J. Evol. Biol. 9: 965–978 (1996)

1010–061X/96/060965–14 $ 1.50 + 0.20/0

Source-sink populations in Mediterranean Blue tits: evidence using single-locus minisatellite probes

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Key words: Asymmetric gene flow; *Parus caeruleus*; population genetics; population structure.

Abstract

Long term studies on population biology of Blue tits (*Parus caeruleus* L.) in Mediterranean habitats have shown that in patchy landscapes life-history traits seem to be adapted to the predominant type of habitat, where reproductive success is higher. The "source-sink hypothesis' suggests that differences in the local production of fledglings result in an asymmetrical gene flow from rich deciduous habitats ("source") to evergreen poor habitats ("sink"), preventing local adaptation in evergreen habitats.

In this study we used single-locus minisatellite DNA probes to test the following predictions of the source-sink hypothesis: 1) source and sink populations are not genetically differentiated; 2) amount of gene flow is ranked in the following decreasing order: between source and sink habitats, among source habitats and among sink habitats; and 3) linkage disequilibrium is higher in sink than in source populations. Results were consistent with these three predictions, and with previous results obtained using other approaches. Results clearly support a source-sink

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functioning of Blue tit populations in southern France mosaic landscapes, and emphasise the need of combining genetic and ecological studies to understand the functioning of natural populations.

Introduction

The balance between local selection pressures and gene flow determines the extent to which local populations can become adapted to heterogeneous environments (Endler, 1977; Slatkin, 1985; Ehrlich and Raven, 1969). Few empirical studies have examined patterns of selection across habitats that may strongly differ in terms of selection regimes. Some examples in the literature suggest that gene flow may prevent small scale local adaptation in different species – e.g. in plants (Keddy, 1982; Kadmon and Schmida, 1990), and in animals (Stearns and Sage, 1980; Nevo and Bar-El, 1976; Hall, 1981; Reiter and Le Boeuf, 1991; Dhondt et al., 1990).

Mediterranean landscape is a mosaic of habitats including patches that strongly differ in quality for insectivorous birds such as tits. A long term study of the between-habitat variation in life-history traits of the Blue tit (*Parus caeruleus* L.) has been undertaken in two regional landscapes that differ in the proportion and size of habitat patches (see review in Blondel et al., 1993 and Dias and Blondel, 1996). One study area is located in the region of Montpellier (southern France) and includes many habitat patches dominated by the deciduous Downy oak *Quercus pubescens*. On the other study site, on the island of Corsica, habitats dominated by the evergreen Holm oak *Q. ilex* are much more abundant than habitats dominated by *Q. pubescens*. There is virtually no gene flow between these two study sites (the Corsican population has been ascribed to the subspecies *Parus caeruleus ogliastrae*).

The Blue tit is a small insectivorous bird whose preferred prey during the breeding season are caterpillars developing in the young leaves of trees (Betts, 1955; Perrins, 1965; 1991; van Balen, 1973; Cowie and Hinsley, 1988; Zandt et al., 1990; Blondel et al., 1991). Thus the yearly leafing patterns of the tree determine the timing and amount of food available for the birds. The leafing process of the Downy oak involves the renewal of all of the foliage and occurs early in the season, whereas that of the Holm oak involves only 30% of the foliage and occurs about three weeks later (Cramm, 1982; Blondel et al., 1992; 1993).

In the mainland habitat mosaic, where deciduous habitat patches are abundant, Blue tits start to breed early and their breeding success is high (Blondel, 1985; Isenmann et al., 1987; Blondel et al., 1992, 1993; Dias et al., 1994). In the island mosaic, where evergreen habitat patches predominate, Blue tits start to breed about three weeks later and their breeding success is also high (Blondel and Isenmann, 1979; Blondel, 1985; Blondel et al., 1993). In both cases, tits start to lay so that their young are about 10 days old (which corresponds to the time of their highest food requirements) when the local caterpillar abundance peaks. Both populations are assumed to be adapted to their habitats, because timing of reproduction is well synchronised with food availability. A strong genetic component of the laying date has been experimentally demonstrated (Perret et al., 1989; Blondel et al., 1990; Lambrechts and Dias, 1993; Lambrechts et al., 1996).

By contrast, evidence of maladaptation has been found in the "non-dominant" habitat patches of the two regional systems, i.e., in the mainland evergreen and in the island deciduous (Zandt et al., 1990; Blondel et al., 1992; 1993; Dias et al., 1994; Dias and Blondel, 1996). In the mainland evergreen patches, Blue tits initiate egg-laying almost at the same time as in nearby deciduous ones so that the period of highest food requirements of the young occurs long before the date of peak caterpillar abundance. The breeding success of tits in such mainland evergreen habitats is low (Cramm, 1982; Blondel et al., 1987; Dias et al., 1994). Similar results are available from Corsica where tits in deciduous habitat patches start to lay later than would be expected based on the leafing process of the trees (Lambrechts and Dias, 1993; Dias and Blondel, 1996), so that they also miss the local peak of food abundance. We concluded from these studies that there are "deciduous genotypes" on the mainland and "evergreen genotypes" on the island (Lambrechts and Dias, 1993; Dias and Blondel, 1996).

These results suggest that a source-sink system (*sensu* Pulliam, 1988; see Dias, 1996 for a review) could operate at the scale of each of these two habitat systems (Blondel et al., 1992, 1993; Dias et al., 1994; Dias and Blondel, 1996). Deciduous habitat patches on the mainland (where tits have "deciduous genotypes") may function as "sources" from which birds emigrate to evergreen habitat patches where the low production of young cannot balance local mortality. This would imply an asymmetrical gene flow from "sources" to "sinks" preventing local adaptation in evergreen habitat patches, i.e. the evolution of "evergreen genotypes" similar to those that have evolved on Corsica.

The validity of the source-sink hypothesis can be assessed by using different approaches, such as capture-recapture methods, analysis of life-history variation, morphometry, or the study of population structure and gene flow. In this study we chose the latter, using four hypervariable single-locus minisatellite DNA probes. The starting point was to determine the geographical range at which Blue tit populations are genetically differentiated. If source populations are not differentiated at the scale studied, the only possible genetic prediction is that the expected lifetime of any particular neutral gene is shorter in a sink than in a source habitat. Testing this prediction requires long term genetic studies which are not currently available. One the other hand, if source populations are differentiated at the scale of the study, then we should expect also a significant differentiation among sink habitats, but not between sink and source habitats as a whole. Several additional predictions are possible. First, estimates of number of migrants between source and sink habitats should be higher than among sources or among sinks, and number of migrants among source habitats should be higher than among sink habitats. Second, linkage disequilibrium should be higher in sink habitats than in source habitats because of immigration of birds from distinct and differentiated source habitats (generalised Wahlund effect, Nei and Li, 1973).

Table 1. Geographical and ecological characteristics of the sampling sites.

Code	Locality	Dominant tree	Latitude	Longitude	Altitude (m)
Continent:					
A	La Rouvière	*Q. pubescens*	43° 40′ N	03° 40′ E	100–300
B	Argelliers	*Q. pubescens*	43° 42′ N	03° 40′ E	100–200
C	Causse de la Selle	*Q. pubescens*	43° 48′ N	03° 37′ E	200–300
D	Liouc	*Q. pubescens*	43° 56′ N	04° 00′ E	100–150
E	Puéchabon	*Q. ilex*	43° 45′ N	03° 35′ E	200–300
F	Titou	*Q. ilex*	43° 43′ N	03° 38′ E	100–200
G	Aigues Vives	*Q-ilex*, *Q. pubescens*, & gardens	43° 44′ N	04° 11′ E	25–50
Corsica:					
H	Pirio	*Q. ilex*	42° 23′ N	08° 45′ E	100–150

Materials and methods

Sampling sites

Eight sites were sampled in 1992 (Tab. 1, Fig. 1): a) Seven sites in Southern France, in different habitat types: four in deciduous Downy Oak (*Quercus pubescens*) habitats (Rouvière, Argelliers, Causse de la Selle and Liouc, hereafter named populations A, B, C and D, respectively); two in evergreen Holm Oak (*Q. ilex*) habitats (Puéchabon and Titou, hereafter called populations E and F, respec-

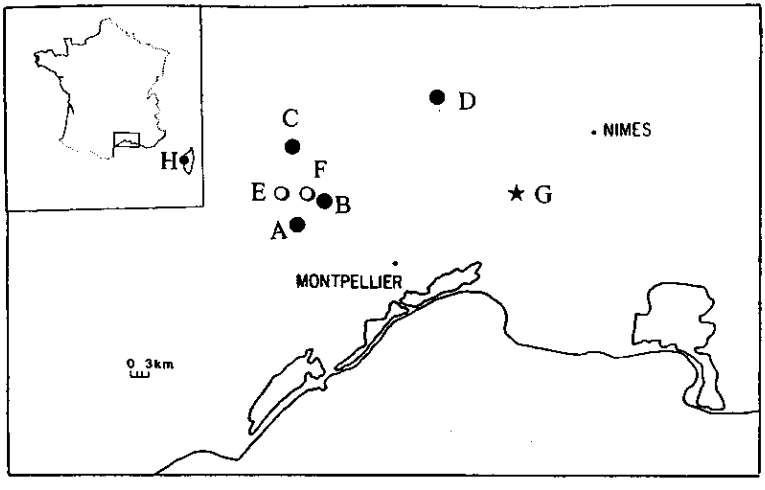

Fig. 1. Location of the sampled areas in deciduous habitats (closed circles), evergreen habitats (open circles) or mixed ones (star) in southern France. Letter codes as in Table 1.

tively); one in a mixed wood with Holm and Downy Oaks, close to gardens (Aigues Vives, called population G); and b) one site in the island of Corsica, in a Holm Oak forest (Pirio, hereafter called population H). Birds were sampled during the breeding period, either as breeding adults or, when the parents could not be sampled, as chicks (one chick per brood). Sample sizes for populations A to H were respectively 27, 3, 4, 17, 12, 8, 3 and 20.

DNA isolation, Southern blotting and hybridisation

DNA was isolated from whole blood samples as described by Verheyen et al. (1994). After digestion with the *Hinf* I restriction enzyme, DNA fragments were separated by electrophoresis, transferred by Southern blotting to nylon membranes. Four single locus minisatellite DNA probes were used (Verheyen et al., 1994). These probes are named (Hanotte et al., 1991) using the prefix cPcaMS (charomid *Parus caeruleus* minisatellite sequence) followed by a number; only the number will be used here: the four probes are hereafter called 1, 3, 11 and 14. Probes were (α-^{32}P)dCTP labelled using a Multiprime labelling kit (BRL) and then hybridised to the filters containing the *Hinf*-I digested DNA. Hybridisation and washing conditions are described in Verheyen et al. (1994). Autoradiography was performed overnight at -70 °C using intensifying screens.

Identification of alleles

For each probe, the band sizes were determined by comparing their migration distance with that of the length markers of known sizes (Analytical Marker System from Promega), using the method described in Duggleby et al. (1981), and were sorted by size over the whole data set. Two bands were considered to represent distinct alleles when they differed by more than 100 bp. Alleles that could not be unambiguously identified (for technical reasons) were not taken into account, which explains that some allelic sample sizes are uneven.

Population differentiation

Genic differentiation between populations was tested using Fisher's exact test on $R \times C$ contingency table for each locus. An unbiased estimation of the exact probability was performed with a Markov chain method (Raymond and Rousset, 1995a), using the Genepop (version 2.0) software (Raymond and Rousset, 1995b). For all tests, the Markov chain was set to at least 100 000 steps, and 1000 steps of dememorisation (see Guo and Thompson, 1992 for details). This test remains unbiased even for small sample sizes or low frequency alleles (see Raymond and Rousset, 1995a for explanations). Overall significance of several tests for each locus was estimated by the Fisher's combined probability test (Fisher, 1970).

Genic differentiation between populations was measured using θ, the Weir and Cockerham (1984) estimator of Fst. The number of migrants (or Nm) between the populations sampled was estimated as $Nm = (1/F_{ST} - 1)/4$ (Wright, 1969), using the F_{ST} estimate calculated above. The formula assumes neutral markers and an island model of population structure (see e.g. Hartl and Clark, 1989).

A lower θ value between than among habitat types was evaluated as follows. All θ values among all possible sample pairs were classified in two categories. The first one (INTRA) contained pairs of samples from the same habitat (either source-source or sink-sink pairs), the second (INTER) contained pairs of samples from the two habitats (source-sink pairs). The slope of the rank regression between θ values and the two classes (INTRA and INTER) indicates which category contains globally higher θ values. As all data points are not independent, the possibility of lower θ values in the INTER class was tested by randomly permuting source and sink habitats across samples, and computing the number of cases when the Spearman rank regression coefficient was lower or equal than the observed one. This computation was performed using the MANTEL program available in Genepop (version 2.0).

Linkage disequilibrium

The unbiased composite linkage disequilibrium, or D_{ij} (Weir, 1990) was computed for all possible pairs of alleles in each of the continental populations (except for population G) using the Linkdos program (Garnier-Gere and Dillman, 1992). A higher linkage disequilibrium in the putative sink habitats than in the putative source ones was expected. To test this hypothesis, all absolute D_{ij} values were compared between habitat types using a Mann-Whitney test (Siegel and Castellan, 1988). As all D_{ij} values for a given locus pair in one population are not statistically independent, one randomly chosen D_{ij} was removed for each locus pair in each populations to insure independence.

Results

Allelic polymorphism

Depending on the probe, the number of alleles detected on the whole data set varied between 21 and 31 for a total of 156 to 186 genes analysed (Fig. 2, Tab. 2). The mean number of alleles detected per locus was positively correlated to sample size (Spearman rank-order correlation coefficient $r_s = 1$, $P = 0.0025$, one-tailed test), indicating that additional sampling is necessary to collect most of the existing alleles in these populations.

Population differentiation

The overall differentiation among populations was highly significant ($P < 10^{-6}$). In order to analyse the differentiation within and between habitats, several tests

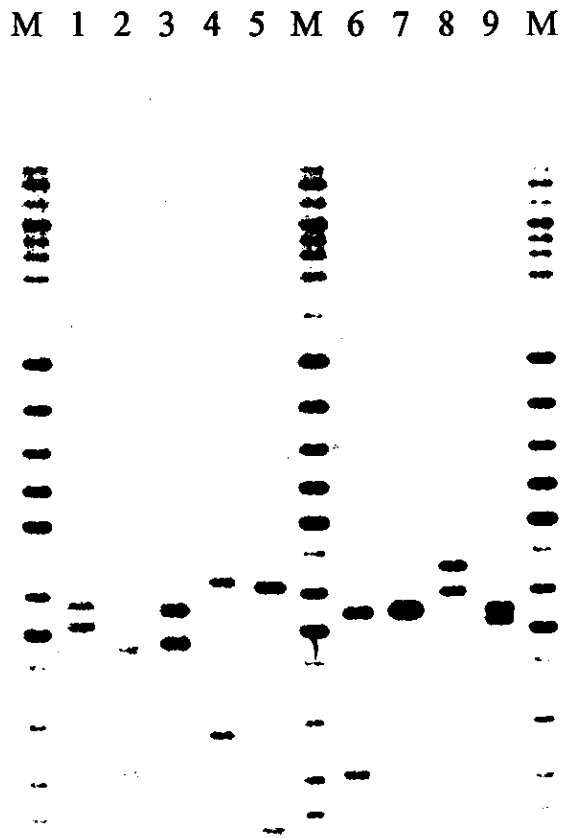

Fig. 2. Example of an autoradiography after hybridisation with probe 1. M refers to the length marker. Each individual is identified by a number. Individual number 7 is homozygous.

were performed. First, the continent-island differentiation was assessed by testing the allelic composition of population H against the allelic composition of the continental group. This resulted in a highly significant test ($P < 10^{-4}$, Tab. 3) and a large differentiation ($\theta = 0.019$), indicating that Corsican Blue tits have diverged from mainland. Second, a possible differentiation within the mainland was tested by excluding population H. A significant ($P < 0.002$, Tab. 3) and moderate ($\theta = 0.0084$, Tab. 4) differentiation was found, indicating that mainland populations are structured at the scale studied. Third, a differentiation within each type of habitat was tested. Deciduous habitats were structured ($\theta = 0.0083$; $P < 0.03$, Tabs. 3 and 4) as were the evergreens too ($\theta = 0.018$; $P < 0.02$, Tabs. 3 and 4). Due to the intra-habitat differentiation, the inter-habitat differentiation cannot be tested by comparing pooled samples across habitats. θ among pairs of source or pairs of sink habitats were higher than among pairs of source and sinks habitats (Rank regression, $b = -0.375$), indicating a lower genic differentiation between than within

Table 2. Allelic count for each probe in each sample. "All" refers to the number of genes analyzed per probe in each population, and "Total" refers to the number of genes analyzed per probe for all populations.

	Probe																													
	1								3								11								14							
Pop:	A	B	C	D	E	F	G	H	A	B	C	D	E	F	G	H	A	B	C	D	E	F	G	H	A	B	C	D	E	F	G	H
Allele																																
1	1	0	0	1	2	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
2	1	0	0	0	0	0	0	0	2	0	0	2	0	0	0	0	0	0	0	0	2	0	0	0	2	0	0	0	0	0	0	0
3	3	1	1	2	0	1	0	1	2	1	1	1	1	0	2	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
4	2	0	0	1	0	0	0	0	5	0	0	5	0	0	0	2	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0	2
5	2	0	0	3	2	2	1	0	2	0	1	7	7	0	0	2	2	0	0	1	0	1	0	0	0	0	0	1	0	0	0	2
6	5	1	0	2	2	0	0	1	5	0	0	2	1	1	1	6	6	0	0	0	0	2	0	0	0	0	0	0	1	0	0	0
7	7	0	0	1	1	0	0	1	4	1	0	5	2	1	0	3	0	1	1	0	0	0	0	0	1	2	0	1	2	0	0	0
8	5	0	0	2	4	1	1	5	2	0	1	1	1	3	0	2	0	0	0	1	0	1	0	0	0	0	0	1	0	0	0	0
9	2	1	1	0	3	1	1	4	3	0	0	1	0	0	1	6	2	0	0	3	0	2	0	2	1	0	0	2	0	0	1	0
10	2	0	0	5	4	0	0	6	6	0	1	1	3	2	1	6	2	0	1	1	2	0	0	2	0	0	0	2	0	0	0	0
11	4	0	1	1	0	0	0	0	1	0	0	0	1	0	0	3	3	1	0	0	1	0	0	0	1	0	0	3	1	0	1	2
12	7	0	1	2	2	2	0	13	4	0	1	0	2	0	0	3	3	0	0	2	1	0	0	0	1	0	0	1	0	0	0	2
13	0	0	0	1	1	1	0	4	0	0	0	0	0	0	0	1	5	1	0	6	1	1	0	3	3	0	1	1	2	0	0	0
14	8	0	0	7	1	2	0	0	2	0	0	0	0	0	0	0	1	1	3	6	0	2	0	5	2	0	0	3	1	0	0	3
15	4	0	1	2	0	3	0	1	1	0	0	0	0	1	0	0	2	1	0	2	5	0	1	3	1	0	0	0	2	1	0	0
16	0	0	2	1	0	1	1	0	1	1	0	1	3	0	0	0	0	0	0	2	1	0	0	2	4	0	2	0	2	0	0	0
17	0	1	0	0	0	0	1	0	2	0	1	2	0	1	0	0	4	0	3	0	3	1	0	5	1	0	0	1	0	0	0	0
18	1	0	0	0	1	0	0	0	2	0	0	1	0	0	0	1	3	1	0	1	3	1	0	3	3	0	2	3	2	0	0	0
19	0	0	0	0	0	0	0	1	2	2	0	0	0	1	0	2	1	0	0	0	0	0	0	8	3	0	1	0	0	1	0	0
20	0	1	1	2	0	1	0	0	1	1	1	2	1	0	0	1	0	0	0	2	0	0	0	1	3	0	0	0	2	0	0	6
21	0	0	0	1	0	1	1	3	0	0	1	0	1	1	1	1	2	0	0	0	0	1	0	0	1	1	0	1	0	0	0	3
22									4	0	0	3	0	0	0	1	0	0	0	0	1	0	0	0	4	1	0	3	1	1	1	3
23																	0	0	0	1	0	0	0	0	7	0	0	1	0	0	0	8
24																	0	0	0	1	0	0	0	0	7	0	0	4	0	0	0	4
25																	1	0	0	0	0	0	1	0	7	2	1	3	5	0	0	1
26																	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	1
27																									7	2	1	3	5	0	0	1
28																									0	0	0	0	0	0	0	1
29																									1	0	0	3	0	0	2	1
30																									0	0	0	0	0	0	1	0
31																									0	0	1	0	1	0	0	1
All	54	5	8	34	23	16	6	40	52	6	8	34	23	11	6	40	40	6	8	30	23	12	2	35	54	6	8	34	22	3	6	40
Total	186								180								156								173							

Table 3. Differentiation between sets of populations: probability of Fisher exact test on contingency table (S.E. in parentheses). Bold characters indicate significant values ($P < 0.05$). Total refers to the Fisher's combined probability test.

Populations	Loci				
	1	3	11	14	**Total**
Corsica-mainland	**<0.001** (<0.001)	0.17 (0.0084)	0.064 (0.0077)	**0.001** (0.00045)	**<0.0001**
Intra-mainland	**0.049** (0.011)	0.076 (0.014)	**0.008** (0.0031)	0.104 (0.018)	**0.001**
Mainland:					
Intra Evergreen	0.15 (0.006)	0.076 (0.0047)	**0.02** (0.0019)	0.39 (0.0098)	**0.017**
Intra Summergreen	0.083 (0.012)	0.32 (0.019)	**0.037** (0.0072)	0.19 (0.017)	**0.029**

Table 4. Values of the theta parameter of Weir and Cockerham (1984) and the corresponding estimates for number of effective migrants (Nm) for different population subdivisions.

	Theta					Nm
Loci:	1	3	11	14	All	
Subdivision:						
Within continent	0.0008	0.0116	0.0112	0.0106	0.0086	29
Within habitat:						
Evergreen	0.0063	0.0275	0.0375	0.0000	0.018	14
Summergreen	0.0035	0.0059	0.015	0.0089	0.0083	30
Between habitats:	−0.0026	0.0063	−0.0057	0.0025	0.0001	>2000

habitats. This tendency was marginally not significant (Mantel test, 50 000 permutations, $P = 0.068$).

The effective number of migrants per generation among either all continental populations, all evergreen or all deciduous was between 14 and 30 (Tab. 4). It was much higher between continental habitat types: more than 2000 migrants per generation were estimated between deciduous and evergreen habitats (Tab. 4).

Linkage disequilibrium

All possible unbiased composite linkage disequilibrium D_{ij} were computed for all pairs of alleles (at distinct loci) but one, in each continental population (except population G), which resulted in 4480 distinct values. They were all transformed to their absolute values. The mean (0.013) of all D_{ij} calculated from populations A, B,

C and D was lower than the mean (0.027) of all D_{ij} calculated from populations E and F (Mann-Whitney, $P < 10^{-5}$).

Discussion

In the present study we wanted to test some predictions of the source-sink hypothesis through a combined approach of population genetics and molecular biology, using four single-locus minisatellite probes. If Blue tit populations in southern France function as a source-sink system between deciduous and evergreen habitats, respectively, then several predictions could be tested on a population genetics ground.

Population differentiation

Southern French populations of Blue tit are significantly ($P < 0.002$, Tab. 3) differentiated at a scale of less than 50 km. This differentiation remains when only one type of habitat is considered, despite the resulting reduction of the data set. Differentiation within source or within sink habitats is detected at a scale of less than 40 and 5 km, respectively. This situation is unusual for birds, but not new (e.g. Randi and Alkon, 1994; Verheyen et al., 1995).

There is a tendency for lower θ values (hence larger number of migrants) among pairs of samples from distinct habitats than among pairs of samples from the same habitat, consistent with a lower genic differentiation between habitat types. The estimated number of migrants between source and sink habitats is very high ($Nm > 2000$), but drops to 30 for between source habitats, and decreases to 14 for between sink habitats (Tab. 4). This is the order expected, but it is not straightforward to statistically test the difference between these values. The last two numbers correspond to θ values significantly different from zero ($P < 0.03$, Tab. 3), indicating that the corresponding numbers of migrants (30 and 14) are not infinite. The number of migrants estimated correspond to successful emigrants, so that immigrants and unsuccessful emigrants are irrelevant on a genetic point of view. *Ne* estimates bear little relationship to the actual movements of birds between the habitats. In addition, direct estimates of number of reproducing migrants among and between habitats using ringed birds could not be used in this case, because of the low number of birds recaptured (unpublished data).

In conclusion, genic differentiation among and between habitat types is consistent with the source-sink hypothesis, although a larger data set covering additional source and sink populations, analysed with a larger number of loci, would allow a better statistical assessment.

Linkage disequilibrium

Linkage disequilibrium could be created by sampling individuals in several differentiated populations (Nei and Li, 1973). Because sink habitats are supposed

to contain mainly birds sampled from nearby source habitats which are differentiated (Tab. 3), a higher linkage disequilibrium is expected in evergreen habitats. This is true even if migrants or their offspring were sampled, as several generations of random mating are needed to suppress the linkage disequilibrium (see e.g., Hartl and Clark, 1989). We found a higher linkage disequilibrium in evergreen than in deciduous habitats, as expected; hence, this prediction is verified and supports the source-sink hypothesis.

Two other genetic consequences of a source-sink functioning might have been tested, but were not applicable to the current study.

First, allelic diversity (Nei, 1978) should be higher in sink than in source habitats, due to the immigration from distinct and differentiated source habitats. But testing this requires that source and sink habitats have the same population size, which is not the case in this study. Evergreen habitats have a lower population size (Blondel et al., 1993; Dias and Blondel, 1996), hence a tendency to present a reduced genetic diversity. The outcome of both opposing effects cannot be predicted without further theoretical work. In addition, the use of hypervariable markers generate high heterozygosity values (in this study the minimum was 0.915, details not shown). With such high values, differences could exist only at the second decimal place, and any statistics would require a large sample size.

Second, in the case of an absolute sink (i.e. no offspring survive in sink habitats), sampling should only capture birds born within source habitats, and this should result in an heterozygote deficiency at each locus (Wahlund, 1928). In the case of non absolute sink (i.e., survival of some offspring is possible), no heterozygote deficiency is expected in offspring as this effect disappears after one generation of random mating, and only direct migrants should be sampled to test this prediction. In the present study, some offspring survival has been observed in sink habitats (P. C. D., pers. observation), but direct migrants could not be correctly identified in samples, hence this last prediction could not be tested here.

Conclusion

This kind of study clearly shows the need of combining genetic with ecological studies to understand how natural populations function and evolve. Source-sink systems are at the crossing of population genetics and population dynamics. Elaborate methods based on such theories are expected to be available in the future. Several results presented here could be explained by an alternative hypothesis. For example, the higher linkage disequilibrium in sink habitats could also be explained by a higher drift if the population size is lower in these habitats than in sources: to disentangle drift and population mixing in the source-sink system requires specific work. However, previous studies using different ecological approaches have shown evidence of adaptation to dominant habitats and maladaptation in non-dominant habitat types in southern France and on Corsica (e.g., studies of life-history traits, morphometry, consequences on nestling condition and breeding success of the synchronisation between breeding date and food supply). All the results of the

present study are consistent with the hypothesis that an asymmetrical gene flow from source to sink habitats maintains maladaptation in sinks and with previous results from different approaches.

Acknowledgements

We thank Jacques Blondel (who initiated the Blue tit program in 1976), Marcel Lambrechts, Marie Maistre and Philippe Perret for their contribution to field work, Marleen Van den Broeck for participating a lot in lab work in Antwerp, Philippe Jarne for help and advice with lab work in Montpellier, Jerzy Banbura, Nick Barton, Jacques Blondel, Philippe Jarne, Marcel Lambrechts, Juha Merilä, Nicole Pasteur, François Rousset, Monty Slatkin and two anonymous reviewers for helpful comments on previous drafts of the manuscript. André Dhondt and Christine Van Broeckhoven received P.C.D. in their labs. G.R.V. was supported by an IWONL grant. The JNICT/Programa Ciência (Portugal), the french Ministère de la Recherche and the Service Commun de Biosystématique de Montpellier are acknowledged for financial support to PCD. This is paper No. 96.112 of the Institut des Sciences de l'Evolution.

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Received 1 January 1995;
accepted 14 May 1996.