Independent colonization of multiple urban centres by a formerly forest specialist bird species

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Urban areas are expanding rapidly, but a few native species have successfully colonized them. The processes underlying such colonization events are poorly understood. Using the blackbird *Turdus merula*, a former forest specialist that is now one of the most common urban birds in its range, we provide the first assessment of two contrasting urban colonization models. First, that urbanization occurred independently. Second, that following initial urbanization, urban-adapted individuals colonized other urban areas in a leapfrog manner. Previous analyses of spatial patterns in the timing of blackbird urbanization, and experimental introductions of urban and rural blackbirds to uncolonized cities, suggest that the leapfrog model is likely to apply. We found that, across the western Palaearctic, urban blackbird populations contain less genetic diversity than rural ones, urban populations are more strongly differentiated from each other than from rural populations and assignment tests support a rural source population for most urban individuals. In combination, these results provide much stronger support for the independent urbanization model than the leapfrog one. If the former model predominates, colonization of multiple urban centres will be particularly difficult when urbanization requires genetic adaptations, having implications for urban species diversity.

Keywords: colonization; dispersal; genetic divergence; genetic diversity; range expansion; urban

1. INTRODUCTION

The rapid growth in urban development and increasing concentration of people in these areas (Cohen 2003; UN 2008) is associated with marked ecological changes, including alterations to climatic regimes, phenology, resource availability and habitat quality (Zhou *et al.* 2004; Grimm *et al.* 2008). Consequently, growth in urban development is a major cause of extinction (Czech & Krausman 1997), with the native species richness of most taxa being markedly reduced in highly urbanized areas (Tratalos *et al.* 2007; Grimm *et al.* 2008). Despite this, a small number of species thrive in urban areas where they occur at higher densities than those in more natural habitats (Blair 1996). The number of these urban-adapted species is gradually increasing as additional species invade urban areas and adapt to them, but the mechanisms underlying these colonization events remain poorly understood (Heyder 1955; Diamond 1986; Luniak *et al.* 1990; Rasner *et al.* 2004; Rutz 2008).

A lack of information regarding the timing of urban colonization events, especially with regard to multiple sites

across a large geographical region, often hinders investigations of the mechanisms driving colonization of urban areas. A notable exception is the European blackbird *Turdus merula*, which was previously confined to forest, but is now one of the most common urban birds across much of the western Palaearctic (Luniak *et al.* 1990; Evans *et al.* 2009a). The availability of numerous dated records of urban colonization events across the blackbird's geographical range reveals that urban colonization was first observed in Germany, in the 1820s, but has occurred much more recently to the north and east (Heyder 1955; Luniak *et al.* 1990). This strong spatial pattern has been interpreted as evidence that, following an initial urban colonization event, additional urban populations were established in a leapfrog manner (Luniak *et al.* 1990). This could have occurred because individuals were either adapted to the novel habitat or imprinted on it (Mabry & Stamps 2008). This leapfrog model of urban colonization is particularly plausible in migratory or partially migratory species, such as the blackbird, in which occasional long-distance dispersal events of over 300 km have been recorded, even though natal and adult dispersal distances are typically much shorter than this, at approximately 3 km (Paradis *et al.* 1998). While plasticity may sometimes be involved in the colonization of urban areas (Slabbekoorn & Ripmeester 2008), urban blackbirds exhibit some adaptations to city life, which have a partly genetic basis, including changes in stress responses, migratory tendencies and reproductive cycles

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(Partecke *et al.* 2004, 2006; Partecke & Gwinner 2007). Therefore, urban blackbirds may be more likely to possess the necessary traits for colonization of unoccupied urban areas than rural blackbirds. Finally, the leapfrog model receives support from experimental introductions of urban and rural blackbirds to cities in eastern Europe that lacked blackbirds, in which only the introductions of urban birds succeeded (Graczyk 1974, 1982). The simplest alternative to the leapfrog model of urban colonization is that urban populations are established through independent colonization events.

Given the relatively recent history of urban colonization by blackbirds, assessment of this species' population genetic structure in urban and rural areas can distinguish whether colonization arose primarily from leapfrog or independent colonization events. Any colonization event is likely to result in the loss of genetic diversity due to founder effects, but this will be particularly marked following cumulative founder events in which individuals from a recently established population subsequently disperse and establish additional populations (Pruett & Winker 2005). The loss of genetic diversity in urban populations relative to rural ones will thus be greater following leapfrog colonization compared with independent colonization. In isolation, this is a rather weak prediction as, without making a number of unsupported assumptions concerning propagule size and the relatedness of colonizers, quantitative assertions of the loss of genetic diversity anticipated under each model cannot be made. However, the leapfrog colonization model also predicts that loss of genetic diversity should increase with distance from the initial urban colonization event, and should be most marked in recently established populations. Such relationships are unlikely to arise if colonization occurred independently. Moreover, three additional and more robust predictions can be made. First, the leapfrog model predicts that urban populations should form a separate genetic cluster from rural ones, but this will not be the case following independent urban colonizations. Second, the leapfrog model predicts that genetic differentiation between urban and rural populations should be greater than that between separate urban populations. By contrast, the independent urban colonization model predicts the opposite pattern. Finally, under a leapfrog colonization model, assignment tests should associate urban individuals with an urban source population, while source populations will be rural under the independent urban colonization model. We assess these predictions using microsatellite data at 22 loci, from 749 breeding adult blackbirds sampled from 25 urban and rural populations located across the western Palearctic.

2. MATERIAL AND METHODS

(a) Sampling

Approximately 30 adult breeding blackbirds were captured, using mist nets, at each of 12 paired urban and rural sites, and an additional rural site in southern France (table 1). This additional rural site was intended to be paired with an urban site, but a fully urbanized population was not present. Rural sampling was, however, completed to ensure that we sampled a population that was just north of the Pyrenees, a potential barrier to gene flow. Sampling was conducted during the main breeding season at each site, with sampling dates

varying from the end of March in Tunisia to late June and early July in Latvia. Blood samples from Munich populations were supplied by Jesko Partecke from breeding adults taken between 1998 and 2000. In all other cases, each urban and rural site within a pair were sampled within 10 days of each other during 2006 or 2007. Blood samples were taken from the brachial vein and stored in absolute ethanol. Paired sites were located between 23 and 45 km apart. This is an order of magnitude greater than the mean adult and natal dispersal distances of this species, which, respectively, are 3.2 and 3.3 km, although dispersal can occur over much greater distances in this species (Paradis *et al.* 1998). All urban sites were located in the core built-up area of each city, defined as localities where the predominant land cover type was impervious surfaces (concrete, tarmac, etc.), and excluded regions on the edge of the city or close to areas of green space that were directly connected to rural areas. Rural sites were undeveloped or contained a few isolated houses.

(b) Genotyping and locus selection

Individuals were genotyped at 24 microsatellite loci that had previously been characterized in the blackbird by Simeoni *et al.* (in press; table S1 in electronic supplementary material 1). Genomic DNA was extracted using an ammonium acetate precipitation method (Nicholls *et al.* 2000) prior to PCR amplification. The 24 loci were amplified in three separate PCR multiplex procedures. Each 4 µl multiplex PCR (Kenta *et al.* 2008) contained approximately 2 ng of DNA, 0.05–2.4 µM of each primer and 2 µl of 2× QIAGEN Multiplex PCR Master Mix. The PCR programme used was 95°C for 15 min followed by 30 cycles of 94°C for 30 s, annealing temperature (49, 55 or 62°C, depending on locus) for 90 s, 72°C for 60 s and finally 60°C for 30 min. Repeated genotyping of 267 individuals using the same extractions revealed a low genotyping error rate (0.4%), and a similarly low error rate (0.2%) was obtained when genotyping was repeated using new DNA extractions for 20 individuals.

Exact tests based on a Markov chain method (Raymond & Rousset 1995) implemented in GENETOP 3.4 indicated that no pair of loci exhibited a consistent signal of linkage disequilibrium. Exact tests for deviations from Hardy–Weinberg (HW) genotypic proportions (Guo & Thompson 1992) and calculation of null allele frequencies for each population in CERVUS v. 3.0 indicated that, following sequential Bonferroni corrections for each population individually, two loci exhibited frequent and significant deviations from HW and high null allele frequencies (table S1 in electronic supplementary material 1). These loci were excluded from subsequent analyses that are thus based on the data from the remaining 22 loci.

(c) Analysis of genetic diversity

We calculated the total number of alleles (A) in each population and mean observed (H_o) and expected (H_e) heterozygosities per locus using the methods of Nei (1978) and implemented in GENETIX 4.05.2 (Belkhir *et al.* 2004). Each population's mean allelic richness per locus was calculated with FSTAT v. 2.9.3 (Goudet 1995) using rarefaction to standardize measures for a population size of 16 individuals (i.e. one less than the size of the smallest sample). These measures of genetic diversity were not consistently normally distributed (Kolmogorov–Smirnov tests, $p < 0.05$), so they were analysed using non-parametric tests in MINITAB (which was used for all statistical tests unless

Table 1. Locations of focal populations and number of genotyped individuals.

	urban			rural		
	latitude	longitude	<i>n</i>	latitude	longitude	<i>n</i>
Berlin	39.48° N	13.42° E	31	52.85° N	13.80° E	30
Groningen	53.22° N	6.56° E	31	53.48° N	6.18° E	31
Krakow	50.06° N	19.94° E	45	50.03° N	19.94° E	27
Madrid	40.41° N	3.69° W	31	40.75° N	3.81° W	33
Montpellier	n.a.			43.66° N	3.66° E	30
Munich	48.12° N	11.57° E	30	47.88° N	11.57° E	30
Prague	50.07° N	14.43° E	30	50.22° N	14.81° E	30
Riga	56.95° N	24.06° W	17	56.99° N	23.32° E	24
Sheffield	53.37° N	1.46° W	31	53.04° N	1.54° E	31
Szczecin	53.42° N	14.54° E	32	53.67° N	14.41° E	31
Tallinn	59.44° N	24.75° E	26	59.06° N	24.71° E	18
Tunis	36.81° N	10.17° E	35	36.53° N	9.99° E	31
Valencia	39.48° N	0.39° W	31	39.69° N	0.25° W	33

otherwise stated). When assessing differences in diversity between urban and rural populations, a paired test (Wilcoxon signed-rank test) was initially used. However, as assignment and exclusion tests revealed that a small number of urban individuals were genetically more similar to distant rural populations, analyses were repeated using the non-paired Mann–Whitney test. This also enabled data from the rural Montpellier population, which lacked a paired urban site, to be used in the analyses. Both sets of tests gave qualitatively identical results and thus we report only the results of the paired tests. Blackbird urbanization was first recorded in the 1820s in Bamberg and Erlangen, two towns located 35 km from each other in central Germany (Heyder 1955; Luniak *et al.* 1990; K. L. Evans, K. J. Gaston & B. J. Hatchwell 2008, unpublished data). We calculated the distance from each focal urban population to the mid-point between these two localities for use in Spearman rank correlations between urban genetic diversity metrics and distance from the initial urban population.

(d) Analysis of genetic clusters

This was conducted in two ways. First, PHYLIP 3.5 (Felsenstein 1993) was used with TREEVIEW 1.6 to construct an unrooted tree from the D_S distance matrix (see below). Second, STRUCTURE v. 2.2 (Pritchard *et al.* 2000) was used to assess the number of subpopulations or clusters (K). Ten independent runs of $K=1$ –10 with 200 000 Monte Carlo Markov chain iterations and a burn-in period of 100 000 iterations were performed, using the model with correlated allele frequencies and assuming admixture. The Dirichlet parameter for the degree of admixture was allowed to vary between runs. For each value of K , the log-likelihood values were averaged and standard deviation was calculated. These averages were then used to calculate the posterior probability for each value of K . The log-likelihood values declined at values of K approaching 10, thus it was considered unnecessary to run STRUCTURE with $K>10$. The software CLUMPP (Jakobsson & Rosenberg 2007) was used to align cluster membership coefficients from the 10 replicate cluster analyses from each K -value chosen using the LargeKGreedy algorithm with 10^5 random input orders. The fractions of ancestry were averaged over individuals within each of the 25 population samples.

(e) Analysis of genetic divergence

SPAGeDI 1.2 was used to calculate F_{ST} (Weir & Cockerham 1984) and Nei's unbiased genetic distance D_S (Nei 1978). Significance was determined by 10 000 permutations of individual genotypes between populations. Pairwise F_{ST} and D_S values were not normally distributed (Kolmogorov–Smirnov test, $p<0.05$) and thus habitat-specific differences in genetic divergence were compared using median values. Classical significance tests are not appropriate here, as each population contributes to many measures of genetic divergence, creating non-independence. We thus assessed significance using a permutation test, with 1000 randomizations, in which each population was randomly assigned to a new location without replacement. We used Pearson correlations to assess the association between genetic divergence and timing of urban colonization, data on which were available for nine sites from Heyder (1955), Luniak *et al.* (1990) and K. L. Evans, K. J. Gaston & B. J. Hatchwell (2008, unpublished data).

(f) Assignment and exclusion tests

With GENECLASS 2.0 (Piry *et al.* 2004), we attempted to assign each urban individual to its most likely population of origin, other than the population from which it was captured. This was achieved using a likelihood-based partial Bayesian assignment test (Ranala & Mountain 1997) in combination with exclusion tests (Paetkau *et al.* 2004). Exclusion probabilities were calculated using a Monte Carlo simulation of 10 000 multilocus genotypes and using an exclusion threshold of $p<0.01$. Given the low genetic divergence between many rural populations (see §3), we could rarely assign individuals to a specific source population with high confidence. Our primary interest was whether a large proportion of individuals were assigned to another urban population, as predicted by the leapfrog model. Therefore, we summed the assignment probabilities for rural and urban origins and classified them according to two standard assignment thresholds (95% and 80%; Manel *et al.* 2005). Individuals for which neither the summed urban nor rural assignment probabilities met the specified threshold were not assigned. We also excluded individuals for which (i) all our sampled populations could be excluded as a potential source (just one individual met this criterion) or (ii) exclusion tests indicated that the source population could

Table 2. Comparison of allelic diversity and heterozygosity metrics averaged over 22 microsatellite loci for (a) urban populations and their paired adjacent rural equivalent, and (b) rural populations south of the Pyrenees relative to more northern ones. (Urban blackbird populations have lower genetic diversity than rural ones and southern rural populations tend to be less diverse than northern rural ones. Genetic diversity metrics for each population are provided in table S2 of electronic supplementary material 2.)

(a) genetic diversity metric	median		mean reduction in urban populations (%)	Wilcoxon signed-rank test
	rural	urban		
number of alleles	152.50	135.00	−11.0	$Z=3.1$; $p=0.002$
allelic richness	6.10	5.45	−10.0	$Z=3.1$; $p=0.002$
observed heterozygosity	0.64	0.62	−2.1	$Z=1.3$; $p>0.05$
expected heterozygosity	0.65	0.62	−2.8	$Z=2.6$; $p=0.009$

(b) genetic diversity metric	median		mean reduction in southern populations (%)	Mann–Whitney U-test
	north	south		
number of alleles	153.00	129.00	−15.7	$W=80.0$; $p=0.11$
allelic richness	6.10	5.20	−14.8	$W=85.0$; $p=0.02$
observed heterozygosity	0.64	0.61	−4.7	$W=76.0$; $p=0.35$
expected heterozygosity	0.65	0.64	−1.5	$W=68.0$; $p=0.47$

not be that with the highest probability of assignment (just two individuals met this criterion and, not surprisingly, in both cases the assignment probabilities were low: 0.47 and 0.36).

3. RESULTS

(a) Genetic diversity

We found statistically significant, but modest, reductions in the genetic diversity of urban populations with regard to the total number of alleles, average allelic richness per locus and expected heterozygosity (H_e); observed heterozygosity (H_o) did not differ significantly between urban and rural populations (table 2a; table S2 in electronic supplementary material 2). The proportional loss of allelic diversity in urban populations, relative to their paired rural ones, was negatively correlated with the year of urbanization (number of alleles: $r_s = -0.64$, $n=9$, $p=0.07$; allelic richness: $r_s = -0.74$, $n=9$, $p=0.02$). Changes in heterozygosity did not exhibit this pattern (H_o : $r_s = -0.159$, $n=9$, $p=0.7$; H_e : $r_s = -0.55$, $n=9$, $p=0.13$). We found no significant correlation between the loss of genetic diversity in urban populations and their distance from the first recorded urban colonization event (number of alleles: $r_s < 0.0001$, $n=12$, $p=1.0$; allelic richness: $r_s = -0.18$, $n=12$, $p=0.6$; H_o : $r_s = -0.13$, $n=12$, $p=0.7$; H_e : $r_s = -0.06$, $n=12$, $p=0.85$).

(b) Genetic clusters

An unrooted neighbour-joining tree based on D_S reveals that urban and rural populations did not form two distinct populations, and suggests divergence between populations located either side of the Pyrenees (figure 1). The STRUCTURE analysis identified a range of K values with similar likelihood values (figure 2). In these situations, caution is required in estimating K (Pritchard *et al.* 2000), and we thus used the *ad hoc* statistic ΔK proposed by Evanno *et al.* (2005) to identify the most appropriate number of clusters. This method clearly identified two clusters (figure 2), which corresponded to populations

located north and south of the Pyrenees (figure 3). Given this structure, we assessed the levels of genetic diversity in the northern and southern rural populations; genetic diversity tended to be lower in the southern populations (table 2b).

(c) Genetic divergence

The global F_{ST} values, regardless of habitat type, were, respectively, $0.019 \pm \text{s.e. } 0.001$ and $0.057 \pm \text{s.e. } 0.007$ for populations north and south of the Pyrenees. In both cases, values were significantly different from zero ($p < 0.0001$), confirming that within each of these two main clusters the populations were not panmictic.

Within the northern cluster, Nei's unbiased genetic distance D_S values (see table S3a in electronic supplementary material 3) were significantly greater among urban–urban comparisons (median = 0.062, $n=36$) than between urban–rural comparisons (median = 0.036, $n=81$, $p=0.002$) and rural–rural comparisons (median = 0.011, $n=36$, $p=0.001$). F_{ST} values exhibited the same pattern, with urban–urban comparisons (median 0.031) being greater than urban–rural comparisons (median 0.018, $p=0.001$) and rural–rural comparisons (median 0.005, $p < 0.001$). The same trend in genetic divergence was exhibited within the southern cluster, in which the median urban–urban D_S value (0.145) was greater than the median urban–rural D_S (0.085, $p=0.08$) and the median rural–rural D_S (0.070, $p=0.09$; table S3b in electronic supplementary material 3). Similarly, the southern median F_{ST} value (0.075, $n=3$) was greater than the median urban–rural F_{ST} (0.046, $n=9$, $p=0.13$), and greater than the rural–rural median F_{ST} (0.035, $n=3$, $p=0.07$).

Genetic divergence between rural and urban populations does not appear to have been eroded following colonization as the F_{ST} and D_S values among urban populations and their paired rural ones were not correlated with the year of urban colonization (Pearson correlations, $n=9$; F_{ST} : $r = -0.454$, $p=0.22$; D_S : $r = -0.408$, $p=0.28$).

Figure 1. A neighbour-joining tree based on Nei's unbiased genetic distance for (a) all populations and (b) those north of the Pyrenees. Urban and rural populations do not form different clusters. B, Berlin; G, Groningen; K, Krakow; M, Madrid; Mo, Montpellier; Mu, Munich; P, Prague; R, Riga; Sh, Sheffield; Sz, Szczecin; T, Tallinn; Tu, Tunis; V, Valencia. Urban and rural populations are, respectively, labelled in grey and black fonts.

(d) Assignment tests

A much greater proportion of urban birds, by an order of magnitude, were assigned to rural populations than to other urban ones (table 3). Exclusion tests showed that only a few individuals that had been assigned to a rural population could not be assigned to the nearest rural one (table 2).

Figure 2. STRUCTURE analysis of genetic population clusters. A wide range of genetic clusters (K) had similar maximum-likelihood values. In this situation, caution is required when estimating K and the *ad hoc* statistic ΔK provides the best estimate for K (Pritchard *et al.* 2000; Evanno *et al.* 2005), indicating that there were two separate genetic clusters. Diamonds represent the rate of change in the log probability of data between successive values of K , crosses represent the mean-likelihood $\ln(X|K)$ values and error bars represent standard deviation.

4. DISCUSSION

Only two previous studies have assessed genetic diversity in urban birds, and they did so for just a single population. The reductions in diversity that we find for the blackbird are fairly comparable with those observed in an urban Eurasian kestrel *Falco tinnunculus* population (allelic richness, -10% ; H_o , -8% ; Rutkowski *et al.* 2006), but much less marked than that observed in an urban dark-eyed junco *Junco hyemalis* population (allelic richness, -41% ; H_o , -21% ; Rasner *et al.* 2004). The loss of genetic diversity following a colonization event will be particularly marked following cumulative colonization events where individuals from a newly established population subsequently disperse and establish additional populations (Pruett & Winker 2005). The moderate reductions in genetic diversity that we observe thus provide more support for the independent urbanization model than the leapfrog urbanization one. Moreover, we find that, in contrast to the predictions of the leapfrog model, loss of genetic diversity is not correlated with the distance from the first recorded urbanization event and tends to be less marked in the most recently established populations. The latter finding is also noteworthy, as it suggests that initial reductions in genetic diversity following urbanization are not alleviated by the arrival of additional colonists, which is opposite to the pattern found in urban populations of other species (e.g. red fox *Vulpes vulpes*; Wandeler *et al.* 2003).

The independent urban colonization model is further supported by the observation that urban and rural populations do not form separate genetic clusters. Instead, blackbird genotypes separated into two clusters north and south of the Pyrenees. The Iberian Peninsula and northern Africa have acted as a glacial refugium for many species, one signature of which is the high genetic diversity in many extant populations in this region (Hewitt 2000; Schmitt 2007). Surprisingly, allelic diversity tended to be lower in rural blackbird populations from south of the Pyrenees compared with more northern ones, suggesting that this region may not have been a major glacial refugium for this species.

Figure 3. The two genetic clusters identified by STRUCTURE are not predominantly located in urban and rural populations, but tend to occur on either side of the Pyrenees (indicated by the grey bar). Pie charts illustrate the proportion of each of the two genetic clusters, indicated by black and grey, in each population. Location codes are the same as provided in figure 1; the U and R following the location code indicate, respectively, urban and rural populations.

Table 3. The percentage of individuals from each urban population assigned to urban and rural populations when summing assignment scores within each habitat type, and the percentage of rural assignments for which the nearest rural population could be excluded as a potential source. (Assignment tests show that, for the vast majority of urban blackbirds, source populations are rural rather than urban.)

focal urban population	95% assignment threshold				80% assignment threshold			
	urban	rural	local rural excluded	not assigned	urban	rural	local rural excluded	not assigned
Berlin	12.9	41.9	7.7	45.2	12.9	58.1	5.6	29.0
Groningen	3.2	38.7	8.3	58.1	6.5	58.1	5.6	35.5
Krakow	2.2	48.9	13.6	48.9	11.1	64.4	10.3	24.4
Madrid	3.2	51.6	0.0	45.2	6.5	71.0	0.0	22.6
Munich	0.0	40.0	16.7	60.0	3.3	56.7	17.6	40.0
Prague	6.7	30.0	0.0	63.3	16.7	43.3	0.0	40.0
Riga	0.0	35.3	0.0	64.7	5.9	41.2	0.0	52.9
Sheffield	3.2	38.7	8.3	58.1	9.7	67.7	9.5	22.6
Szczecin	3.1	40.6	0.0	56.3	15.6	65.6	9.5	18.8
Tallinn	0.0	46.2	0.0	53.8	3.8	65.4	0.0	30.8
Tunis	2.9	68.6	0.0	28.6	8.6	74.3	0.0	17.1
Valencia	0.0	80.6	0.0	19.4	0.0	90.3	3.3	9.7

There was no correlation between the timing of urban colonization and the magnitude of genetic divergence between paired urban and rural populations. It thus seems unlikely that habitat-specific patterns in the magnitude of genetic divergence have been altered following the initial colonization of urban areas by subsequent gene-flow

between urban and rural populations. The greater differentiation among urban populations than that between urban and rural populations thus also supports the independent urban colonization hypothesis. The differentiation of urban populations is likely to have arisen, at least in part, from founder effects, which could also have

contributed to the stochastic nature of morphological divergence between the paired urban and rural populations (Evans *et al.* 2009b). The increased sedentary behaviour in urban blackbirds compared with rural ones (Partecke & Gwinner 2007; K. L. Evans, J. Newton, K. J. Gaston, S. P. Sharp, A. McGowan & B. J. Hatchwell 2009, unpublished data) may also contribute to the greater differentiation between urban blackbird populations.

Some urban individuals were assigned to other urban populations, suggesting that a small proportion of urban-adapted birds may have contributed to the establishment of novel urban populations. However, the assignment tests demonstrate that a greater proportion of urban individuals, by an order of magnitude, have rural source populations rather than urban ones, providing further support for the individual colonization of urban areas.

Each of our four tests thus provides much greater support for the independent colonization of multiple urban areas by the blackbird than for the alternative leapfrog model. Despite previous evidence supporting the latter (including the strong spatial pattern in the timing of urban colonization, successful experimental introductions of urban birds to cities but failed introductions of rural birds, and evidence for genetic adaptations to urban environments), it thus seems highly unlikely that dispersal of urban-adapted individuals has contributed greatly to the establishment of additional urban blackbird populations. Our results illustrate the importance of assessing colonization processes using genetic tools. More importantly, they set a challenge to identify which of the numerous ecological factors that covary with latitude and longitude have generated the strong spatial pattern in the timing of urban colonization events in this species. Reduced genetic diversity can severely limit the potential of recently established populations to adapt to novel environments (Pujol & Pannell 2008). The fact that the genetic diversity of urban blackbird populations exhibits only moderate reductions may partly explain how this former forest specialist has managed to become one of the most common urban birds across its range. Comparisons of the population genetic structure of multiple urban and rural populations of other taxa are required to determine whether independent urban colonization is general in urban colonists. If independent colonization events predominate then, in species where urbanization requires genetic adaptations, colonization of multiple urban centres will be particularly difficult, potentially having implications for urban species diversity.

Appropriate licences and permits for capture, ringing and sampling of birds were obtained from the relevant authorities in the ten countries where fieldwork was conducted. Samples were imported into the UK under licence.

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