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The role of immigration and local adaptation on fine-scale genotypic and phenotypic population divergence in a less mobile passerine

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Abstract

Dispersal and local patterns of adaptation play a major role on the ecological and evolutionary trajectory of natural populations. In this study, we employ a combination of genetic (25 microsatellite markers) and field-based information (seven study years) to analyse the impact of immigration and local patterns of adaptation in two nearby (< 7 km) blue tit (*Cyanistes caeruleus*) populations. We used genetic assignment analyses to identify immigrant individuals and found that dispersal rate is female-biased (72%). Data on lifetime reproductive success indicated that immigrant females produced fewer local recruits than their philopatric counterparts whereas immigrant males recruited more offspring than those that remained in their natal location. In spite of the considerably higher immigration rates of females, our results indicate that, in absolute terms, their demographic and genetic impact in the receiving populations is lower than that in immigrant males. Immigrants often brought novel alleles into the studied populations and a high proportion of them were transmitted to their recruits, indicating that the genetic impact of immigrants is not ephemeral. Although only a few kilometres apart, the two study populations were genetically differentiated and showed strong divergence in different phenotypic and life-history traits. An almost absent inter-population dispersal, together with the fact that both populations receive immigrants from different source populations, is probably the main cause of the observed pattern of genetic differentiation. However, phenotypic differentiation (P_{ST}) for all the studied traits greatly exceeded neutral genetic differentiation (F_{ST}), indicating that divergent natural selection is the prevailing factor determining the evolutionary trajectory of these populations. Our study highlights the importance of integrating individual- and population-based approaches to obtain a comprehensive view about the role of dispersal and natural selection on structuring the genotypic and phenotypic characteristics of natural populations.

Introduction

Dispersal is a life-history trait that plays a major role in the demographic and evolutionary dynamics of species, determining gene flow and the persistence and diversification of populations (reviewed in Clobert *et al.*, 2012). At the individual level, social pressures (e.g. competition among relatives) have been hypothesized

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to be one of the proximate factors prompting dispersal (Greenwood, 1980). This phenomenon may have an adaptive significance, and some studies have found that individuals obtain territories of better quality after dispersal (Calabuig *et al.*, 2008; Valcu & Kempenaers, 2008; García-Navas & Sanz, 2011a). In this sense, several authors have pointed out that breeding dispersal is promoted by breeding failure or low breeding performance (e.g. after occupying a poor-quality territory), which suggests that this decision – disperse or stay – could be closely related to the individual's own breeding experience (Haas, 1998; Calabuig *et al.*, 2008). By moving away from their natal territory, individuals can also reduce the chance of encountering and mating with kin (e.g. Lambin, 1994) and increase the probability of obtaining a genetically less similar partner (van de Casteele & Matthysen, 2006; Ortego *et al.*, 2008). Dispersal – in particular, natal dispersal for its higher frequency and importance on populations – has been suggested as a behavioural mechanism that allows individuals to avoid close inbreeding (see Szulkin & Sheldon, 2008 and references therein). However, in spite of some potential advantages related to dispersal, the movement from natal to breeding areas can also have negative consequences on the fitness of dispersers. Accordingly, some previous studies have shown that the reproductive output of immigrants is often equal or inferior to that of philopatric individuals (Julliard *et al.*, 1996; Verhulst & van Eck, 1996; Wheelwright & Mauck, 1998; Orell *et al.*, 1999; Marr *et al.*, 2002; Hansson *et al.*, 2004; Calabuig *et al.*, 2008, 2010), suggesting that site experience gives native individuals an advantage over immigrants (Pärt, 1995; Bensch *et al.*, 1998). Other factors may also contribute to explaining the existence of potential differences in performance between immigrant and philopatric individuals. For instance, immigrants may have unique genetic adaptations or life-history strategies that may impair their fitness when exposed to a novel environment (i.e. local maladaptation; e.g. Dias & Blondel, 1996; Postma & van Noordwijk, 2005; Nosil *et al.*, 2005). Selection against migrants has been suggested as a powerful force that can lead to a substantial reduction in realized gene flow among populations (Hendry, 2004). Thus, in spite of the fact that dispersal is the proximate cause determining the genetic exchange among populations, immigration cannot be strictly equated with gene flow because the arrival of immigrants into a given population does not always guarantee the successful establishment of the alleles that they carry (Slatkin, 1985; Mallet, 2001; Yu *et al.*, 2010).

The role of gene flow in shaping the genetic composition of populations and its influence on different adaptive processes has been addressed by some authors (see Garant *et al.*, 2007; Garroway *et al.*, 2013). Gene flow can counteract the effect of natural selection by introducing foreign alleles into locally adapted populations, a process that is likely to prevent local population differentiation in response to divergent selection pressures (Slatkin, 1987; Langerhans *et al.*, 2003; Postma & van Noordwijk, 2005). However, the importance of gene flow in preventing the evolution of local adaptations will depend on the strength of natural selection against immigrants and the alleles that they carry (Lenormand, 2002). In this regard, few studies have revealed the existence of intra-specific differentiation on breeding and/or morphological traits at a small scale (but see Blondel *et al.*, 1999; Senar *et al.*, 2006; Ortego *et al.*, 2012), and the primary evidence comes from studies performed at large spatiotemporal scales (García *et al.*, 2008; Milá *et al.*, 2009; Smith *et al.*, 2011; Edelaar *et al.*, 2012) or conducted on islands, where the homogenizing effects of gene flow may be more limited (Losos & Ricklefs, 2009; Bertrand *et al.*, 2013). In spite of the fact that gene flow can potentially prevent the maintenance of local adaptations, this phenomenon is also fundamental to avoid loss of genetic diversity, ensure the viability of small and isolated populations and reduce inbreeding and its negative consequences (Keller & Waller, 2002). Immigration is generally considered as the preponderant process that prevents the loss of genetic diversity, or contributes to its recovery ('genetic rescue') in bottlenecked populations (Keller *et al.*, 2001; Vilà *et al.*, 2003; Ortego *et al.*, 2007). In this sense, a limited number of immigrants can have a strong restorative impact on the genetic and demographic viability of a population (Vilà *et al.*, 2003; Bensch *et al.*, 2006). The arrival of foreign individuals can induce heterosis, and positive selection on immigrant alleles can lead them to be present in future generations at a higher frequency than predicted from neutral expectations (e.g. Ingvarsson & Whitlock, 2000; Ebert *et al.*, 2002; Bensch *et al.*, 2006). Thus, under some circumstances, the influx of new or rare alleles can result in a disparity in fitness between immigrant and local genotypes as already commented earlier (Bensch *et al.*, 2006).

In this study, we combine molecular and capture–mark–recapture data to study the consequences of immigration and local adaptation on fine-scale phenotypic and genotypic divergence between two nearby blue tit populations located close to the southern edge of the species distribution range (Illera *et al.*, 2011). We monitored these populations over seven consecutive years, genotyped nearly all breeding individuals across 25 microsatellite markers and used this information to identify immigrants and local individuals, determine their genetic characteristics and estimate patterns of gene flow. First, we studied the consequences of immigration at the individual level, analysing differences between immigrants and locally born individuals in terms of phenotype, genotype, characteristics of obtained mates and different components of fitness. This allowed us to address the following specific questions: (i) Do dispersing individuals exhibit a superior phenotype or genotype compared to philopatric individuals? (ii) Do dispersing individuals mate with genetically more compatible individuals compared to immigrants? (iii) Do philopatric individuals have a higher fitness in comparison with their philopatric counterparts? Second, we study the impact of dispersal and local patterns of adaptation at the population level in terms of genotypic and phenotypic differentiation and evaluate the underlying evolutionary scenario shaping the observed patterns. A preliminary study showed that the two analysed populations show a virtually non-existent exchange of individuals (Ortego *et al.*, 2011a), and we hypothesize iv) the presence of genetic and phenotypic differentiation between them due to limited dispersal. Finally, v) we study the relative role of genetic drift and natural selection on observed patterns of population differentiation in different quantitative traits (viz. life-history and morphological traits; Merilä & Crnokrak, 2000).

Material and methods

Study area and general field methods

The study was carried out in two nest-box populations located at Quintos de Mora (Montes de Toledo, central Spain, Fig. 1): Gil García (39°22′N 4°07′W) and Valdeyernos (39°26′N 4°05′W). Both study areas are dominated by deciduous oak forest, and each one contains 100 wooden nest boxes available for hole-nesting passerines since 2006. The density of breeding pairs is similar in both woodlots (~4 pairs/ha) while nesting opportunities for blue tits beyond these study plots are expected to be low due to the absence of nest boxes and the shortage of natural cavities (V. García-Navas, pers. obs.). These populations are separated by 7 km (see Fig. 1), and the connecting landscape is an unsuitable breeding habitat for blue tits: a river floodplain dominated by grassland and scattered trees in a dehesa-like configuration (Tornero, 2003). See García-Navas & Sanz (2011b) for more details about the study area.

During seven study years (2007–2013), we monitored the breeding activity of blue tits from early April to mid-June. Frequent inspections of nest boxes allowed us to determine general breeding parameters: laying date, clutch size, hatching success and fledgling success. Routine fieldwork also included the capture of parents while they were feeding their young (day 8 post-hatching) by means of spring traps. Adults were banded with metal rings (if they were not already) and their sex and age (yearling or older breeder) determined based upon the presence/absence of a brood patch and plumage characteristics (see Svensson, 1992), respectively. Birds were weighed to the nearest 0.1 g using an electronic portable balance, and their tarsus length was measured to the nearest 0.01 mm using a digital calliper. The same parameters were measured in nestlings on day 13 after hatching. Each nestling was also marked with an individual metal ring. Blood samples (20–30 μL) from the parents were collected by puncturing the brachial vein and stored on FTA reagent-loaded cards

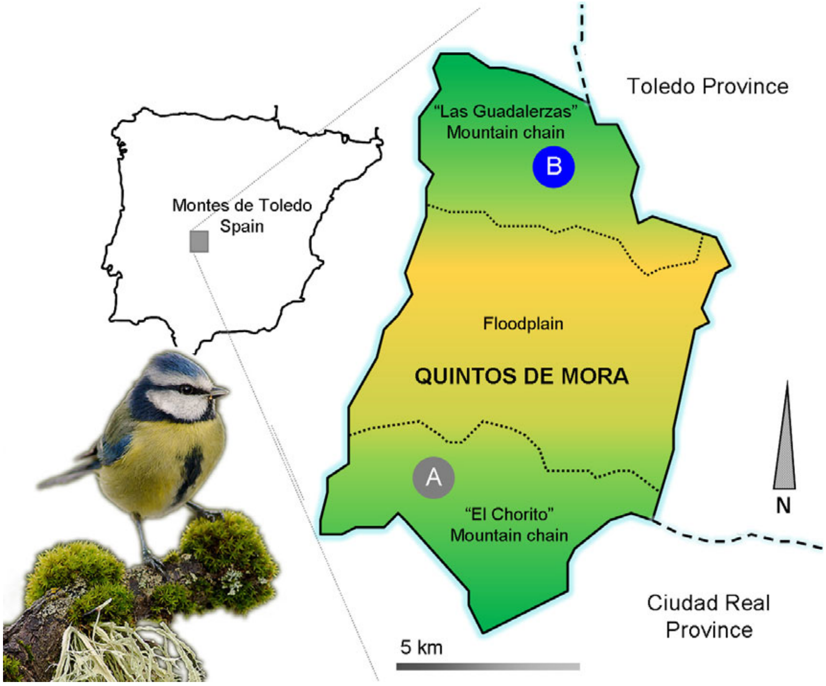

Fig. 1 Map of the study area, which consists of a floodplain (denoted in yellow) flanked by two mountain ranges (denoted in green) in which both populations (A: Gil García, B: Valdeyernos) are located (photograph: J. Caballero).

(Whatman Bioscience, Florham Park, NJ, USA) or in Eppendorf tubes with 96% ethanol. In 2012, a subsample of nestlings (265 from 44 families) was also bled for sex identification.

Molecular analyses

Birds were genotyped at 25 microsatellite markers (see details in Electronic Supplementary Material). Genomic DNA was isolated using a commercial kit (NucleoSpin Blood Kit, Macherey-Nagel GmbH & Co, Duren, Germany). PCRs were carried out in a 10 μL volume containing 1× reaction buffer (67 mM Tris–HCl, pH 8.3, 16 mM $(NH_4)_2SO_4$, 0.01% Tween-20; EcoStart Reaction Buffer, Ecogen, Barcelona, Spain), 2 mM $MgCl_2$, 0.2 mM of each dNTP, 0.15 μM of each dye-labelled primer (FAM, PET, NED or VIC), 0.1 U of *Taq* DNA EcoStart Polymerase (Ecogen) and 1 μL of template DNA. The PCR profile consisted of 9 min of initial denaturing at 95 °C followed by 40 cycles of 30 s at 94 °C, 45 s at the annealing temperature (see Electronic Supplementary Material) and 45 s at 72 °C, ending with a 10-min final elongation stage at 72 °C. Amplification products were run on an ABI 310 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA), and fragment size was determined using GENEMAPPER 3.7 (Applied Biosystems). Nestling sex was determined by PCR amplification of the CHD1-W and CHD1-Z genes using the primers 0057-F and 0002-R (Round *et al.*, 2007). Products were separated on 2% agarose gels that were stained with ethidium bromide and visualized under UV light.

Identification of immigrants

We used the software GENECLASS 2.0 (Piry *et al.*, 2004) to exclude or identify individuals as immigrants following two complementary methods. The first one ('frequency-based procedure') allows the assessment of the probability of each individual that is rejected from reference populations. The second one ('Bayesian assignment procedure') allows us to ascertain population membership of individuals after the inclusion of predefined (or candidate) populations. In other words, this last method allows us to test whether the origin population of a given individual matches with the population in which it was sampled. First, we detected first-generation migrants following the frequency-based method developed by Paetkau *et al.* (2004). Likelihood computation was performed by randomly generating 10 000 genotypes in each population based on their allelic frequencies (Monte Carlo resampling procedure, see Paetkau *et al.*, 2004). Only individuals assigned to one of the two populations with a probability lower than 0.01 were considered as potential immigrants. Complementarily, we also performed an assignment test using the Bayesian method with the leave-one-out option for dealing with missing alleles. Assignment tests calculate the likelihood that the genotype of an individual matches the allelic profile of the population in which it was sampled (L_{home}, Paetkau *et al.*, 1995). Thus, GENECLASS is particularly useful to avoid false positives because it considers the possibility that an individual does not originate from a given sampled source (Sunnucks, 2011). Results obtained from both methods were collated with ringing data gathered in the field (the 'nonringed as nestling' criterion, see Verhulst & van Eck, 1996; Orell *et al.*, 1999). When determining our immigrant pool, we opted for a conservative approximation and we only considered as first-generation immigrants those individuals identified as such by GENECLASS and whose *a priori* population (i.e. the population in which they were sampled) did not match with the most likely population assigned by the program. All unringed adults that were not identified as first-generation immigrants by GENECLASS were not included in the 'immigrant vs. local' analyses, because we cannot rule out the possibility that they are individuals born outside the border of the study plot or unringed nestlings from overlooked natural nests. Thereby, we compared only individuals for whom we had high confidence about their origin: individuals ringed as nestlings and recruited as breeding adults (local individuals) and individuals consistently identified as immigrants using both genetic and capture–mark–recapture methods.

Phenotypic and genotypic characteristics of immigrants vs. locally born individuals

We compared the phenotypic and genotypic characteristics of immigrants and locally born individuals to determine whether dispersing individuals are superior in terms of individual genetic diversity, size or body mass in comparison with those that remain in their natal site. We used homozygosity by loci (*HL*) as an estimate of individual genetic diversity. This measure improves heterozygosity estimates in open populations by weighting the contribution of each locus to the homozygosity value depending on their allelic variability (see Aparicio *et al.*, 2006 for more details). *HL* values were calculated using CERNICALIN, an Excel spreadsheet available at https://sites.google.com/site/joaquinortegolozano/software-1. For morphometric data, we calculated an average value from measures obtained in different years, which were taken by the same observer (VGN) and were highly repeatable ($r_{\text{tarsus}} = 0.67$; $r_{\text{mass}} = 0.48$, P values < 0.01; Lessells & Boag, 1987). Immigrants and locally born individuals (immigrants: Gil García ♀ $n = 26$, ♂ $n = 7$; Valdeyernos ♀ $n = 10$, ♂ $n = 7$; locally born individuals: Gil García ♀ $n = 24$, ♂ $n = 48$; Valdeyernos ♀ $n = 47$, ♂ $n = 48$) were compared using *t*-tests.

Complementarily, we compared pairwise relatedness between immigrants and locally born individuals to ascertain the possible existence of an advantage of immigrants on philopatric individuals in terms of mate quality (viz. genetic similarity). Pairwise relatedness (*r*, Goodnight & Queller, 1999) is an estimate of the coefficient of kinship between two individuals (i.e. the proportion of alleles shared between them) and was computed using the program COANCESTRY (Wang, 2011).

Fitness of immigrants vs. locally born individuals

We compared components of seasonal and lifetime reproductive success between immigrants and locally born individuals. We analysed the following components of seasonal reproductive success (SRS): laying date, clutch size, hatching success (= ratio of number of hatchlings to clutch size), fledgling success (= ratio of number of fledglings to clutch size) and nestling condition (= mean size-corrected body mass of nestlings on day 13). As a measure of lifetime reproductive success (LRS), we used the number of local recruits produced over an individual's lifetime. LRS was determined for all birds that bred in 2007–2011. Those birds that bred for the first time in 2011 might still have a few years ahead to increase the number of recruited young. However, given the short lifespan of this species (mean inter-annual survival rate: ~50%; mean life expectancy: 2 years) and the fact that this source of bias is expected to affect equally both immigrant and philopatric individuals, we included all breeding individuals regardless of whether they were at the beginning or end of their reproductive life. Due to low sample size, we pooled recruitment data from both populations for this particular analysis.

To test for differences in SRS and LRS between immigrants and locally born individuals, we constructed models fitting the origin of individuals (local or immigrant) as a fixed factor. All models were built separately for males and females. In models of SRS, we also considered a series of potential explanatory terms (covariates: laying date, clutch size, brood size, tarsus length; fixed factor: age; see Table 3 for details). We had multiple records for the same individual in different years, so we also included female/male identity and study year as random effects in our models to avoid pseudoreplication. Linear models were carried out with the PROC MIXED module in SAS (SAS Institute, Cary, NC, USA) using a normal error structure and an identity link function. When random effects were present in a model, denominator degrees of freedom for tests of fixed factors were calculated using the Satterthwaite method (Littell *et al.*, 1996). The distribution of the number of recruits resembles a Poisson distribution, and therefore, LRS data were transformed [*log* $(x + 1)$] and analysed by fitting a logarithmic model with Poisson error distribution. We started modelling by entering all independent variables into our null model. All non-significant terms were progressively removed in inverse order of significance until we obtained a final model that included all predictors whose addition improved model fit.

We also analysed potential differences in inter-annual survival rates between immigrants and locally born individuals. We estimated adult survival to the next breeding season during 2007–2013 using the Cormack–Jolly–Seber (CJS) method as implemented in MARK (White & Burnham, 1999). Our predictions of interest focused on the effects of origin (immigrant vs. locally born) on survival probabilities. Therefore, to test the relative importance of adult origin on survival probability, we compared the fit of survival modelled as a function of this factor vs. a model considering time dependence in survival probability. See Electronic Supplementary Material for more details.

Genetic and phenotypic population divergence

We examined inter-population differentiation at neutral loci and phenotypic traits to determine whether limited dispersal between these populations results in discernable genetic and/or phenotypic differentiation between them. The amount of genetic differentiation between populations was quantified using Weir and Cockerham's standardized F_{ST} (Weir & Cockerham 1984). We calculated the pairwise F_{ST} value between the two studied populations and tested its significance with a Fisher's exact test after 9 999 permutations as implemented in GENALEX version 6.5 (Peakall & Smouse, 2012). For each locus and for each population, we also determined the number of private alleles (alleles found in only one population) and allelic richness using GENALEX 6.5 and FSTAT 2.9.3 (Goudet, 2002). In addition, we examined the proportion of immigrants that carry novel (those observed in the population for the first time) or rare (frequency < 2%) alleles into our study populations and the frequency with which they are transferred to their recruiting progeny. Subsequently, we tested for differences in phenotypic traits (reproductive and morphological traits) between the two populations. Only locally born individuals were included in these analyses.

Finally, we compared F_{ST} and Q_{ST} ($\sim P_{ST}$) estimates to examine the relative role of natural selection and genetic drift in explaining the potential existence of variation in life-history and morphological traits between the two studied populations (Merilä & Crnokrak, 2000; Brommer, 2011; Oneal & Knowles, 2013). F_{ST} estimates the extent of population genetic differentiation, and Q_{ST} is an analogous measure of differentiation in quantitative genetic traits (Spitze, 1993). Q_{ST} estimates can be approximated by the phenotypic divergence in a trait across populations (P_{ST} or 'phenotypic' Q_{ST}). We quantified phenotypic differentiation (P_{ST}) for three typical quantitative traits: laying date, clutch size and tarsus length. This approach (F_{ST}–P_{ST} comparison) allows us to study local adaptation and to test the relationship between molecular and quantitative genetic variation among populations. When $F_{ST} = P_{ST}$, there is no evidence for geographically varying natural selection, and the relative effects of drift and selection on population differentiation cannot be separated. Higher relative divergence in quantitative traits than in neutral markers ($F_{ST} < P_{ST}$) is indicative of directional selection favouring different phenotypes in different populations, whereas if $F_{ST} > P_{ST}$, it means that the same phenotypes are favoured in different populations such that divergence in additive genes is smaller than expected on the basis of neutral divergence (i.e. stabilizing selection). P_{ST} values were estimated as described in McKay & Latta (2002). We tested the difference between Q_{ST} and F_{ST} by comparing Q_{ST} values with the distribution of values of F_{ST} (Whitlock, 2008). See Electronic Supplementary Material for more details.

Results

Immigration patterns

A total of 675 adults were genotyped at 25 microsatellite markers and included in our initial genotype data set used for the identification of immigrants. We detected 50 first-generation immigrants using GENECLASS. We found that 92% (46/50) of individuals identified as first-generation immigrants using the frequency method were detected as ‘assignment mismatches’ in the population assignment analysis, that is, individuals assigned to a population other than the one in which they were sampled. Thus, the degree of agreement between the two methods was high. Except for two cases, all individuals identified as immigrants involved unringed birds. These exceptions corresponded to two cases of dispersal between the studied populations: one female ringed as nestling in Valdeyernos and captured as breeding adult in Gil García and a male that dispersed from Gil García to Valdeyernos. Both cases were identified as nonlocal individuals using both first-generation immigrants and assignment mismatch analyses in GENECLASS. Immigration was female-biased (binomial test, $P < 0.01$), with immigrants accounting for 10% (36/359) of females and 5% (14/316) of males present in the studied populations (Table 1). The immigration rate for Gil García was higher than that reported for Valdeyernos (10% vs. 4%; Table 1).

Phenotypic and genotypic characteristics of immigrants vs. locally born individuals

Immigrant females arriving to Gil García present slight morphological differences with respect to local females (Table 2a). We found that immigrant females are marginally larger and tend to show a better body condition than those locally born (Table 2a). In Valdeyernos, there was no evidence that immigrant birds present any phenotypic difference with respect to locally born individuals (Table 2a). In Gil García, immigrant females tended to be more heterozygous than local females (Table 2a). We did not find a significant difference between immigrant and local males with respect to their level of genetic diversity (Table 2a). Immigrants and locally born individuals settled in Valdeyernos did not differ in terms of genetic diversity (see Table 2a).

Regarding mate relatedness, our results indicate that immigrant birds did not mate with more genetically dissimilar individuals than those who did not disperse and breeding in their natal area in any of the studied populations (Table 2a).

Fitness of immigrants vs. locally born individuals

In Gil García, immigrant males seemed to do better than locally born individuals in terms of offspring quality: nestlings raised by immigrant males fledged in better condition than those raised by their local counterparts (Table 2b). We did not find differences with respect to the bird origin (local or immigrant) for the rest of the analysed parameters in Gil García (Table 2b). The performance of female immigrants in Valdeyernos differed from natives in terms of clutch size; they laid on average 0.58 more eggs (Table 2b). The remaining SRS parameters were similar for immigrants and locally born individuals (Table 2b).

When analysing survival data, we found that the model including origin of individuals as factor did not have a better fit to the data than the time-dependent model (Gil García: ΔAIC, 288.34 – 290.60 = −2.26; Valdeyernos: ΔAIC, 367.34–367.92 = −0.58). Thus, adult origin (immigrant; locally born) did not have a relevant effect on survival probabilities.

The number of local recruits ranged between 0 and 3 offspring and did not differ between origin categories (Wald $\chi^2 = 0.01$, $P = 0.96$). However, there was a significant origin × sex interaction (Wald $\chi^2 = 4.83$, $P = 0.03$). Post hoc comparisons revealed that immigrant females produced a lower number of local recruits than philopatric females (mean number of local recruits, immigrants: 0.14 ± 0.08, locally born: 0.46 ± 0.09; $t_{103} = -1.99$, $P = 0.048$), whereas males exhibited the opposite pattern (immigrants: 0.69 ± 0.24, locally born: 0.25 ± 0.06; $t_{115} = 2.37$, $P = 0.02$). When analysing both populations separately, we obtained very similar results; there was no difference in LRS between immigrants and locally born individuals in any of the two study areas (Gil García: Wald $\chi^2 = 0.30$, $P = 0.58$; Valdeyernos: Wald $\chi^2 = 0.02$, $P = 0.89$), but the origin × sex interaction remained significant (or marginally significant) for both populations (Gil García: Wald $\chi^2 = 4.80$, $P = 0.03$; Valdeyernos: Wald $\chi^2 = 3.77$, $P = 0.052$). The number of local recruits produced by immigrant males

Table 1 Number of immigrants arriving at each population over the study period. Only new settlements are listed, and thus, the total pool of immigrants for each year was greater than indicated, that is, it was composed of new settlements or 'newcomers' plus those individuals who arrived in previous years and survived to the following breeding season.

	Gil García				Valdeyernos			
	Immigrants				Immigrants			
Year	Males	Females	Total	Rate	Males	Females	Total	Rate
2007	0	3	20	0.15	4	1	25	0.02
2008	2	6	66	0.12	2	5	79	0.09
2009	1	3	67	0.06	0	0	72	0
2010	1	6	73	0.10	0	0	56	0
2011	2	4	72	0.08	1	0	43	0.02
2012	1	4	56	0.09	0	4	46	0.09
Overall	7	26	354	0.10	7	10	321	0.07

was significantly larger than that of immigrant females ($t_{40} = 2.63$, $P = 0.01$). In absolute terms, immigrant males produced the double of local recruits (8 vs. 4) than immigrant females.

Genetic and phenotypic population divergence

The F_{ST} value between the two studied populations across the 25 typed loci and only considering locally born individuals was significantly different from zero ($F_{ST} = 0.033$, $P = 0.01$). F_{ST} values calculated separately for each locus ranged between 0.002 and 0.095 and were significantly different from zero in all typed loci ($P < 0.01$) but one (*Pca4*, $F_{ST} = 0.002$, $P = 0.06$).

Thirty-one of 50 (62%) individuals identified as immigrants carried new or rare alleles. Female immigrants carried the majority of novel or rare alleles (23/33, 69.7%), but the proportion of male immigrants carrying new or rare alleles was slightly greater than that of the females (males: 10/14, 71.4%; females: 23/36, 63.8%). Immigrants introduced into the studied populations seven novel alleles from six loci (*Pdo5*, *Pca2*, *Pca3*, *Pca7*, *Pca8* and *CcaTgu28*), of which three (two from two males and one from one female) were transmitted to the recruiting offspring ($n = 12$ descendants from immigrants). The occurrence of novel or rare alleles within the pool of immigrants did not vary significantly between populations (Gil García: 22/34, 64.7%; Valdeyernos: 10/17, 58.8%; Chi-square = 0.16, $P = 0.68$). Most of these new or rare alleles (21/33, 63.6%) were specific to each population, that is, they constituted private alleles (see Electronic Supplementary Material). Multilocus F_{ST} data revealed a marginally significant genetic differentiation between immigrants arriving to Gil García and those settled in Valdeyernos ($F_{ST} = 0.006$, $P = 0.059$), which suggests that the source populations of immigrants for each of the two studied population are genetically differentiated (see also Electronic Supplementary Material).

We found significant differences in several phenotypic and reproductive traits between locally born individuals from the two studied populations (summarized in Table 3). Both adult and nestling males from Gil García had larger tarsi in comparison with those from Valdeyernos (Table 3). Clutches were earlier (around 6 days) and smaller in Gil García than in Valdeyernos (Table 3). Mean nestling condition at day 13 after hatching also differed significantly between populations, being lower in Gil García than in Valdeyernos (Table 3). Differences in mean values for nestling measurements can be partially explained taking into account that offspring sex ratio (estimated as the proportion of males) was higher in Gil García than in Valdeyernos (Table 3) and that nestling blue tits are sexually dimorphic (males being larger than females; Table 3, see also Mainwaring *et al.*, 2011).

Comparison of genetically based quantitative trait differentiation (P_{ST}) with its expectation under neutrality (F_{ST}) revealed evidence of divergent selection ($P_{ST} \gg F_{ST}$). When considering the null assumption where $g/h^2 = 1$ (i.e. the proportion of variation due to additive genetic effects between populations, g, equals the proportion within populations h^2), we obtained higher P_{ST} estimates (P_{ST}, tarsus length: 0.66, laying date: 0.78, clutch size: 0.78) than the global F_{ST}. For all traits (tarsus length, laying date and clutch size), the estimates for P_{ST} were higher than the global F_{ST} when altering the assumptions about heritability and the magnitude of additive genetic proportion (see Electronic Supplementary Material). We compared the values of P_{ST} with the expected distribution of F_{ST} *sensu* Whitlock (2008). The mean bootstrap value of F_{ST} ($F_{ST} = 0.032$) was very similar to the observed mean, which indicates that P_{ST} values for the traits are significantly different from the distribution of neutral F_{ST} values. Our results thus were robust to conclude that neutral genetic differentiation is not sufficient to explain geographical differentiation in some qualitative traits and suggest adaptation to local different conditions in the presence of gene flow.

Discussion

Genetic assignment methods and capture–mark–recapture data indicate that both populations show a moderate rate of annual immigration. When comparing the characteristics of immigrant and philopatric individuals, we found that immigrant females tend to be more heterozygous and larger than resident-hatched females in one of our studied populations. A few studies have shown that dispersers possess a higher phenotypic or genotypic quality than philopatric individuals, which could make them more skilled to travel (e.g. if genome-wide heterozygosity increases exploratory and dispersal propensity) or allow them to face the costs

Table 2 Differences (mean ± SE) in (a) phenotypic and genotypic characteristics, and (b) seasonal reproductive success between immigrants and locally born individuals. Some individuals contributed data in multiple years (Gil García: ♀ immigrants n = 49, ♀ locally born n = 45; ♂ immigrants n = 13, ♂ locally born n = 90, Valdeyernos: ♀ immigrants n = 13, ♀ locally born n = 101; ♂ immigrants n = 11, ♂ locally born n = 108) except for those variables denoted with an asterisk (see Material and methods). Only statistics for the main effect (female/male origin) are shown. Final models including all the explanatory terms are provided as Electronic Supplementary Material.

		Females				Males			
Population	Trait	Locally born	Immigrants	Test	P	Locally born	Immigrants	Test	P
Gil García	(a) Individual characteristics								
	Mean adult size (mm)*	15.59 ± 0.41	15.86 ± 0.55	t_{50} = 1.93	0.058	15.96 ± 0.50	16.09 ± 0.58	t_{53} = −0.61	0.21
	Mean adult body mass (g)*	9.65 ± 0.60	9.93 ± 0.48	t_{50} = 1.81	0.07	9.25 ± 2.00	9.76 ± 0.72	t_{53} = −0.66	0.51
	Genetic diversity (HL)*	0.237 ± 0.08	0.195 ± 0.08	t_{50} = −1.84	0.07	0.214 ± 0.09	0.260 ± 0.08	t_{53} = −1.26	0.21
	Pairwise relatedness	−0.026 ± 0.02	−0.016 ± 0.02	$F_{1,36.4}$ = 0.30	0.58	0.009 ± 0.02	−0.038 ± 0.07	$F_{1,69.5}$ = 0.28	0.60
	(b) SRS measures								
	Laying date (1 = 1st April)	17.44 ± 1.63	14.57 ± 1.56	$F_{1,42.2}$ = 2.58	0.11	16.10 ± 1.04	16.45 ± 3.26	$F_{1,65.1}$ = 0.14	0.70
	Clutch size	7.47 ± 0.26	7.42 ± 0.25	$F_{1,42.5}$ = 0.22	0.64	7.44 ± 0.17	7.54 ± 0.53	$F_{1,57.1}$ = 0.10	0.75
	Hatching success (%)	76.26 ± 3.54	75.55 ± 3.47	$F_{1,39.1}$ = 0.11	0.74	79.71 ± 1.77	81.83 ± 5.56	$F_{1,64.9}$ = 0.13	0.72
	Fledgling success (%)	71.34 ± 4.77	65.60 ± 4.72	$F_{1,41.8}$ = 0.80	0.37	76.56 ± 2.30	75.26 ± 7.82	$F_{1,46.6}$ = 0.67	0.42
	Mean nestling mass (g)	10.09 ± 0.11	10.06 ± 0.12	$F_{1,38.6}$ = 0.02	0.88	10.07 ± 0.06	10.31 ± 0.20	$F_{1,56.3}$ = 4.03	0.048
Valdeyernos	(a) Individual characteristics								
	Mean adult size (mm)*	15.48 ± 0.52	15.47 ± 0.55	t_{55} = 0.05	0.96	15.90 ± 0.54	15.98 ± 0.96	t_{53} = −0.30	0.76
	Mean adult mass (g)*	9.58 ± 1.55	9.24 ± 0.33	t_{55} = 0.69	0.49	9.95 ± 0.65	9.45 ± 0.76	t_{53} = 1.74	0.09
	Genetic diversity (HL)*	0.193 ± 0.09	0.213 ± 0.09	t_{55} = 0.60	0.55	0.212 ± 0.09	0.199 ± 0.08	t_{53} = 0.32	0.75
	Pairwise relatedness	−0.031 ± 0.15	−0.008 ± 0.09	$F_{1,45.5}$ = 0.01	0.96	−0.027 ± 0.15	−0.031 ± 0.15	$F_{1,45.2}$ = 0.09	0.93
	(b) SRS measures								
	Laying date (1 = 1st April)	19.98 ± 1.03	23.76 ± 2.71	$F_{1,92.7}$ = 2.19	0.14	20.63 ± 0.95	17.46 ± 2.65	$F_{1,44.1}$ = 0.06	0.80
	Clutch size	8.03 ± 0.19	8.61 ± 0.51	$F_{1,65.7}$ = 4.55	0.036	8.22 ± 0.16	8.54 ± 0.44	$F_{1,57.4}$ = 0.02	0.90
	Hatching success (%)	76.26 ± 3.54	75.55 ± 3.47	$F_{1,40.8}$ = 2.33	0.13	79.71 ± 1.77	81.83 ± 5.56	$F_{1,100}$ = 0.07	0.78
	Fledgling success (%)	71.34 ± 4.77	65.60 ± 4.72	$F_{1,87}$ = 1.62	0.21	76.56 ± 2.30	75.26 ± 7.82	$F_{1,46.6}$ = 0.06	0.80
	Mean nestling mass (g)	10.11 ± 0.08	10.02 ± 0.22	$F_{1,50.3}$ = 0.03	0.85	10.09 ± 0.06	10.02 ± 0.17	$F_{1,46.9}$ = 0.27	0.61

Table 3 Differences (mean ± SE) in morphological and reproductive traits between the two studied populations. Only local recruits were included in these analyses (see Material and methods).

Trait	Gil García	Valdeyernos	Test	*P*
Female adult size (mm)	15.50 ± 0.06	15.46 ± 0.10	$F_{1,73} = 0.11$	0.73
Female nestling size on day 13 (mm)	15.54 ± 0.11	15.37 ± 0.06	$F_{1,128} = 1.51$	0.22
Female adult mass (g)	9.79 ± 0.08	9.71 ± 0.13	$F_{1,71} = 0.27$	0.60
Female nestling mass on day 13 (g)	10.84 ± 0.13	10.09 ± 0.06	$F_{1,127} = 21.41^{a}$	<0.001
Male adult size (mm)	16.12 ± 0.08	15.89 ± 0.07	$F_{1,98} = 5.60$	0.03
Male nestling size on day 13 (mm)	16.14 ± 0.13	15.76 ± 0.05	$F_{1,131} = 7.30$	<0.01
Male adult mass (g)	9.65 ± 0.08	9.98 ± 0.07	$F_{1,97} = 9.25^{a}$	<0.01
Male nestling mass on day 13 (g)	11.09 ± 0.16	10.34 ± 0.06	$F_{1,130} = 5.85^{a}$	0.02
Laying date (1 = 1st April)	14.07 ± 11.34	20.46 ± 11.11	$F_{1,49.8} = 8.61^{b}$	<0.01
Clutch size	7.51 ± 1.68	8.02 ± 1.68	$F_{1,128} = 17.39^{c}$	<0.001
Hatching success (%)	75.65 ± 20.55	81.19 ± 22.56	$F_{1,132} = 1.52^{c}$	0.22
Fledgling success (%)	66.30 ± 28.93	78.16 ± 27.12	$F_{1,139} = 3.58^{d}$	0.06
Mean nestling size (mm)	15.73 ± 0.04	15.54 ± 0.05	$F_{1,122} = 1.56^{d}$	0.21
Mean nestling mass (g)	9.99 ± 0.07	10.13 ± 0.07	$F_{1,122} = 11.51^{e}$	<0.001
Sex ratio (male/female ratio, %)	32.46 ± 23.65	53.81 ± 25.36	$F_{1,42} = 5.70$	0.02

After controlling for: (a) tarsus length, (b) female age, (c) laying date and female age, (d) laying date and brood size, (e) laying date, brood size and tarsus length. Female/male identity and study year were included as random effects except for adult size and condition (only one value or an average value per individual).

associated with dispersing long distances. Our data are concordant with that previously reported in Eurasian eagle-owls (*Bubo bubo*; Delgado *et al.*, 2010), naked mole-rats (*Heterocephalus glaber*; O'Riain *et al.*, 1996) and roe deer (*Capreolus capreolus*; Debeffe *et al.*, 2012), finding that heavier individuals are more likely to disperse than lighter individuals. A genotypic and phenotypic superiority of immigrants may contribute to offset the energy constraints related to dispersal and reduce the potential costs associated with the lack of site experience that they might face when settling in a new breeding place. Dispersal has been also suggested as a mechanism that allows individuals to avoid mating with relatives (Szulkin & Sheldon, 2008; Ortego *et al.*, 2010). However, we did not find significant differences in mate genetic relatedness between immigrants and philopatric individuals although the former are expected to come from somehow genetically differentiated populations and a large proportion of them carry novel alleles.

In concordance with the general pattern of female-biased dispersal in birds, we observed that immigrants were more frequent among females (Greenwood, 1980; Orell *et al.*, 1999; Mabry *et al.*, 2013; but see Verhulst & van Eck, 1996). Data on LRS indicate that immigrant females produced fewer local recruits than philopatric females, whereas immigrant males recruited more offspring than those that remained in their natal area. In absolute terms, our results also indicate that the demographic and genetic impact of females in the receipting populations is lower than that of immigrant males. Thus, the importance of female immigrants in terms of their contribution to future generations and population dynamics is lower than expected according to the observed immigration rate and indicates that sex-biased dispersal does not necessarily equal sex-biased realized gene flow (McCleery & Clobert, 1990; Verhulst & van Eck, 1996; Orell *et al.*, 1999; Prugnolle & de Meeus, 2002; Li & Merilä, 2010). The fact that immigrant males raise heavier nestlings than locally born individuals in one of the study populations suggests that the former may settle in better territories and produce higher-quality offspring, which could increase their survival prospects after fledgling and explain the observed patterns of local recruitment (Monrós *et al.*, 2002; see also Orell *et al.*, 1999; Julliard *et al.*, 1996). The lower LRS of immigrant females in comparison with those locally born is counterintuitive with the fact that the former laid larger clutches in one of the studied populations. Unfamiliarity with breeding habitat and overestimation of local resources could result in immigrant being *too optimistic* when making decisions related to reproductive investment (Dhondt *et al.*, 1990; Dias & Blondel, 1996; Postma & van Noordwijk, 2005), which could explain the discrepancy between the higher fecundity of immigrant females and their poorer performance in terms of number of recruited offspring. The low recruitment rate exhibited by female immigrants may be linked to a shorter lifespan (Verhulst & van Eck, 1996; Pärn *et al.*, 2009; but see Clobert *et al.*, 1988; Hansson *et al.*, 2004). However, we found that adult survival was not associated with adult origin. An alternative hypothesis to explain this finding is that immigrant females may be more likely to produce young that disperse over large distances before establishment (Orell *et al.*, 1999). Regarding this, it has been suggested that LRS estimates of dispersing and philopatric individuals may be subject to systematic biases due to within-individual consistency and parent–offspring resemblance in dispersal behaviour, which may have a genetic basis (Dingemanse *et al.*, 2002; Pasinelli *et al.*, 2004; Korsten *et al.*, 2010). Unfortunately, we have no information about the frequency with which young travelled beyond the limits of our study area, which could lead to an underestimation of LRS estimates of dispersing parents (Bélinchon *et al.*, 1996; Doligez & Pärt, 2008).

Capture–mark–recapture data showed that individuals very rarely travelled the 7 km that separates the two studied populations, and microsatellite data also indicate that gene flow between them is restricted (see also Ortego *et al.*, 2011a). We found a significant genetic differentiation between the two populations, with a pairwise F_{ST} value (0.03) similar to that reported in a previous study analysing patterns of genetic structure between a mainland population of blue tit (the nominal form *C. c. caeruleus*) and Corsican populations of the *C. c. ogliastrae* subspecies ($F_{ST} = 0.03–0.04$) separated ~ 400 km apart (Porlier *et al.*, 2012). Thus, the observed level of genetic differentiation reported here is much higher than expected on the basis of the short distance that separates our study populations (7 km). In spite of their geographical proximity, the suboptimal habitat separating both populations could prevent dispersal between them or increase the energy expenditure and risk of predation as a result of having to travel through an unsuitable matrix (Harris & Reed, 2002; McRae, 2006; Smith & Batzli, 2006; Baguette & Van Dyck, 2007). Unfavourable or inhospitable habitats may restrict dispersal and lead to genetic differences between populations separated by short distances (Postma *et al.*, 2009; Oliveras de Ita *et al.*, 2012).

When analysing population divergence in phenotypic and life-history traits, we found significant differences in terms of male and offspring body size, female fecundity and offspring sex ratio. To the best of our knowledge, few studies have previously reported a similar pattern of differentiation at such fine spatial scale, and most have been carried out considering insular populations (Chan & Arcese, 2003) or subpopulations differing in habitat quality and leading to asymmetric or nonrandom gene flow (Shapiro *et al.*, 2006; Camacho *et al.*, 2013). In a recent study with forest thrushes (*Turdus lherminieri*) on Guadeloupe (Lesser Antilles), differences in body size were found between forest thrush populations from the western and eastern part of the island (Arnoux *et al.*, 2013). A similar case was reported on Reunion Island where Mascarene grey white-eyes (*Zosterops borbonicus*) inhabiting the highlands were shown to be larger than those inhabiting the lowlands (Milá *et al.*, 2010). In previous studies on Corsican blue tits, Blondel and colleagues revealed the existence of marked differences in morphological traits (wing and tarsus length, body mass) between mainland and insular populations from 25 to 40 km apart (Blondel *et al.*, 1999). This scenario differs from that reported here because in addition to the inter-landscape variation (mainland vs. island), there is also an inter-habitat variation (evergreen vs. deciduous woodland) (Blondel *et al.*, 1999; Blondel & Charmantier, 2006). According to the 'divergence-with-gene flow' model of speciation (Maynard Smith, 1966), divergent selection pressures may lead to local population differentiation because habitat-specific selection regimes are presumably strong enough to outweigh the effects of gene flow (Lambrechts *et al.*, 1997; Schluter, 2001; Porlier *et al.*, 2012), a pattern that has been reported in previous studies (Postma & van Noordwijk, 2005; Senar *et al.*, 2006). Accordingly, our comparison of quantitative and molecular genetic variation suggests that the studied traits are differentiated between populations to a greater extent than expected due to genetic drift alone. Evidence of phenotypic differentiation exceeding the level of genetic divergence in birds is primarily based on large-scale studies (e.g. see Leinonen *et al.*, 2008 for a review), and to the best of our knowledge, this is the first study to show evidence of divergent selection between populations located a very short distance from each other. However, a number of important caveats underlie P_{ST}–F_{ST} comparison (Kekkonen *et al.*, 2012); it means that our inferences regarding local adaptation as a causal factor behind the observed geographical variation should be interpreted cautiously, and hence, such conclusions remain tentative.

Overall, our study shows that the impact of immigrant males is higher than that of females in terms of relative and absolute number of recruited offspring, indicating that the most dispersing sex does not necessarily contribute more to realized gene flow. To the best of our knowledge, this is the first study showing such counter-intuitive disparity between observed sex-biased dispersal and its consequences on demography and realized gene flow. Immigrants carried novel alleles into the receiving populations, and a considerable proportion of them (42.9%) were effectively transmitted to their recruiting offspring, which suggests that the genetic impact of immigrants is not ephemeral. The continuous influx of immigrants may thus contribute to maintain local levels of genetic diversity even when the absolute number of immigrant's offspring recruiting into the population is small (e.g. Vilà *et al.*, 2003; Ortego *et al.*, 2007, 2011b). A low rate of inter-population dispersal, together with the fact that both populations receive immigrants from genetically differentiated source populations, has probably resulted in the observed pattern of genetic differentiation. However, inter-population phenotypic differentiation exceeds the level of genetic differentiation, indicating that the impact of divergent natural selection is not counterbalanced by the homogenizing effects of gene flow. Our study highlights the importance of integrating individual- and population-based approaches to understand the importance of immigration and local selective regimes in driving fine-scale genetic and phenotypic divergence.

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Appendix S1 Estimation of survival probabilities using CJS mark-recapture models in MARK.

Appendix S2 Differences in genetic quality and phenotypic traits between immigrants arriving to both populations.

Appendix S3 Phenotypic differentiation between populations in comparison with the neutral expectations for divergence (F_{ST} - P_{ST} comparisons).

Table S1 Survival models (binomial error distribution and logit link function) for both blue tit populations

Table S2 Percentage (%) of immigrant and locally-born individuals that (a) survived to the following breeding season.

Table S3 P_{ST} values for tarsus length, laying date and clutch size under different assumptions.

Table S4 Panel of 25 microsatellite markers used in the present study.

Table S5 GLMMs for genetic similarity and fitness-related traits in relation to origin of adult blue tits breeding at Gil García.

Table S6 GLMMs for genetic similarity and fitness-related traits in relation to origin of adult blue tits breeding at Valdeyernos.

Figure S1 Tarsus length resemblance estimated by parent-offspring regressions in the two studied blue tit populations.

Figure S2 P_{ST} sensitivity analysis for variable estimates of the additive genetic proportion of among population differences in laying date, clutch size and tarsus length (g).

Figure S3 P_{ST} sensitivity analysis for variable estimates of the additive genetic proportion of among population differences in tarsus length (g) calculated using three different heritability estimates.

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