

# Recent spread of Blue tits into the Barcelona urban environment: morphological differences and the role of balanced dispersal

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## Abstract (N=208 words)

Rates of phenotypic change are greater in cities than in any other habitat. Consequently, urban habitats are an ideal experimental area to study contemporary evolution and adaptation. A key question related to phenotypic changes in urban animals relates to the patterns of gene flow between the city and the natural habitats surrounding cities, but the results obtained thus far have been contradictory. The aim of this study was to analyze genetic differentiation and patterns of gene flow and size variation in urban blue tits *Cyanistes caeruleus* in Barcelona city, using a panel of 26 microsatellites. We compared birds from one locality at the city center, one at the suburban area, and one in a nearby forest. We found a significant genetic differentiation between the populations, which has evolved over a period of about 35 generations (50 years), based on historical data. There was significant gene flow between all populations. There was no significant asymmetric gene flow between the forest and the other two populations, which is consistent with balanced gene flow and dispersal. City birds were smaller than individuals from the forest. Simulations showed that the selection required to achieve these phenotypic differences would have to be high, which in turn suggests that phenotype-dependent dispersal may be involved.

Key words: Blue tit; body size; gene flow; local adaptation; urbanization

## Introduction

Traditionally, it had been argued that evolution proceeds slowly. This view was based on the fact that most populations seemed to have reached an equilibrium and hence, phenotypic change for most traits is generally quite slow in natural populations. However, we now know that this is not the case, and that in some situations, evolution can proceed rapidly (Hendry 2017). One of the most famous and oldest examples of so-called “rapid” evolution was the dramatic change in frequency of melanic moths in the United Kingdom and elsewhere in Europe due to industrial pollution (Kettlewell 1973; Majerus 1998). In the 1960s, several papers supported the idea of rapid evolution, such as studies on changes in color and body size in house sparrows (*Passer domesticus*) introduced to North America (Johnston and Selander 1964) and the change in skeletal characters and body size in mice (*Mus musculus*) introduced to islands (Berry 1964). Since then, many more papers on the subject have been published, giving rise to the area of research termed *contemporary evolution* (Carroll et al. 2007; Hendry 2017; Hendry and Kinnison 1999, 2001; Reznick and Ghalambor 2001).

Contemporary evolution is especially common in response to global change (Carroll et al. 2007) and is very often associated with human activities (Stockwell et al. 2003), because this forces animals to adapt to the rapid changes in the landscape. This explains why these “fast” evolutionary adaptations are increasingly described. Rates of recent phenotypic change seem to be greater in urban areas than in any other habitat type, including non-urban anthropogenic contexts (Alberti et al. 2017; Thompson et al. 2018). Consequently, urban habitats are an ideal experimental area to study contemporary evolution and adaptation (Rivkin et al. 2019).

A key question in relation to phenotypic changes in urban animals is related to patterns of gene flow between the city and the natural habitats surrounding cities. Patterns of gene flow have a strong effect on the outcome of divergent selection and hence, local adaptation (Garant et al. 2007; Kawecki and Ebert 2004). The role of gene flow patterns is even more important in urban environments, because high rates of gene flow from natural populations homogenize allele frequencies between natural and urban populations, preventing loci under selection from becoming fixed in the urban populations (Johnson and Munshi-South 2017; Miles et al. 2019; Munshi-South and Richardson 2020; Rivkin et al. 2019). Asymmetric movements from natural habitats to the city, for instance, can lead to a source-sink structure, in which the sink populations may not be able to evolve local adaptations because they are flooded with immigrants. Results obtained thus far in urban ecology, however, are contradictory. Previous work has found a higher gene flow from forest or rural habitats to city populations; this is the case in blackbirds *Turdus merula* (Evans et al. 2009a), burrowing owls *Athene cunicularia* (Mueller et al. 2018), and wild boars *Sus scrofa* (Stillfried et al. 2017). However, a higher gene flow from the city to the forest or rural habitat has also been described, as in great tits (Björklund et al. 2010), natal multi-mammate mice (Gryseels et al. 2016) and Cooper’s hawks *Accipiter cooperii*

(Millsap 2017). Balanced dispersal, with equal numbers of immigrations and emigrations between different habitats (Doncaster et al. 1997), surprisingly, has not been described between urban and rural environments. Another phenomenon not yet described in urban setting is matching habitat choice. In this case, individuals disperse to habitats that best match their phenotypes, thus comprising a mechanism of directed gene flow that may ultimately promote population differentiation and adaptation. This could be an alternative mechanism to increase local adaptation (Bolnick and Otto 2013; Edelaar and Bolnick 2012). Clearly, more work is needed specifically focusing on the study of dispersal in urban environments.

An interesting pattern repeatedly found in urban settings is that the size of different morphological traits is smaller in city-dwelling individuals (Caizergues et al. 2018; Liker et al. 2008; Senar et al. 2014; Yeh 2004). How this pattern has come about is far from clear. Urban great tits in Montpellier were smaller than their forest counterparts, but the difference in body size between urban and forest great tits was not the result of divergent selection when measuring fitness from the number of chicks raised (Caizergues et al. 2018). Variation in the size of the black breast stripe of the great tits in Barcelona was the result of divergent selection associated with a differential survival of the birds in relation to stripe size according to the habitat: larger stripes were favored in the forest, while the urban habitat favored small stripes (Senar et al. 2014). Hence, the size of urban great tits in Montpellier could be modulated by differential survival rather than by differences in reproductive success. However, differences in size between urban and natural populations could also be modulated by matching habitat choice processes, which has never been tested. This test requires the integration of the study of trait variation with patterns of gene flow, an approach rarely attained in urban ecological studies.

The aim of this study was to analyze genetic differentiation and patterns of gene flow and size variation in urban blue tits *Cyanistes caeruleus* in Barcelona city, using data from the city center and suburban area and comparing that to a nearby forest. The blue tit is an especially good model species for this purpose. Recently, Olano-Marín et al. (2011b) identified a panel of microsatellites distributed across 25 chromosomes, which enables the use of a high number of molecular markers to more accurately examine patterns of gene flow (Olano-Marin et al. 2011a, 2011b). Blue tits in Mediterranean areas, such as Barcelona, are also especially suited for these studies because they are mostly sedentary and show relatively low rates of dispersal (<0.5 km) (Ferrer et al. 2016), with reduced gene flow among geographically close populations facilitating processes of local adaptation (Ferrer et al. 2016; García-Navas et al. 2014; Porlier et al. 2012a; Porlier et al. 2012b). An additional advantage of carrying out this analysis in Barcelona is that we know that blue tits entered the city in the 1970s. Previous data described the blue tit as only inhabiting Barcelona city in harsh winters (Balcells and Domènech 1965). Later, in the 1980s, the species had become common in Barcelona urban parks (Batllori and Uribe 1988). This knowledge allows us to test how much time may be needed to achieve genetic differentiation

between urban and forest birds, which is the second aim of this paper. Altogether, this will allow us to test how and when blue tits have become adapted to the urban environment. Our initial hypothesis was that, similar to great tits, blue tits in Barcelona city would be smaller than their forest counterparts, would be relatively isolated from forest birds and gene flow would be higher from the city to the forest habitat than the reverse, with the suburban in between. However, and contrary to expectations, we found that although urban birds were certainly smaller than forest birds, we found no evidence of biased gene flow in either direction.

## METHODS

### **Field methods**

Blue tits were captured from 2013 to 2015 using special funnel traps adapted to the capture of tits (Senar et al. 1997). Forest birds (n= 54) were captured at the Can Cata Field Station located in Collserola National Park (henceforth referred to “Forest”), 3 km from Barcelona city. This area consists of a mixed forest, dominated by pure oak (*Quercus ilex* and *Quercus cerrioides*) stands at the bottom of the valleys and Aleppo pine (*Pinus halepensis*) forests in the hills. Urban birds were captured at two parks in Barcelona city, Ciutadella Park (42 birds) and Desert de Sarria-Setmenat Park (42 birds). Ciutadella Park is situated in the city center, is one of the largest (30 ha) and oldest parks in Barcelona, and is surrounded by buildings on all sides. It is an open-plan park with a high number of daily visitors, and thus does not resemble a natural forest. Throughout the text, we refer to this park as the ‘City’. Desert de Sarria-Setmenat Park is located on the northwest outskirts of Barcelona city (a suburban area). It is partly a natural forest, but there are several buildings within the grounds, as well as some orchards, and many visitors. In the text, we refer to this park as ‘Suburban’ (Figure 1).

Birds were aged according to Svensson (1992), distinguishing between birds in their first year (Young birds) (EURING ages 3 and 5) and older birds (Adults) (EURING ages 4 and 6). For each bird, we measured wing length (maximum cohort), tarsus length and body mass according to Svensson (1992). We also collected blood samples for DNA extraction and samples were stored in pure ethanol at -20°C. Birds were sampled, handled and ringed with special authorization (001501-0402.2009), following Catalan regional ethical guidelines for the handling of animals in research, from Servei de Protecció de la Fauna, Flora i Animal de Companyia, according to Decree 214/1997/30.07, Generalitat de Catalunya.

### **Genetics**

DNA was extracted using an Ecogen Master Pure DNA Purification Kit (MCD85201). We used 26 microsatellite loci taken from Olano-Marin et al. (2011a, 2011b); Ase18, PAT, Pdo5, Mcyae4, Pca3, PK12, PmaGAn45, Pca8, Cdi31, POCC1, CcaTgu19, CREB1, CcaTgu4, TG05-046, Pca9, Tgu01, Pca4, ADCYAP1, CcaTgu13,

CcaTgu17, DkiB119, CcaTgu14, Gf06, TguEST09-021, MSLP4, and PmaGAn40. The samples were run using the PCR conditions given in the original papers.

We tested Hardy-Weinberg equilibrium using a log-likelihood test for multiple alleles (Weir 1996): 105-106). We tested for linkage disequilibrium using Arlequin 3.5.2.2 (Excoffier et al. 2005). We found one significant deviation from Hardy-Weinberg equilibrium in one locus out of  $3 \times 26 = 78$  locus-population combinations (1.3 %). Thus, we can assume there are no null alleles at any scale that might affect the results. Given the large number of tests for linkage disequilibrium for all possible allele combinations in all three populations, we counted the number of significant cases and compared it to the expected number at the  $P = 0.05$  level:  $0.05 \times (26 \times 25) / 2 = 16$  cases in each population. We found 18, eight and two cases, respectively, which is in line with that expected by chance alone. We therefore conclude that linkage disequilibrium is highly unlikely to be a problem in subsequent analyses.

The basic population genetic summary statistics (number of alleles and observed heterozygosity) and estimates of  $F_{ST}$  and population-specific  $F_{IS}$  were done in Arlequin 3.5.2.2 using the locus-by-locus approach and are given in S1.  $F_{ST}$  has a very strong theoretical underpinning, but unfortunately it is dependent on the within-population heterozygosity (by definition), which is clearly related to the type of markers used. Thus,  $F_{ST}$  values cannot be compared between studies but only among populations with the same markers. This also means that  $F_{ST}$  is not ideal as a measure of population differentiation. To remedy this, Hedrick (2005) developed a standardized measure of differentiation ranging from 0 (no differentiation) to 1 (total differentiation), hence allowing for a more intuitive interpretation and comparison with other studies. Thus,

$$G'_{ST} = \frac{G_{ST}(k - 1 + H_S)}{(k - 1)(1 - H_S)}$$

where  $G_{ST}$  equals  $F_{ST}$  as used here, and  $H_S$  is the average within population heterozygosity. We used the randomization procedure in Arlequin to obtain 95 % confidence intervals for each  $F_{ST}$  and recalculated to  $G'_{ST}$ . We used Arlequin for an assignment test of individuals to each population.

Effective population size ( $N_e$ ) was calculated assuming a metapopulation model where the different productivity of the demes (defined as number of offspring produced) is taken into account:

$$N_e = \frac{N}{(1 + F_{IS})(1 + F_{ST}) - 2F_{IS}F_{ST}}$$

(Nunney 2016). The population size ( $N$ ) of City and Suburban was set to 40 each (unpublished data), whereas the forest population of Forest is unknown but much

larger so we used values drawn from a uniform distribution ranging between 200 and 500. Population size in the two city populations was estimated from long term monitoring, including capture/recapture.  $N$  for the combined population was estimated as the harmonic mean of the separate  $N$ -values. We estimated  $N_e$ -values for each locus separately and calculated a mean value over loci. These values were then bootstrapped 5000 times to get a mean and a variance of  $N_e$ .

Family relations among individuals were estimated using MLRelate (Kalinowski et al. 2006) to ensure that the individuals used were independent. If there are many families with close relatives in the samples, this would compromise the analyses to some unknown extent. Only likelihoods of half-sib and higher relatedness-values that were two times larger than the likelihood of the unrelated alternative were considered.

We estimated gene flow by calculating the degree of differentiation in the absence of gene flow using equation 6b in Hedrick (2005):

$$G_{ST(t)} = \frac{\left(1 - \frac{1}{k}\right) (1 - H_S) \left[1 - e^{-\frac{tH_S}{N_e(1-H_S)}}\right]}{H_S + \left(1 - \frac{1}{k}\right) (1 - H_S) \left[1 - e^{-\frac{tH_S}{N_e(1-H_S)}}\right]}$$

where  $k$  is the number of populations,  $N_e$  effective population size as estimated above, mean expected within-population heterozygosity ( $H_S$ ), and  $t$  is time since divergence. This formula is used to calculate the expected degree of genetic differentiation. We know that this occurred about 50 years ago, and with a generation time of 1.5 years,  $t$  corresponds to 33 generations. This gives us an expected degree of differentiation with no gene flow during this time. If the observed values are smaller than the expected, then gene flow is the most likely explanation, while if the observed values are larger, the most likely explanation is divergent selection. We used this form rather than the most common one,  $F_{ST} = 1 - e^{-t/N_e}$ , because it can incorporate uncertainty in the  $H_S$ -estimates. We did a simulation and drew values from a normal distribution with the mean and error variance equal to that estimated for  $H_S$  over all loci and populations (in Arlequin) and  $N_e$  (see above). This was done 1000 times. The observed distribution was taken from Arlequin transforming the  $F_{ST}$  values to  $G'_{ST}$  according to Hedrick (2005) as above.

To further elucidate the degree of population differentiation and migration in this and previous generations, we used Structure 2.3.4 (Pritchard et al. 2000). We carried out two different types of runs. First, we were interested in the migration patterns and ancestry of individuals. We used the sampled populations as prior information, and correlated allele frequencies since all populations have the same ancestry (Falush et al. 2003). Second, we used an admixture model with the sampled population as prior information, and correlated allele frequencies. We used 10 000 burn-in runs, followed by 50 000 runs with sampling (running a much smaller number of runs did not change the results). Secondly, rather than only using the putative number of populations ( $K =$

3) as with the analysis of migration, we ran  $K = 1$  to 6, i.e. with three sampled sites and up to three “ghost populations” to determine if there has been migration from more distant and unknown populations. All models were run ten times each. We compared the models by means of weighted AIC-scores, which provides the relative strength of support (Burnham and Anderson 2002).

To estimate gene flow we used the following recursion:

$$F_{ST}(t) = \frac{1}{2N_e} (1 - m)^2 + \left(1 - \frac{1}{2N_e}\right) * (1 - m)^2 * F_{ST}(t - 1)$$

We tested values of  $m$  to determine which value minimized  $(F_{ST}(\text{obs}) - F_{ST}(\text{sim}))^2$ . We used  $m$ -values ranging from 0.05 to 0.6 with an interval of 0.001. We ran 500 samples with the uncertainty of  $N_e$  and  $F_{ST}$  taken into account by drawing values from the distributions of  $N_e$  and  $F_{ST}$  from above. In this way, we obtained a standard error of the  $m$ -values. We did this for all three populations as well as for the pairwise comparisons. We used the online software *divMigrate* to test for asymmetric gene flow (Sundqvist et al. 2016).

### **Morphology and selection**

We calculated the mean values of three traits—wing length, tarsus length and mass—for the two different age classes (young and adults) and compared them using a one-way ANOVA with population as a factor, and testing the differences of means using the Tukey test.

We used two models from Hendry et al (2001) to estimate the amount of selection required to account for any observed differences in phenotypic traits among birds of the same age group and, from different localities, under a scenario of gene flow. The first assumes that migration occurs first and then selection, and we added the assumption that the source population (Forest) did not experience any directional selection after the City population was founded. We confined the comparison to Forest and the City since that is where we found the main differences between populations (see section Morphology in the Results). The difference is then

$$\Delta D = V_A \beta - mD$$

where  $V_A = h^2 V_P$ ,  $D$  is the difference in mean values at the start of the simulation,  $\Delta D$  is the change in the difference in trait means,  $m$  is the migration rate, and  $\beta$  is the selection gradient. We used the phenotypic variance,  $V_P$ , observed in the populations, while heritability values,  $h^2$ , were used as below. The second model assumes that selection occurs first and then migration and is given as

$$\Delta D = (1+m)V_A \beta - mD$$

We used these equations recursively starting from  $D = 0$ , for different levels of  $h^2$ , ranging from 0.2 to 0.8 for 33 generations and compared the estimated and observed



difference in mean values. This will be expressed as the percent absolute difference between the two populations. The selection will be expressed as the variance standardized selection gradient (Walsh and Lynch 2018). This is done to make the estimated selection comparable to other studies of actual selection. We used the values for first-year birds because selection is most easily seen in this group. The adults are a larger heterogenous group of different ages where other sources of selection have occurred in addition to movement between the populations. Ideally, we would have tested for the neutral divergence in these morphological traits, and there are some tests available. However, their usefulness is questionable when  $N_e$  is small as in our case, and the time since divergence in relation to  $N_e$  is short (Walsh and Lynch 2018). Thus, we refrained from running this test.

## RESULTS

### *Genetic markers*

The markers differed in the number of alleles but there was no difference between populations (Kruskal-Wallis 0.1,  $P = 0.95$ , S1). There was no significant difference in  $H_s$  between populations (Kruskal-Wallis 0.050,  $P = 0.78$ , S1). Relatedness was low in each of the populations with on average 3.8 % of the dyads (pairs of individuals) having a relatedness equal to half-sibs in Forest, 3.3 % in the City center and 3.0 % in the Suburban area. We found in total ten full-sib and two parent-offspring unambiguous relations out of 3153 dyads. These few pairs of relatives did not affect the analyses in any way. The effective population size ( $N_e$ ) was 65 (95 % confidence interval: 61-69) for the Forest-City combination, 66 (62-70) for Forest-Suburban combination, and 38 (37-39) for the City-Suburban combination. The population-specific  $F_{IS}$  values did not differ in their locus-specific  $F_{IS}$  values (Kruskal-Wallis = 3.6,  $P = 0.17$ , S1).

The mean (95 % confidence interval) observed values of  $G'_{ST}$  varied between 0.063 (0.028-0.11) (Forest-City) to 0.028 (0.008-0.048) (Suburban-City) with the Forest-Suburban value in between with a  $G'_{ST}$  of 0.044 (0.011-0.084). The expected values were clearly higher with no overlap with the observed values (Fig 2). Of 54 individuals in the Forest, 52 were assigned to this population with the remaining two assigned to the City and ambiguously to City or Suburban. All City birds were assigned to the City, and all but one of the Suburban birds were assigned to the Suburban population, with the remaining individual assigned to the City population.

The analysis using Structure showed the same results assigning individuals to the three populations with an accuracy of over 97 % (Fig 3). When we used several populations and an admixture model, the best model was four populations with an evidence ratio (the ratio of best versus next best Akaike weights) of 1211 in relation to the next best model, which was five populations (difference in AIC-values = 14.2).

This means that the four-population model was 1211 times more likely than the next best model. The model with three populations had considerably less support (difference in AIC = 163.4). Group 1 was represented in all three populations but at a much higher frequency in the City than in the other populations, with over 50 % of the individuals assigned to this group (Fig 3). About 30 % of the individuals in the Forest was assigned to group 2, while almost none of the individuals in the City and the Suburban populations were assigned to this group. Slightly more than 10 % of the individuals in the City were assigned to group 3, while almost none from the other populations. Group 4 was represented in all populations, but individuals in the Suburban population were almost exclusively assigned to this group, more than Forest individuals and far more than birds from the City population.

Using the recursive equation given in Methods, we found that the estimated gene flow between the Forest and the City was significantly larger than the gene flow between the Forest and the Suburban population ( $t = 2.0$ ,  $P = 0.024$ ; Fig 4), but not between any other combination. There were no indications of asymmetric gene flow ( $P > 0.40$  for all directions based on 1000 bootstraps).

### ***Morphology***

There were significant differences between populations in mass, wing length and tarsus length, and birds from the city were significantly smaller for every tested trait than forest birds (Table 1).

There is a window where the combination of heritability and selection will result in a difference in mean values as that observed (i.e.  $D = 0$ , blue areas, Fig 5). This window is quite narrow and selection that is too large or too small will result in a difference larger or smaller than expected. If we assume a heritability of 0.5 then the selection gradient would need to be around 0.45 for wing length, 0.20 for mass and 0.30 for tarsus length.

## **DISCUSSION**

We found a significant genetic differentiation between the different populations evolving over a period of about 35 generations (50 years). The assignment tests and the significant  $G'_{ST}$  values showed a clear genetic pattern, with the City population being the most differentiated. Interestingly, the analyses of genetic structure revealed that the most likely number of groups was in fact four rather than the three we sampled from. This means that there are birds entering from other areas to different degrees. This is not surprising given that movements are well-known, albeit rare but high enough to give rise to the patterns we observed.

There was no difference in heterozygosity between the populations, or in terms of the inbreeding coefficient. If we assume that  $F = 0$  at time  $t = 0$ , the observed inbreeding coefficient would be attained at  $t = 35$  with an effective population size of around 200-500. Since this is an order of magnitude larger than that observed, the only explanation is that there is gene flow between the populations, which is what we found. This is strengthened by the simulations of expected genetic differentiation assuming no gene flow that returned values significantly larger than that observed. The estimated gene flow was higher for Forest and City, while the other directions were about the same level (Fig 4), but with no indications of asymmetric gene flow. This pattern contrasts with previous studies, which have either found a higher gene flow from forest to city populations or *vice versa* (see introduction for examples). Data from blue tits are consistent with a pattern of balanced dispersal (Diffendorfer 1998; Doncaster et al. 1997; H. Tattersall et al. 2004), rarely found in urban settings. This is interesting, because asymmetric gene flow is generally stated as a source of local adaptation processes (Pavlacky et al. 2012). However, as stated in the introduction, although gene flow can be symmetric in relation to the number of individuals dispersed, it could be asymmetric in relation to the phenotype of the individuals, provided the phenotypes are unrelated to the genetic markers used. This phenotype-dependent dispersal, in which both forest and urban populations move to better match their habitat (Bolnick and Otto 2013; Edelaar and Bolnick 2012), could explain the levels of local adaptation to the urban habitat that we detected. This assumes that dispersing individuals settle into the new population. For this mechanism to work, dispersal should be equivalent to gene flow.

The birds in the City were on average smaller than the young Forest and Suburban birds. This pattern of smaller traits in urban animals is similar to other species (Caizergues et al. 2018: 6; Liker et al. 2008; Senar et al. 2014; Yeh 2004: 171). In the simulations of divergence with gene flow, we found that the selection needed to obtain the observed difference for different levels of heritability was substantial, ranging from around  $\beta_{\sigma} = 0.20$  (mass) to 0.45 (wing). To put this into perspective, the median  $\beta_{\sigma}$  in a number studies compiled by Hereford et al. (2004) was 0.17. However, this is an upper limit and four factors could act to reduce the importance of selection. First, there could be an effect of plasticity such that young birds in the City grow less than in the Forest due to less food availability, a warmer climate or some other external factor (Caizergues et al. 2018; Evans et al. 2009b). Nevertheless, the gene flow detected between the city/forest populations should erase local differentiation. The only way to solve this would be through experiments and common garden-raising of chicks. Second, an alternative potential cause for these patterns could be density-dependent processes, such that a substantial proportion of smaller birds could be outcompeted by larger ones in the forest and forced to emigrate to sub-optimal patches (such as those found in the city). This, however, assumes asymmetric gene flow from the forest to the city, which was not the case. A third factor that needs to be considered is that we actually found support for four population among the three populations studied. That is, there is immigration from an

unknown source and if these birds are smaller than the forest birds this can explain the small size of the City birds. However, the presence of the unknown population was most marked in the Forest population so if this mechanism is operating it must be an influx of large birds to the forest. Even though we have no direct evidence in either direction, we view this explanation as less likely. The fourth factor, which has only recently gained interest, is the previously cited phenotype-dependent dispersal (Bolnick and Otto 2013; Edelaar and Bolnick 2012). If smaller birds migrate to the City in larger proportions than random then this would mimic selection for a smaller size in the City. Morphological data could therefore additionally support phenotype-dependent dispersal as a mechanism to increase the patterns of local adaptation of blue tits to the urban environment found. The best way to test for the differential effect of phenotype-dependent dispersal and natural selection would be a multistate or multi-event capture-recapture approach (Choquet et al. 2009; Desprez et al. 2013; Senar et al. 2002), where both survival and movement are modelled according morphology. This approach, however, requires suitable populations in both habitats that exchange sufficient numbers to allow parameter estimations. Hence, until movement data (e.g. mark-recapture or telemetry) is available, phenotype-dependent dispersal as a mechanism to promote urban local adaptation will remain an untested hypothesis.

Overall, and similar to other studies (Gryseels et al. 2016), our data show that contemporary evolution can proceed rapidly, especially during the invasion of a novel environment such as a city (Mueller et al. 2018; Yeh 2004). However, it is not clear whether this is only the result of natural selection. Future work should test the relative contribution of natural selection, plasticity and phenotype-dependent dispersal to the adaptation of animals to the urban environment.

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Table 1. Differences in morphology between the three populations of adults and young blue tits.

Trait	F	df	P	City vs Forest	Forest vs. Suburban	City vs. Suburban
<b>Body mass</b>						
Adults	4.8	2,362	0.0086	0.020	0.068	0.59
Young	3.3	2,452	0.037	0.044	0.18	0.44
<b>Wing length</b>						
Adults	11.2	2,381	< 0.001	< 0.001	0.0040	0.21
Young	18.9	2,472	<0.001	< 0.001	<0.001	0.99
<b>Tarsus length</b>						
Adults	4.7	2,329	0.0095	0.010	0.19	0.33
Young	13.8	2,432	<0.001	<0.001	0.41	< 0.001

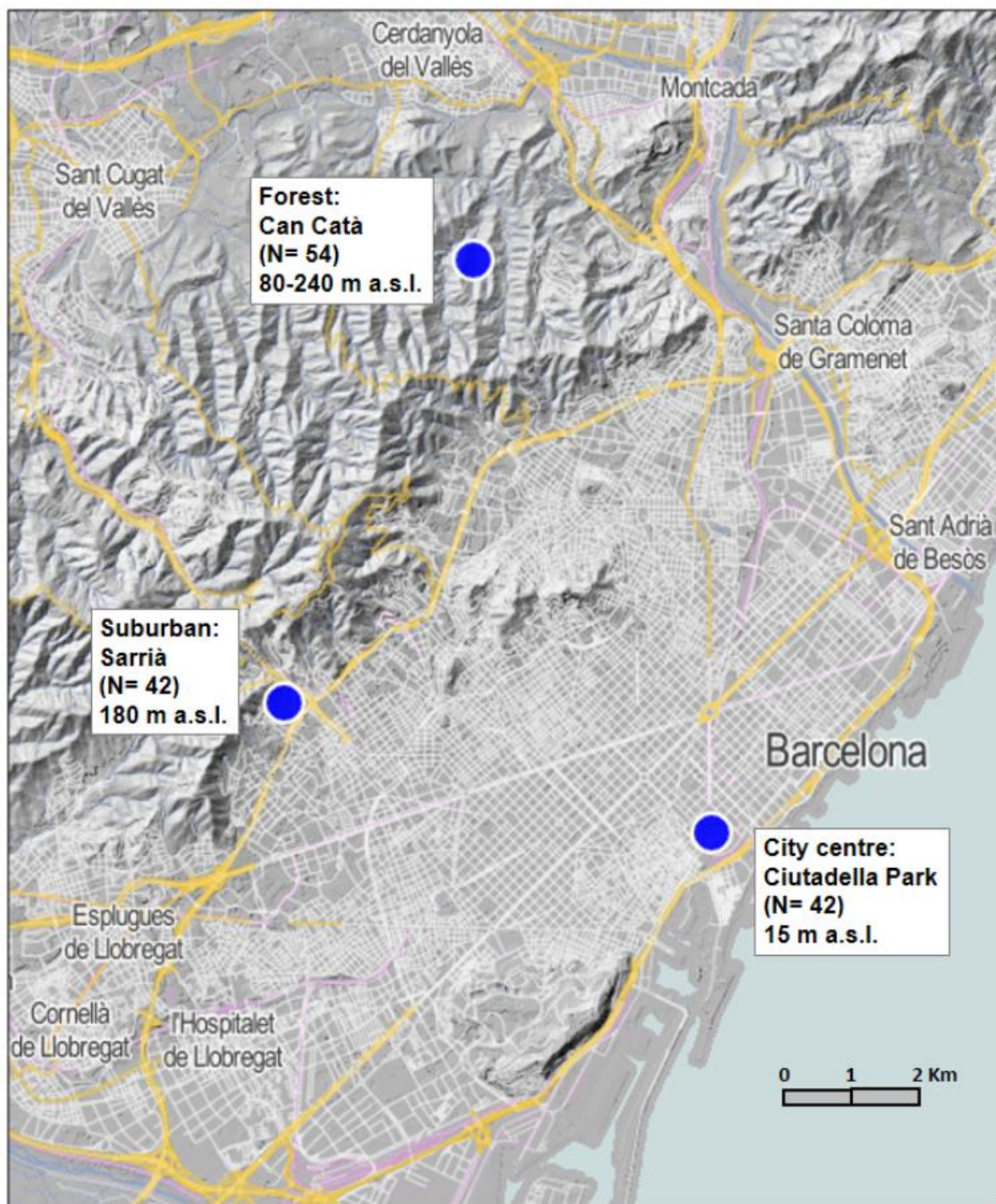
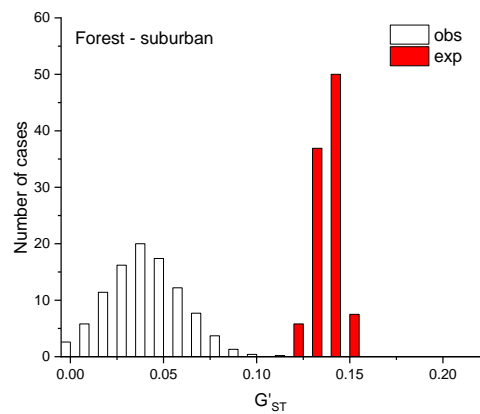
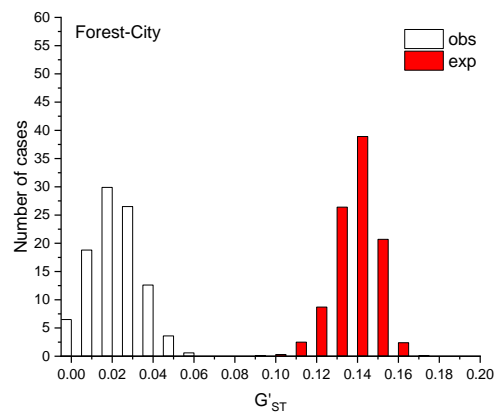
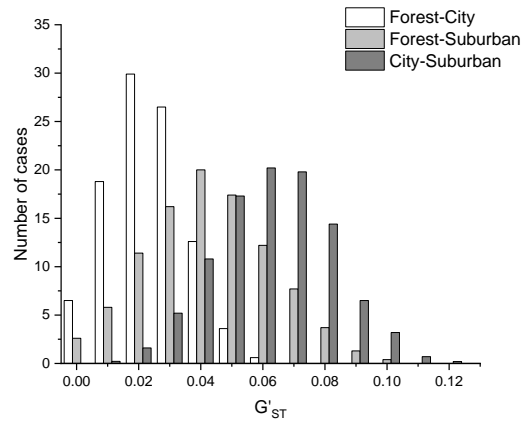
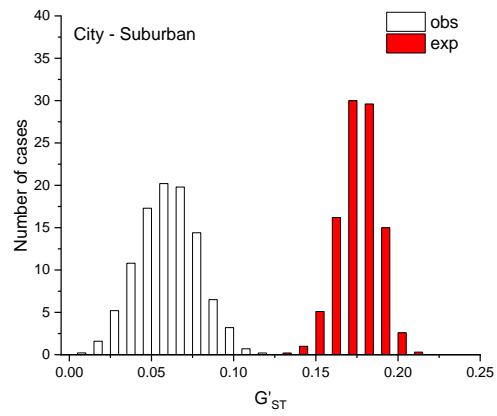


Figure 1. Map of the study area showing the locations of the three sampling areas: City Park (city center), Desert de Sarrià (suburban area) and Can Catà Field Station (forest). Altitude of the three areas above sea level and sample size is also provided.

Figure 2. Population differentiation. a) Observed  $G'_{ST}$  values for all population comparisons. b-d) Observed and expected  $G'_{ST}$  values for the three different comparisons.





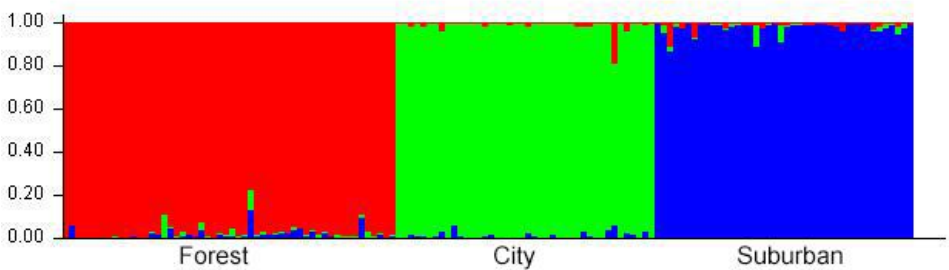
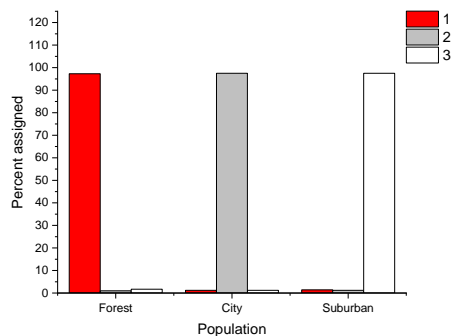
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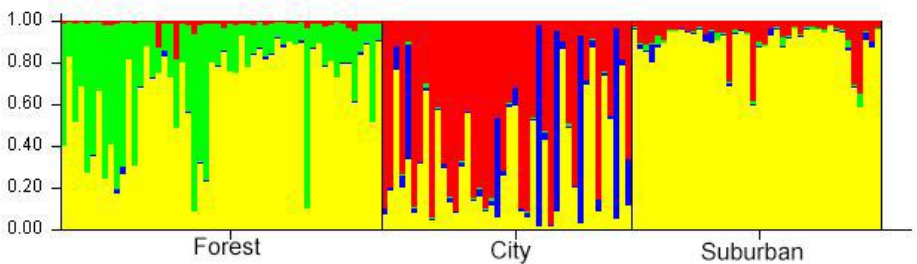
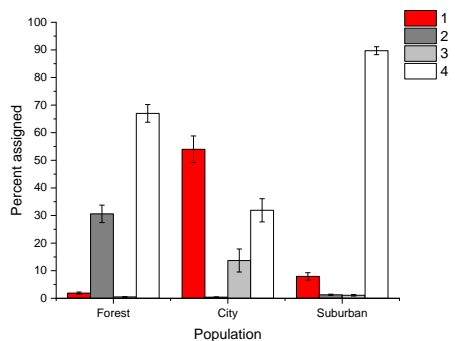
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Figure 3. Genetic structure using STRUCTURE 2.3.4. a) Assignment of individuals to each population using predefined populations. b) Assignment of individuals to estimated populations. See Methods for more details.

a)

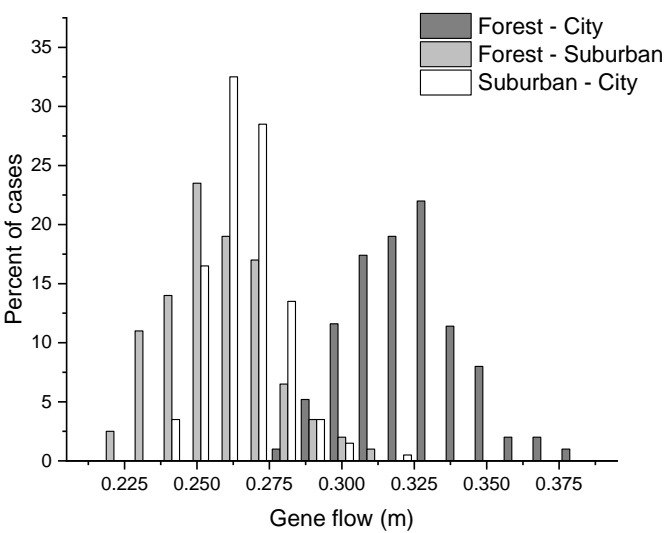


b)



626 Figure 4. Estimated gene flow. See text for details.

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Fig 5. Pattern of morphological differences between populations. Means + SE.

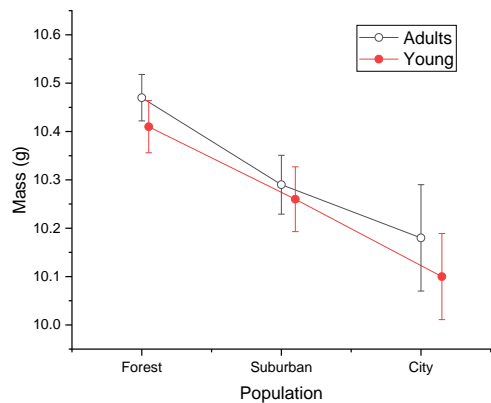
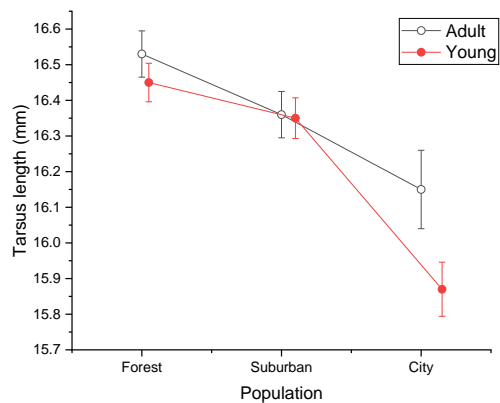
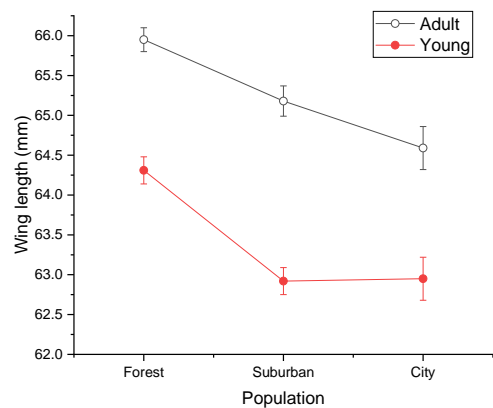
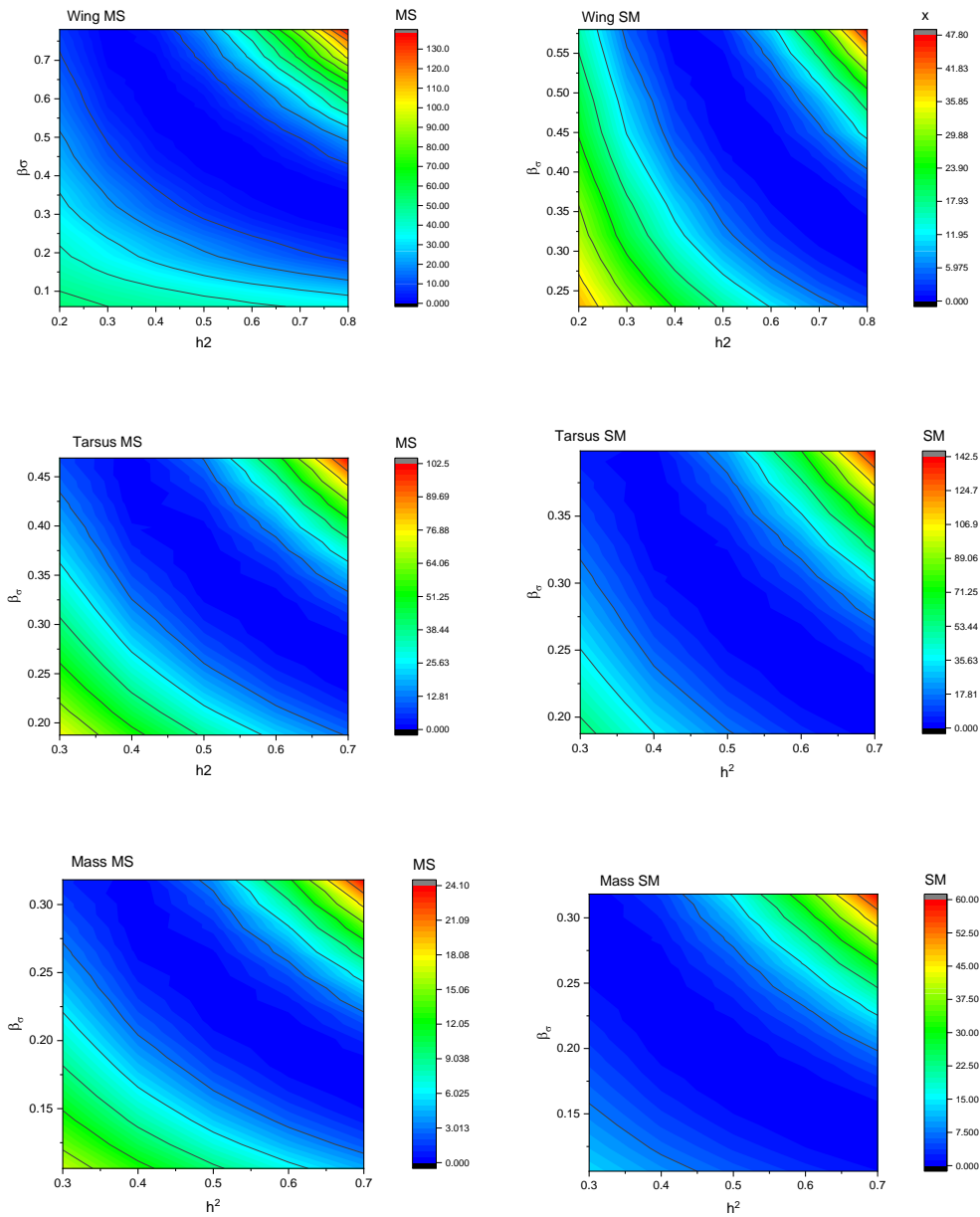


Fig. 6. The combination of heritability and selection in relation to the difference observed. The Z-scale is the difference between the actual and the simulated difference given as percent deviation. SM stands for Selection, then Migration, and MS stands for Migration, then Selection. The colors refer to the difference between expected and observed trait means; thus the blue color refers to the area where there is no difference ( $D = 0$ ). See text for more details.





Locus	# Alleles			Hs			FIS		
	Forest	Suburba n	City	Forest	Suburba n	City	Forest	Suburba n	City
Ase18	11	13	12	0.87	0.83	0.8	0.0064	0.032	0.058
PAT	7	8	7	0.58	0.5	0.48	0.079	0.04	0.063
Pdo5	18	18	25	0.81	0.83	0.95	0.097	0.077	-0.03
Mcye4	17	17	17	0.89	0.95	0.93	0.034	-0.048	-0.021
Pca3	23	19	18	0.77	0.76	0.9	0.13	0.12	-0.052
PK12	16	14	16	0.74	0.79	0.67	0.16	0.085	0.23
PmaGAn4 5	22	21	23	0.85	0.83	0.81	0.07	0.1	0.12
Pca8	26	22	26	0.83	0.8	0.81	0.12	0.14	0.14
Cdi31	9	7	11	0.39	0.34	0.5	0.22	-0.042	-0.034
POCC1	19	11	11	0.9	0.76	0.88	-0.01	0.14	-0.011
CCaTgu19	15	15	18	0.85	0.93	0.76	0.053	-0.047	0.16
CREB1	6	6	5	0.38	0.26	0.26	0.033	-0.078	0.22
CcaTGU4	3	1	3	0.076	na	0.14	-0.022	na	-0.045
TG05-046	2	2	2	0.53	0.52	0.4	-0.079	-0.21	0.18
Pca9	10	10	10	0.65	0.67	0.81	0.12	0.087	0.042
Tgu01	6	3	3	0.19	0.14	0.4	0.27	0.45	0.047
Pca4	11	10	9	0.77	0.83	0.79	0.078	-0.069	0.042
ADCYAP1	5	5	7	0.49	0.6	0.6	0.14	-0.11	0.016
CcaTGU13	5	6	4	0.63	0.67	0.48	0.054	0.011	0.27
CcaTGU17	4	3	6	0.44	0.36	0.54	0.24	0.22	0.13
DkiB119	8	6	5	0.68	0.78	0.71	-0.001	-0.08	0.019
CcaTgu14	11	13	14	0.63	0.79	0.76	0.075	0.073	0.076
Gf06	5	5	5	0.6	0.71	0.71	0.13	0.012	-0.008
TguEST09- 21	3	2	1	0.019	0.048	na	0.66	-0.012	na
MSLP4	9	8	7	0.63	0.79	0.6	0.025	-0.14	0.094
PmaGAn4 0	5	5	6	0.46	0.51	0.57	0.13	0.089	0.047
Mean	10.62	9.96	10.8	0.6	0.64	0.65	0.108	0.034	0.070
SE	0.30	1.19	1.39	0.047	0.048	0.042	0.027	0.026	0.018

Pairwise Fst Below diagonal) with 95 % confidence intervals (above diagonal)from Arlequin

	Forest	City	Suburba n
Forest		0.0018, 0.011	0.0026, 0.019
City	0.0064		0.0063, 0.024
Suburban	0.099	0.0144	