Local differentiation in the presence of gene flow in the citril finch *Serinus citrinella*

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It is well known theoretically that gene flow
can impede genetic differentiation between
populations. In this study, we show that in a
highly mobile bird species, where dispersal is
well documented, there is a strong genetic and
morphological differentiation over a very short
geographical scale (less than 5 km). Allocation
tests revealed that birds caught in one area
were assigned genetically to the same area
with a very high probability, in spite of
current gene flow. Populations were also mor-
phologically differentiated. The results suggest
that the relationship between gene flow and
differentiation can be rather complicated and
non-intuitive.

Keywords: gene flow; genetic differentiation;
morphological differentiation; dispersal; finches

1. INTRODUCTION

Populations are unlikely to adapt locally if there is
strong gene flow from other populations with different
environmental conditions, which creates a conflict
between gene flow and local differentiation (Mayr
1963). Despite this, population divergence with gene
flow has been observed in several taxa. These cases
share common features. One set of studies show
divergence at small spatial scales, where movements
are not necessarily restricted by physical barriers
(Blondel *et al.* 1999; Postma & Van Noordwijk 2005;
Garant *et al.* 2005). These populations can broadly
be described as parapatric but at different spatial
scales. Another set of studies concern populations
that spend most of their time in allopatry (ecotypes),
but become sympatric during breeding (e.g. Skulason
et al. 1999), hence maximizing probabilities for gene
flow. However, in both of these cases, selection within
habitats can overcome gene flow if there is a long
period of selection relative to gene flow. This indicates
that the timing between selection and gene flow can
be important in creating differences in spite of gene
flow. Based on this, we should hypothesize that
populations that face diversifying selection at the time
of gene flow should not be able to differentiate to the
same extent.

The citril finch (*Serinus citrinella*) is a small (12 g)
granivorous non-territorial Cardueline finch breeding
at high densities in the Pyrenees. In this area, the
interaction between a Mediterranean influence and
high altitudes creates a significant contrast between
north- and south- facing slopes, so that patches of
highly differentiated habitats appear at very close
proximity. Populations leave the breeding areas in
winter and gather at overwintering sites, where they
are found together. In spring, they leave the over-
wintering areas and again settle in the different
subalpine habitats. We studied two populations that
differ significantly in survival rate, breeding success
and body mass, and where there is also substantial
dispersal between the populations (Senar *et al.* 2002).
This means that the citril finch populations spend a
substantial part of the year in sympatry, but diverge
only in terms of breeding habitat choice. The citril
finch is, therefore, an ideal model species for a study
of divergence with gene flow, since the periods of
selection and gene flow (breeding) are the same.

2. MATERIAL AND METHODS

We studied two populations in different habitats in the Spanish
Pyrenees, both situated at high altitudes (2000 m), but on two sides
of a mountain slope differing in direction and vegetation structure
(Senar *et al.* 2002). La Vansa (42°12′ N, 1°35′ E) faces north and is
moist and cool, and has a high abundance of mountain pines (*Pinus
uncinata*), which is a main food source for citril finches. La Bofia
(42°10′ N, 1°32′ E) is situated 5 km from La Vansa and faces
south, is drier and sunnier and has a lower abundance of pines.
Citril finches were trapped with mist nets at drinking vessels and
individually marked. We recorded sex and age, and measured wing
and tarsus lengths (Borràs *et al.* 1998). Morphological analyses are
based on data obtained from 1996 to 2003 (Bofia: $n=798$; Vansa:
$n=1.509$). We extracted DNA from blood samples from birds
captured at random during 1998 in the field in the two locations
($n=19$ from each population) by means of Chelex extraction. We
only used blood from breeding adult birds, therefore avoiding
catching family groups.

For microsatellite analysis, we used the following primers: *Lox1,
HrU2, HrU7, Pocc6, Ppi2, Pca2, Pca9* (Primmer *et al.* 1996; Piertney
et al. 1999; Dawson *et al.* 2000). These primers were amplified
using a PCR-cycle of 94 °C for 2 min, followed by 30×(94 °C for
30 s, 50 °C for 30 s, 72 °C for 30 s), and 72 °C for 10 min using
standard PCR conditions.

The microsatellite data were analysed using the GENEPOP
(Raymond & Rousset 1997) and ARLEQUIN software (Schneider
et al. 2000) for estimation of heterozygosity, test of Hardy–
Weinberg (HW) equilibrium, F_{ST}-values and test for genetic
differentiation. We performed several different multilocus assign-
ment tests, which all differ in terms of method and assumptions.
We did this to make sure that our conclusions are robust for details
regarding algorithms and assumptions. We used three different
approaches based on an extensive resampling of individuals and a
calculation of genotype likelihoods from these resampled datasets,
using GENECLASS2 (Paetkau *et al.* 2004). We also used the Bayesian
clustering method developed by Pritchard *et al.* (2000) and
implemented in the software STRUCTURE. Briefly, this method
assigns individual genotypes to different populations based on the
allele frequency distributions in each population and returns
probabilities of the assignment of a genotype to the different
populations. We also used the software DOH (http://www2.ualberta.
ca/jbrzusto/Doh.php) that calculates an assignment index with the
highest probability of an individual's genotype in any population.
Finally, we used MIGRATE 1.7.6 (Beerli & Felsenstein 2001) to
estimate $M=m/\mu$ (where m is the migration rate), simultaneously,
with effective population size ($N_e\mu$). This program was run 10
times to secure accurate estimates.

3. RESULTS

The number of alleles differed between different loci
and between populations, as did the frequencies of
alleles. In La Vansa, three loci (*Lox1, HrU7, HrU2*)
differed from HW expectations, which show a

heterozygote deficiency. In La Bofia, two loci (*HrU7*, *HrU2*) differed from HW expectations; in all cases, we found a heterozygote deficiency. La Vansa had a slightly higher level of heterozygosity (mean ± s.e.m. = 0.39 ± 0.067) than La Bofia (mean ± s.e.m. = 0.27 ± 0.074), but the difference was not significant ($p > 0.24$, Mann–Whitney U-test).

Rates of gene flow were low between the two populations based on simulations using BAYESASS and MIGRATE. The gene flow from La Bofia to La Vansa was 0.023 (s.e. = 0.039) and from La Vansa to La Bofia was 0.013 (s.e. = 0.020). The estimates of effective population sizes were highly consistent among runs in MIGRATE (La Vansa $N_e\mu = 0.224$ (CV = 0.11); La Bofia $N_e\mu = 0.305$ (CV = 0.13); mean values over 10 runs), with La Bofia having the larger effective population size in all runs. Taken together this means that gene flow (N_em) can be estimated to be around five to ten individuals every generation in each direction, assuming a mutation rate of 10^{-3}.

Most of the genetic variation was found within individuals (62.9%), but 9.4% was found among populations, which was highly significant (locus-by-locus AMOVA, $p = 0.002$), corresponding to an F_{ST} of 0.093 ($R_{ST} = 0.043$). An exact test of population differentiation using GENEPOP gave the same result (combined $\chi^2_{14} = 52.89$, $p < 0.001$). The different population assignment programs all classified individuals to their original population to a very large extent, with the number of correctly classified individuals ranging between 73.6 and 78.9%. The results from STRUCTURE show that La Vansa birds were assigned to La Vansa with a very high probability, whereas La Bofia birds were assigned to La Bofia with a very high probability (figure 1).

Citril finches differed significantly in morphology between the localities when sex, age and year had been accounted for in a factorial ANOVA (Wilks' lambda = 0.96, d.f. = 2,925, $p < 0.001$). Birds in Bofia had longer tarsus and shorter wings than birds in Vansa (wing length: $F_{1,2243} = 44.92$, $p < 0.001$, tarsus length: $F_{1,2243} = 17.97$, $p < 0.001$; figure 2).

4. DISCUSSION

The results show that there was significant and substantial genetic differentiation between the La Vansa and La Bofia. This result was based on a variety of methods and we therefore regard the result as being robust. This shows that despite the short distance, the well-documented movements and gene flow between the localities (Senar *et al.* 2002), strong genetic differences can still occur. In addition to differences in allele frequencies, we also found differences in morphology. This is consistent with differences in the feeding ecology and habitat structure between the two localities: La Vansa birds, which rely more often on pine seed obtained directly from cones in trees in hanging positions, had shorter tarsus lengths than La Bofia birds, which rely more on grasses and, hence, ground feeding (Senar *et al.* 2002). This is in accordance with the general view that ground-feeding species have longer tarsus than clinging and hanging ones (Newton 1967; Moreno &
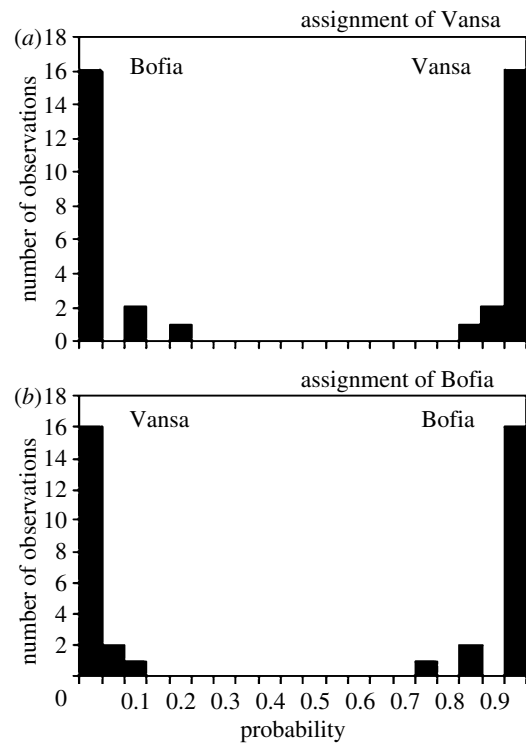
Figure 1. The probability of assigning an individual captured in one locality to each of the two localities. (*a*) assignment to La Vansa. (*b*) Assignment to La Bofia. The text in the figures refers to where the individuals were captured.
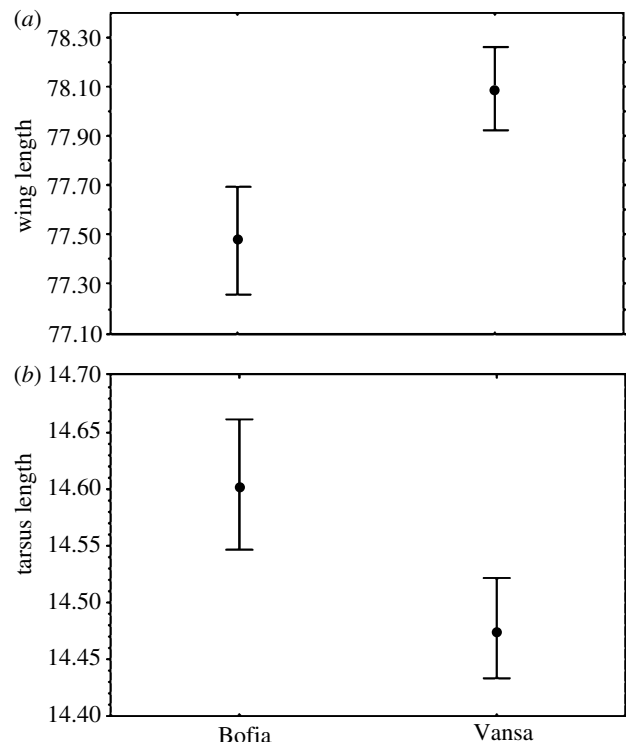

Figure 2. (*a*) Wing and (*b*) tarsus length variation (mean ± s.e.) of citril finches comparing Bofia versus Vansa localities. Values have been standardized for sex, age and yearly variations with factorial ANOVA.

Carrascal 1993). The shorter wings of La Bofia citril finches may be related to a higher presence of aerial predators in this area (A. Borcas 2005, personal observation), as shorter wings are more manoeuvrable

and, hence, may be more adaptative (Alatalo *et al.* 1984; Winkler & Leisler 1985; Rayner 1988). We have to point out that, although the differences in biometry between localities were small, differences between localities were in the opposite directions: wing length was larger in La Vansa, while tarsus length was larger in La Bofia.

These results suggest that even when selection and gene flow occur at the same time, morphological and genetic divergence can still occur. The degree of genetic divergence was high and the results from the assignment tests show that individuals could be accurately assigned genetically to the population where they were caught. The mechanism behind this pattern is not currently known, but two non-mutually exclusive factors can be imagined. First, there might be selection acting on individuals with the 'wrong' genotype. This assumes that there is a connection between the diversity at the microsatellite level and phenotype. We know that there are morphological differences (Senar *et al.* 2002), but whether these are based on genetic differences, or are only a result of plasticity, is not known. Second, we might have phenotype-dependent dispersal, so that individuals facing the wrong environment are more likely to disperse than others, which then might act to promote the differences (Postma & Van Noordwijk 2005). Again, this assumes genetic differences at a large scale and not only in a few microsatellite loci.

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