Selection-Based Biodiversity at a Small Spatial Scale in a Low-Dispersing Insular Bird

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The blue tit is a highly mobile small passerine found in deciduous and evergreen oaks. In mainland populations, gene flow results in local maladaptive timing of breeding in evergreen oak forests, the rarer habitat. However, on the island of Corsica, two populations only 25 kilometers apart are highly specialized and differ between the two habitat types in breeding and morphological traits. In contrast to theoretical predictions about the homogenizing effects of gene flow, this highlights evolutionary consequences of habitat diversification and isolation at a small spatial scale in insular organisms, which should be taken into account in conservation policies.

In spite of a surge of interest in the evolution of local adaptation and the consequences of human-induced habitat modifications in populations, surprisingly few studies have demonstrated the relationships between phenotypic variation and variation of selection pressures on a microgeographic scale. In contrast to many observations in plants (*1*), population differentiation of phenotypes at a microgeographic scale has rarely been proven to be adaptive in animals (*2*). Intraspecific, genetically based microgeographic variation of suites of covarying traits in relation to environmental factors has been found in some species of insects, fishes, and mammals (*3*). In such mobile organisms as birds, local differentiation of fitness-related traits as a response to variation in known environmental factors is supposed to be rare at a microgeographic scale because of the homogenizing effects of gene flow that override local adaptation (*4*).

We provide an example of a close match between several life-history traits and local selection regimes in two populations of blue tits, *Parus caeruleus*, 25 km apart on the Mediterranean island of Corsica. These two populations live in oakwoods which are dominated by deciduous oaks (*Quercus pubescens*) for one population (the Muro population, hereafter called M) and by evergreen oaks (*Q. ilex*) for the other (the Pirio population, hereafter called P). Spring development of foliage and of leaf-eating caterpillars (the key food for tits) occurs 4 weeks earlier in deciduous than in evergreen oaks (*5*). Contrary to expectations from previous findings in similar geographic configuration of habitats on the mainland, which predicted small differences in breeding time because of the homogenizing effects of gene flow across habitats within a landscape (*6*), population studies using nest boxes showed that the M population started breeding more than 1 month earlier than the P population (*7*). As a result, each population closely matches the local short period of maximum food availability (Fig. 1). Moreover, the two populations consistently showed, over a 6-year study period, significant differences in all measured demographic and morphometric traits (Table 1). Blue tits have much better breeding performance at M, where food is plentiful early in spring, than at P, where food is about one-tenth as abundant and has peak availability 1 month later. Three lines of evidence indicate that natural selection in very different environments is the primary cause of a phenotypic divergence, which is so large that the two populations have different niches and independent population dynamics.

First, reproducing immigrants in each population, be they adults or yearlings, are recruited locally within their own valley, as shown by their morphometry compared to that of the local breeders (Fig. 2A).

Second, common garden experiments designed to test whether population differences in laying date result from genetic differences or from phenotypic plasticity have shown from five independent samples that blue tits from the P and the M sites maintained in the same environmental conditions consistently showed contrasting reaction norms (Fig. 2B). This contrasts sharply with the demonstrated gene flow of tits at a much larger spatial scale on the mainland based on demographic (*5*) and molecular (*8*) data, as well as from experiments in aviaries (*9*). In life-history studies of several mainland populations of tits, maladaptive responses were attributed to extensive gene flow from optimal toward suboptimal habitats (*10*). The very late laying date of the M captive birds may be explained by changes in the physical environment (aviaries) completely reshaping the outcome of interactions between genotype and environment, resulting in different reaction norms (*11*). The key point is that the reaction norms

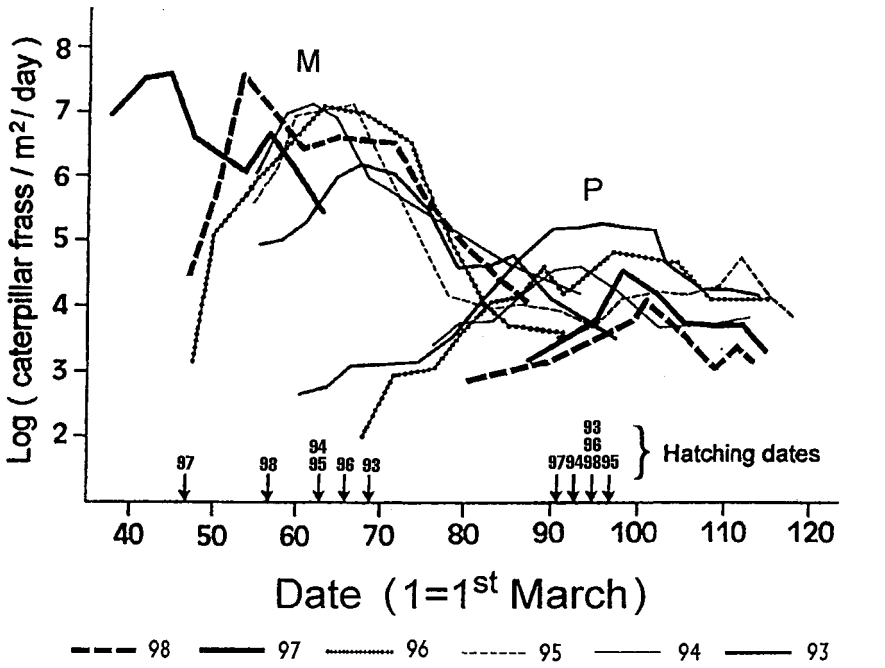

Fig. 1. Differences in the timing and abundance of caterpillars between the deciduous (M) and the evergreen (P) oakwoods. Over a 6-year study period (1993–98), the timing of hatching of blue tits (arrows on the *x* axis) was closely synchronized within each population to the peak in caterpillar availability.

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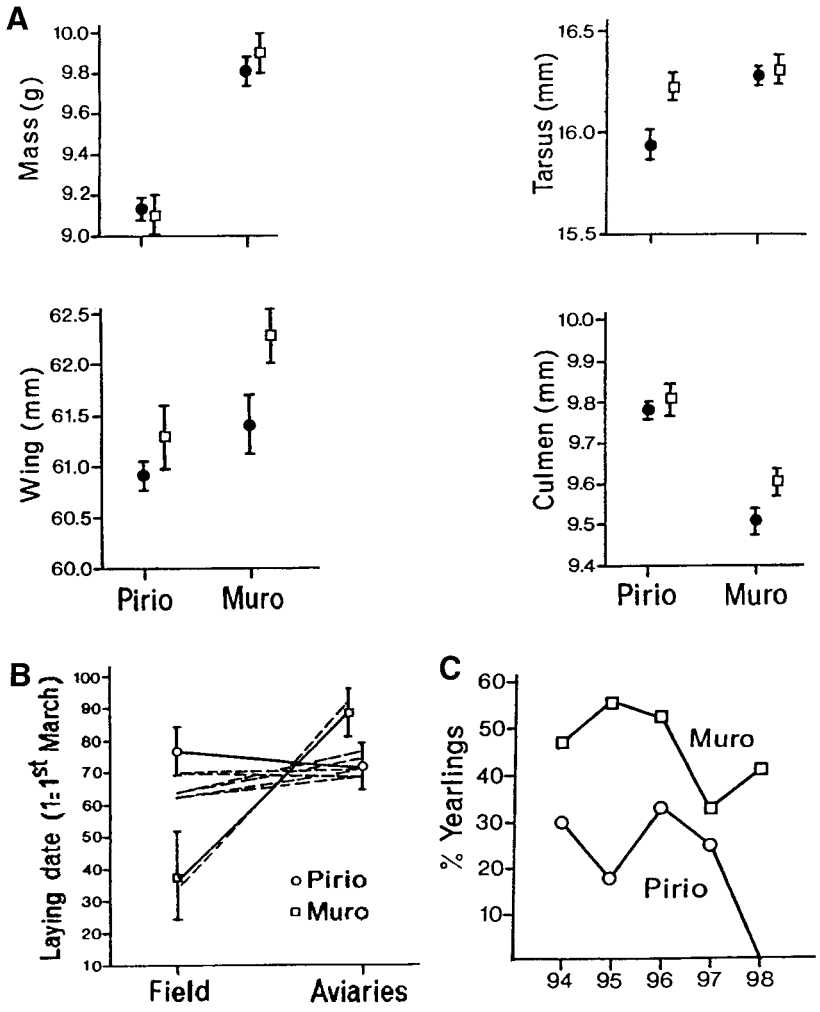

Fig. 2. (**A**) Comparison of the morphometry of Muro and Pirio yearling blue tits. Controlling for sex differences, yearlings have a larger body size (body mass, tarsus length, and wing length) but a shorter bill at Muro than at Pirio [site effect for body mass, $F(1,303) = 142$, $P < 0.001$; wing length, corrected for overdispersion, $\chi^2 = 12.97$, $P < 0.001$; tarsus length, $F(1,298) = 13.21$, $P < 0.001$; culmen, $F(1,305) = 24.28$, $P < 0.001$]. All data ±1 SE. Similar between-population differences of morphological traits in yearlings as those in adult breeders (Table 1) mean that yearlings immigrated from similar sites, that is, their respective local populations (*25*). Although philopatric yearlings (born in the nestboxes, white squares) have slightly higher values for all traits than unknown yearlings (black circles), between-population differences in these traits are significantly higher than between-class (philopatric versus immigrants) differences within each population. (**B**) Comparison of the laying dates (±SD) of Muro and Pirio blue tits in the field and in aviaries ($\chi^2 = 16.84$, $P < 0.001$). Captive Pirio birds started laying at about the same time in aviaries as in the wild, whereas captive Muro birds started laying much later (solid lines). In four cases (three at Pirio, one at Muro) where laying dates were known for both daughters and their mother (dashed lines), captive birds (which have been trapped in five different sessions in 5 years) laid at approximately the same date as their mothers. (**C**) Comparison of the proportion of yearlings in the two populations. A higher proportion of yearlings at Muro than at Pirio, combined with a different yearly variation of this proportion between the two populations, indicates that they function independently. Although about 24% of the birds that breed annually in the nestboxes are yearlings, not a single young born at Pirio was recruited in the population in 1998 as a consequence of a devastating storm at this site in 1997 during the nestling stage. At the Muro site, the young had already fledged at the time of the storm so that this event had no population consequences in 1998.

of the two populations differ but do not overlap, which could only arise through reduced gene flow (*12*).

Third, an exceptional rainstorm (~300 mm accumulation) on 5 to 6 June 1997 caused widespread breeding failure in the evergreen (P) population. The weights of those young which did fledge were so low that they had almost no chance of surviving to breed (*13*). Nestbox occupancy was about one-quarter lower than usual in the following year, and not a single yearling recruited to the breeding population (Fig. 2C), although this habitat type extends over the whole P valley and is the most common habitat type on Corsica. This demonstrates that there is little or no gene flow from the deciduous (M) population, where breeding was normal in 1997. The combination of all these approaches points out population differentiation at this small spatial scale.

Differences in the timing of reproductive events as a response to differences in the timing and abundance of food resources (the food schedule) are a major component of adaptation to different environments (*14*). A close adaptation of the breeding time to local food resources results in suites of traits such as body size and shape, life history, and demographic traits that do not evolve independently even though they may be genetically independent. Traits vary either directly (for example, laying date) or as an indirect consequence of interactions among them. Theory predicts that if the environment is relatively constant in space, which is true of both P and M populations, maximizing mean fitness results in a specialized phenotype because environmental constancy favors the evolution of specialization (*15*), even if gene flow is in excess of a few migrants per generation (*16*). The response of the P and M populations to strikingly different but constant habitat-specific food schedules is clearly adaptive, and the observed population patterns are expected from life-history theory under these contrasting regimes of selection (*16*).

How much of the observed population differences are genetic? The two populations are so different in phenotype and overall demography that they function independently of each other as a result of alternative adaptations to different ecological niches (*17*). Population crashes such as that which occurred in 1997 at P, combined with the absence of immigration, are likely to maintain local specialization. In birds, no study has so far addressed the question of dispersal among populations that exhibit large differentiation in life-history traits over such a small spatial scale. The absence of observed migration from one population to the other does not mean that dispersal between them does not occur. We argue that, whatever the extent of dispersal, the amount of gene flow between the two populations is too low to prevent strong selection on traits that diverge in association with the exploitation of different resources (*4*). Potential immigrants from one population to the other may not even succeed in entering the population, because divergent selection is sufficiently strong to outweigh the effects of gene flow and random factors. Dispersal may even be very small because in temporally stable but spatially heterogeneous environments, selection favors habitat fidelity and a reduced propen-

Table 1. Demographic and morphometric parameters of the Muro and Pirio blue tit populations of Corsica. Statistical analyses showing the effects of explanatory variables (site and year) and their interaction (site*year) were made using generalized linear models and MANOVA (SAS Institute Inc.) (*22*). As compared to the P population, the M population shows (i) significantly higher breeding performance (clutch size, nesting success, breeding success, mass, and tarsus length of the fledglings), (ii) a larger body size of adults except for culmen length which is shorter, and (iii) a higher ratio of yearlings (birds born the previous year)/adults. Local habitat specialization is also reflected on body size and shape. A MANOVA analysis showed that the two populations are morphologically different (Lawley test, $F_{3,402} = 64.51$, $P < 0.0001$). Variation in body size reflects the abundance of food, and variation in bill size and shape relates to foraging habits. Birds have longer bills in harsh substrates (that is, evergreen sclerophyllous leaves) than in soft substrates (that is, summergreen deciduous leaves) (*23*). This pattern of large birds with short bills at M and small birds with long bills at P fits, at the microgeographical scale of this island, the trend described at the scale of the whole distributional range of the blue tit (*24*), that is, between populations living in deciduous broad-leaved habitats in central Europe and populations living in the Mediterranean part of the species range.

Parameter	Pirio (P)	Muro (M)	Site* year	Site	Year
Density†	0.49 ± 0.07 (681)	0.76 ± 0.05 (233)		$\chi^2 = 52.3$***	$\chi^2_5 = 14.9$**
Breeding performance					
Laying date‡	75 ± 5.5 (360)	41 ± 13.8 (204)		$\chi^2 = 397.6$***	$\chi^2_5 = 11.5$*
Clutch size	6.3 ± 1.24 (360)	8.3 ± 1.73 (204)	$\chi^2_{11} = 15.6$***		
Nesting success§	0.72 ± 0.10 (360)	0.89 ± 0.05 (204)		$\chi^2 = 29.2$***	$\chi^2_5 = 28.7$***
Breeding success‖	0.52 ± 0.13 (294)	0.82 ± 0.04 (194)	$\chi^2_{11} = 14.0$**		
Fledging mass (g)	9.3 ± 0.83 (212)	10.4 ± 0.53 (146)	$F(11,346) = 4.74$***		
Tarsus length (mm)	15.89 ± 0.61 (211)	16.28 ± 0.40 (148)	$F(11,357) = 4.82$***		
Adult morphometry					
Male: Wing (mm)	63.6 ± 1.04 (145)	64.1 ± 1.27 (91)	$F(11,235) = 1.94$*		
Tarsus (mm)	16.26 ± 0.48 (145)	16.50 ± 0.49 (91)		$F(1,229) = 11.68$***	
Culmen (mm)	9.69 ± 0.34 (145)	9.53 ± 0.37 (91)		$F(1,234) = 12.43$***	
Mass (g)	9.4 ± 0.50 (145)	10.1 ± 0.53 (91)	$F(11,224) = 5.28$***		
Female: Wing (mm)	60.9 ± 1.14 (112)	61.6 ± 1.45 (60)	$F(11,171) = 2.94$**		
Tarsus (mm)	15.85 ± 0.54 (112)	16.00 ± 0.44 (60)		$F(1,165) = 4.12$*	
Culmen (mm)	9.87 ± 0.38 (112)	9.72 ± 0.46 (60)		$F(1,170) = 5.16$**	
Mass (g)	9.2 ± 0.53 (112)	9.8 ± 0.43 (60)	$F(11,160) = 2.85$*		
Proportion of yearlings¶	0.23 ± 0.13 (577)	0.41 ± 0.14 (334)	$\chi^2_{11} = 39.6$***		

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. †Proportion of nestboxes occupied. ‡Dates given in "Marchdate," for example, 32 = 1st of April. §Proportion of nests with at least one fledgling. ‖Number of fledglings/clutch size. ¶Young birds born the previous year.

sity for dispersal (*18*). Our data support the divergence-with-gene-flow model that has been documented in birds by Smith *et al.* (*19*), but that model was at a much larger spatial scale.

What sets the Corsican populations apart from those on the mainland is a reduced dispersal which is a component of an insular syndrome (*20*) that has been shown to occur in Corsican birds (*21*). A stronger habitat fidelity in Corsica than on the mainland explains why population differentiation of blue tits has been possible on the island but not on the mainland. Indeed, long-term studies conducted in a similar geographic configuration of deciduous and evergreen habitats on the mainland, near Montpellier (France), showed a much smaller difference in the timing of reproduction between the two types of habitats than in Corsica (about 1 week) (*5*). This results in the nestling stage not being synchronized with the peak of abundance of caterpillars in the mainland evergreen habitat. This mismatch, a local maladaptation, has been attributed to gene flow between habitat patches preventing local specialization, a hypothesis supported by a genetic analysis (*8*). Moreover, of the 1297 and 1276 nestlings banded at the P and the M sites, respectively, not a single exchange has been observed during a 6-year study period whereas in a similar configuration of habitats on the mainland, several birds have been reported to immigrate from one habitat to the other. Thus, the two adjacent populations on the island behave in exactly the same way as do isolated populations between deciduous habitats on the mainland and evergreen habitats on Corsica. Between the latter two populations, there is a difference of 1 month in breeding time, which common garden experiments have shown to be genetically based (*9*).

Fine-scale population adaptation of blue tits within a single island emphasizes that geographic proximity does not necessarily imply similar adaptation, as is often assumed in bird populations, and that adjacent populations may be very different. Most vertebrate populations in natural landscapes are open, dynamically interacting entities, which is a key aspect of their persistence in the face of environmental variation. Population differentiation of Corsican blue tits, a case of "nested insularity," highlights the importance of considering evolutionary pathways of populations on islands for assessing biodiversity patterns in heterogeneous landscapes, which is of importance for conservation issues.

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GTP Binding by Class II Transactivator: Role in Nuclear Import

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Class II transactivator (CIITA) is a global transcriptional coactivator of human leukocyte antigen–D (HLA-D) genes. CIITA contains motifs similar to guanosine triphosphate (GTP)–binding proteins. This report shows that CIITA binds GTP, and mutations in these motifs decrease its GTP-binding and transactivation activity. Substitution of these motifs with analogous sequences from Ras restores CIITA function. CIITA exhibits little GTPase activity, yet mutations in CIITA that confer GTPase activity reduce transcriptional activity. GTP binding by CIITA correlates with nuclear import. Thus, unlike other GTP-binding proteins, CIITA is involved in transcriptional activation that uses GTP binding to facilitate its own nuclear import.

Class II transactivator (CIITA) is a transcription factor that controls the expression of multiple genes, including class II major histocompatibility complex (MHC), human leukocyte antigen–DM and invariant chain, involved in the exogenous pathway of antigen processing and presentation (*1*). Constitutive and inducible expression of class II MHC requires CIITA, as shown by analysis of immunodeficient patients who lack functional CIITA (*2*) as well as analysis of gene knockout mice (*3*). CIITA is a transcriptional coactivator in that, although it does not bind DNA per se, fusion of CIITA with the GAL4 DNA binding domain results in transactivation of promoters bearing the GAL4 target DNA (*4*). CIITA interacts with the MHC promoter binding factor RFX5, components of the basal transcription complex, including the transcription factor TFIIB and a subset of TATA-binding protein (TBP)–associated factors (TAFs), the transcription factors B cell octamer-binding protein (Bob1) and CREB-binding protein (CBP) (*5*).

Structural and mutational analyses have shown that CIITA contains acidic activation and proline-serine-threonine–rich (*6*) domains required for transcriptional activation (*1, 4, 7*). In addition, CIITA contains three putative GTP-binding protein motifs: a P-loop or Walker A motif [G1, consensus sequence $GX_4GK(S/T)$: ^{420}GKAGQGKS^{427}], a magnesium coordination site (G3, DXXG: ^{461}DAYG^{464}), and a site that confers specificity for guanosine [G4, (N/T/S)KXD: ^{558}SKAD^{561}] (*1, 7, 8*). A mutation in any of these three motifs reduces the transactivation function of CIITA (*7*). Hence, CIITA may represent a GTP-binding protein; however, the function and significance of these GTP-binding motifs have not been shown.

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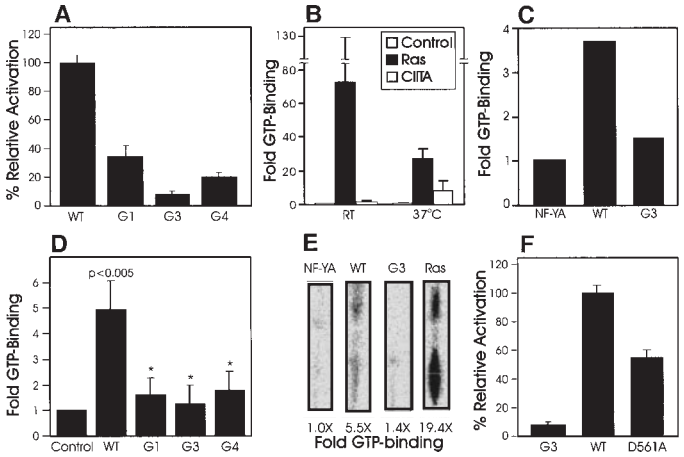

Fig. 1. Ability of CIITA and mutants to activate transcription correlates with GTP binding. (**A**) GTP-binding motif mutations decrease activation of transcription. CIITA-defective RJ2.2.5 cells were cotransfected with 1 μg of DNA encoding FLAG-tagged wild-type CIITA (WT) and CIITA mutants GTP1(ΔGK) (G1), GTP2(ΔDAYG) (G3), and GTP3(ΔSKAD) (G4) and 1 μg of the HLA-DR promoter/luciferase reporter DRA300luc (*24*). Luciferase activity was determined 24 hours after transfection. Results are expressed as percent relative luciferase activity compared with CIITA-induced activity (140-fold) over the reporter alone. Means of two independent experiments in triplicate (±SEM) are shown. (**B**) CIITA binds GTP. In vitro translated and immunoprecipitated CIITA and RasQ61L were tested for GTP binding at 25°C (RT) and 37°C (*31*). Fold increase of GTP-binding is shown and represents three independent determinations (±SEM). (**C**) GTP binding by CIITA is specific. CIITA (WT), the mutant G3, and the non–GTP-binding protein NF-YA were tested in the GTP-exchange assay as described above. Binding was corrected for protein expression; mean of two experiments is shown. (**D**) GTP-binding motif mutations decrease GTP binding. CIITA, G1, G3, and G4 were tested for their ability to bind GTP at 37°C as described above. GTP-binding activity was corrected for protein expression and is expressed as fold increase above control (±SEM) for three independent experiments. *t* -test values were calculated for each data set. *P* values: CIITA versus control (shown), $P < 0.005$; mutants versus CIITA (*), $P \leq 0.01$. CIITA averaged 32,800 ± 11,200 counts per minute (cpm) (about 9.4×10^{16} cpm per mol of CIITA assuming 50 ng of in vitro translated product per immunoprecipitation). (**E**) CIITA binds GTP in vivo. COS-7 cells were transfected with 1 μg of the indicated construct and labeled as described (*32*). Representative TLC results are shown for guanine nucleotides eluted from the indicated proteins. The mean fold GTP-binding above background is shown for three separate labeling experiments. (**F**) D^{561} is important for activation of transcription. RJ2.2.5 transfected with DRA300luc reporter and either CIITA or D561A as in (A). Activation with G3 is shown for comparison. Means of two independent experiments in triplicate (±SEM) are shown.