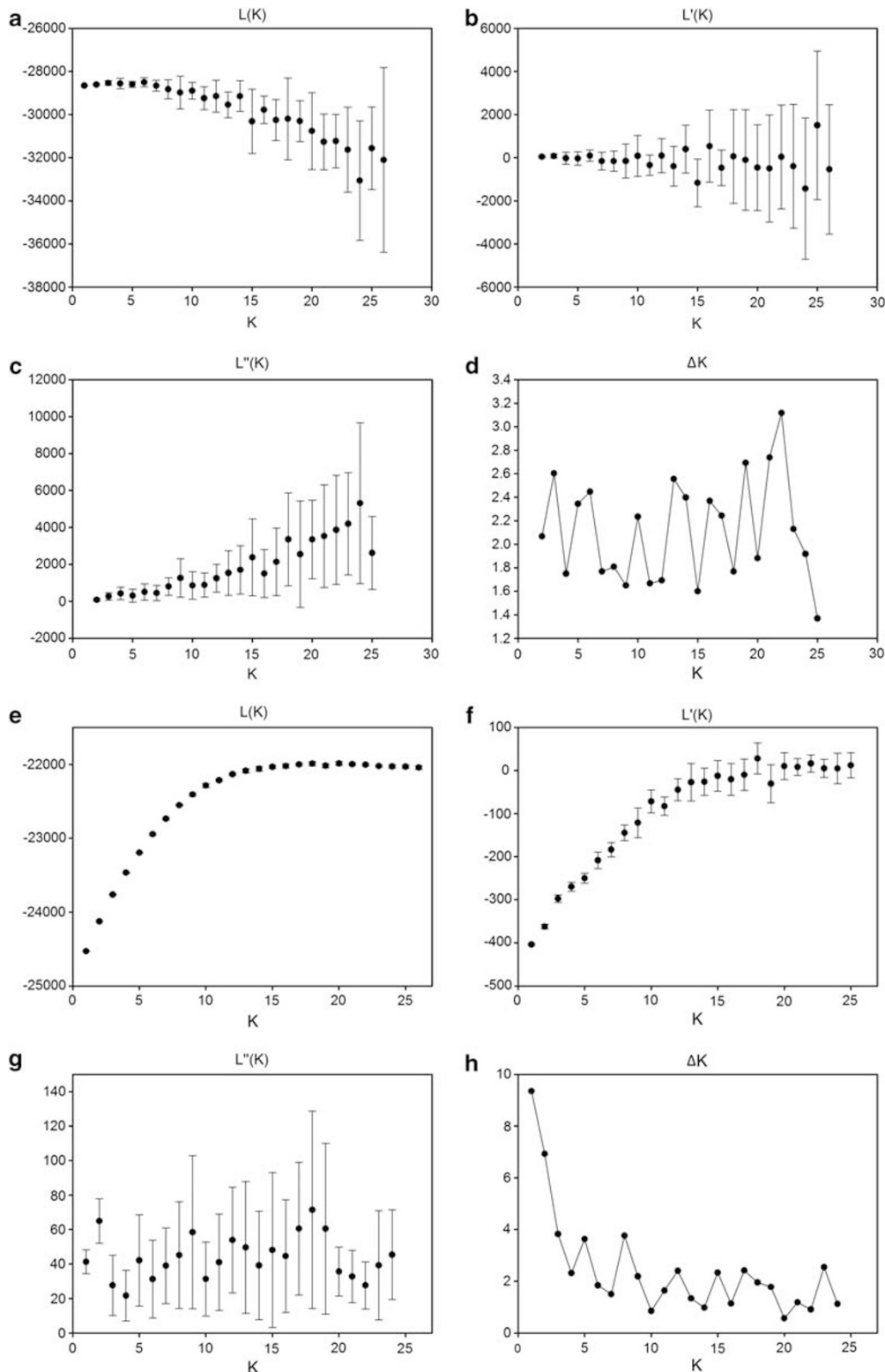


Figure 2 Graphical display of the four steps to detect the true number of clusters (following Evanno *et al.*, 2005). (a) and (e): $L(K)$, mean posterior likelihood (\pm s.d.) over 10 runs for each K value. (b) and (f): $L'(K)$, first order rate of change in the posterior likelihood (\pm s.d.). (c) and (g): $L''(K)$, absolute value of the second order rate of change in the posterior likelihood (\pm s.d.). (d) and (h): ΔK , mean absolute values of $L'(K)$ over s.d. of $L(K)$. (a–d): STRUCTURE analysis (Pritchard *et al.*, 2000), (e–h): INSTRUCT analysis (Gao *et al.*, 2007).

population sizes (and increase in genetic drift), or both. Whereas a small number of additional immigrants may be sufficient to replenish genetic variation and reduce inbreeding depression ('genetic rescue'

sensu Ingvarsson, 2001), larger numbers of conspecifics are generally needed to positively affect population growth or vital rates and promote population stability (Taylor and Dizon, 1996) ('demographic

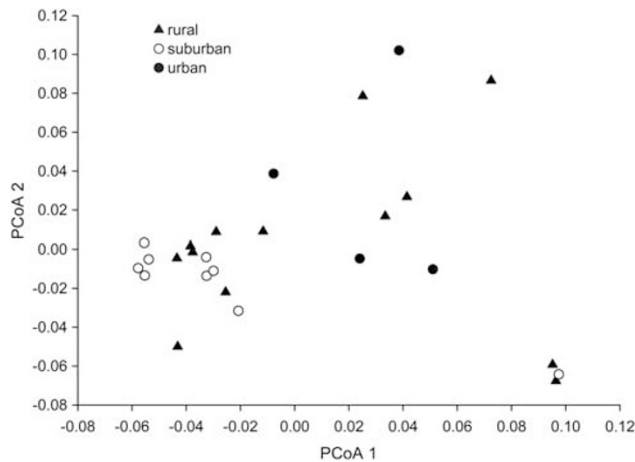


Figure 3 Scatter plot of the first two PCoA axes of genetic variation (based on pairwise F_{ST} values) between 26 house sparrow populations. Filled circles (urban populations), open circles (suburban populations), filled triangles (rural populations).

Table 3 Number of individuals assigned to a single population cluster (' N_{unique} ') and their most likely cluster of origin ('assigned site') are given for each sampling site

Sampling site	N	Assigned site			N_{uniquely}
		Rural	Suburban	Urban	
Rural	345	19 (0.61)	12 (0.39)	0	31 (0.09)
Suburban	256	7 (0.22)	25 (0.78)	0	32 (0.13)
Urban	89	4 (0.43)	3 (0.57)	0	7 (0.08)

Corresponding percentages are given between brackets. An individual was excluded from a population cluster if its genotype likelihood was less than 0.01 when compared with a likelihood distribution of 10000 simulated genotypes. See text for details.

rescue' *sensu* Brown and Kodric-Brown, 1977). Hence, demographic independence can be based on genetic independence, but not the other way around (Taylor and Dizon, 1999). Along these lines, a study of reproduction in British house sparrows has shown that, in 2 of 3 years, local recruitment in urban populations was insufficient to reach the predicted threshold required for population stability (Peach *et al.*, 2008). As this demographic deficit was not counterbalanced by ample immigration from suburban or rural populations, this resulted in a population decline of 28% over the 3-year study period (Vincent, 2005). Unfortunately, estimates on genetic differentiation were not available to simultaneously measure genetic rescue effects.

Several other studies have quantified the genetic population structure of birds across gradients of urbanization. A study on common kestrels (*Falco tinnunculus*) did not reveal differences in levels of allelic richness or genetic differentiation between urban and rural areas (Riegert *et al.*, 2010; Rutkowski *et al.*, 2010). Likewise, pairwise levels of differentiation indicated weak structuring between urban and coastal populations of song sparrows (*Melospiza melodia*) (MacDougall-Shackleton *et al.*, 2011). Yet, in both species and in house sparrows near the city of Ghent, levels of relatedness were consistently higher in urban populations (Riegert *et al.*, 2010; MacDougall-Shackleton *et al.*, 2011; Vangestel, 2011). Although such results could indicate subtle differences in connectivity between both areas, highly synchronized postnatal dispersal directions of siblings through parental control (Matthysen *et al.*, 2010) or area-dependent levels of complete nest predation (MacDougall-Shackleton *et al.*,

2011) might increase genetic similarity between individuals as well. In contrast, urban great tit (*Parus major*) populations showed higher genetic variation compared with forest populations, possibly indicating higher gene flow from urban parks to forests than *vice versa*. While levels of relatedness between urban great tits were also higher than expected based on random mating, comparative data of nearby forest populations are currently lacking (Björklund *et al.*, 2010). Genetic variation across gradients of urbanization may reflect adaptive responses to heterogeneous environments evolved during the urbanization process, rather than differences in demographic features (Partecke and Gwinner, 2007; Badyaev *et al.*, 2008; Evans *et al.*, 2009). One of the most striking and best documented examples of such urban adaptation has been described in blackbirds (*Turdus merula*). Although originally a forest specialist, this species started to colonize urban environments during the early nineteenth century and showed a clear genetic basis for at least a number of behavioral and phenotypic trait shifts in urban populations (Partecke *et al.*, 2006a, b, Partecke and Gwinner, 2007). In free-ranging populations of house sparrows, Liker *et al.* (2008) showed that individuals from more urbanized areas were consistently smaller and in worse body condition than individuals from more rural ones. This difference in body mass remained significant when birds were housed in a common captive environment. Rather than reflecting reduced access to food for adults (for example, due to strong competition), or short-term responses to high food predictability (for example, by strategic mass regulation), Liker *et al.* (2008) considered this phenotypic variation to be under genetic control and possibly adaptive in urban environments. Genetic population connectivity, or lack thereof, has a central role in evolutionary ecological processes. Although evolutionary theory predicts high levels of gene flow to mitigate local adaptive responses (Hendry *et al.*, 2001), they do not necessarily eliminate the adaptive potential of a population (Storz, 2005). For instance, non-random dispersal with respect to phenotypes has been held responsible for the evolution of differences in body mass in different avian populations (Garant *et al.*, 2005; Storz, 2005; Postma and Van Noordwijk, 2005). Adaptive differences in body weight in house sparrows might persist under genetic admixture if smaller or lighter birds would either be forced or attracted to highly built-up areas and larger or heavier birds would tend to settle in more open areas. Although we cannot fully ascertain that the observed genetic structure in our study was caused by differences in demography across the urbanization gradient, we consider it unlikely to be driven by urban adaptations, as we have not yet identified phenotypic or behavioral patterns matching the urban-rural dichotomy. Indeed, as opposed to the study by Liker *et al.* (2008), urban and rural house sparrows did not differ in mean body size and condition in our study area. Urban individuals did show evidence of higher nutritional stress than individuals from less built-up areas, however, this variation was earlier shown to reflect constraints on home range behavior (Vangestel *et al.*, 2010).

Earlier genetic studies on house sparrow populations were either conducted pre-decline or in non-urban environments. Hence, to our knowledge, this is the first study that provides a detailed description of the population genetic structure of contemporary house sparrow populations along an urbanization gradient after the onset of the decline. As pointed out in previous studies (Kekkonen *et al.*, 2010), these results grant us the opportunity to monitor and evaluate future genetic changes in urban house sparrow populations and further improve conservation efforts by assembling and integrating our current knowledge on demography, behavior and population genetics in this species.

DATA ARCHIVING

Data have been deposited at Dryad: doi:10.5061/dryad.kt145km5.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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