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Habitat type does not affect population genetic structure in sympatric great tits (*Parus major*) and blue tits (*P. caeruleus*)

Jochen Blank[1], Michael J. Stauss[1], Jürgen Tomiuk[1] Joanna Fietz[2] & Gernot Segelbacher[3,4]

[1)] *Group of General Human Genetics, Department of Medical Genetics, Institute of Human Genetics, University of Tübingen, Wilhelmstrasse 27, D-72074 Tübingen, Germany*
[2)] *Department of Experimental Ecology (Biologie 3), University of Ulm, Albert-Einstein-Allee 11, D-89069 Ulm, Germany*
[3)] *Department of Wildlife Ecology and Management, University of Freiburg, Tennenbacher Str. 4, D-79085 Freiburg, Germany*
[4)] *Vogelwarte Radolfzell, Max Planck Institute of Ornithology, Schlossallee 2, D-78315 Radolfzell, Germany*

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Populations of two sympatric *Parus* species, the great tit (*Parus major*) and the blue tit (*Parus caeruleus*), were studied in deciduous and mixed-coniferous forests in southwestern Germany. We analysed the genetic variation of microsatellite and allozyme loci within and among populations to infer dispersal and potential source-sink dynamics in populations of both species that live in different habitat types. The genetic structure was found to be homogeneous across local populations of both species within years and genetic variation differed among years. The homogeneous genetic pattern among habitats suggests high migration and dispersal rates. Despite considerable differences in the breeding performance of individuals from different habitat types, there is no strong genetic evidence for source-sink dynamics in southwestern German populations of the great tit and the blue tit.

Introduction

In many ecological studies spatial dispersal patterns of organisms in heterogeneous environments are extensively discussed (for review *see* Kareiva 1990, Dias 1996). In this context the source-sink model is one of the most popular scenarios for individuals' dispersal in environments that are subdivided into patches of high and low quality (Pulliam 1988). Source-sink models assume constraints on migrants: e.g., (1) There can be density-dependent dispersal due to habitat selection (Pulliam 1988), or (2) a viable population living within a source habitat randomly releases a large number of individuals into sink habitats in which viability of populations is reduced and can only be maintained through the permanent input from source habitats (density-independent dispersal: Shmida & Ellner 1984, Holt 1985).

In Mediterranean blue tit populations genetic differentiation between local populations has been explained by source-sink dynamics and asymmetrical gene flow between different habitat types (Blondel *et al*. 1992, Dias *et al*. 1996) whereas genetic differences among local Belgian populations of blue tits (*Parus caeruleus*) were suggested to be the result from increased

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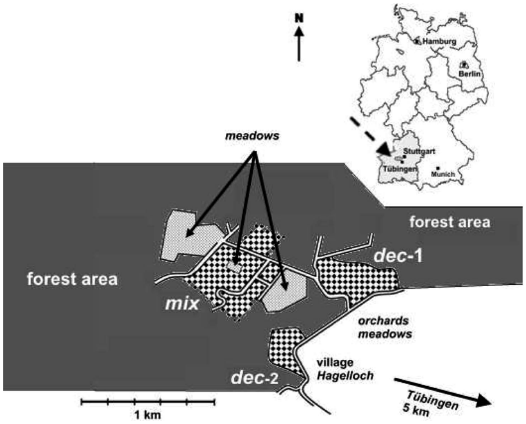

Fig. 1. Map showing the forest area in southwestern Germany and the location of three populations sampled in this study. Localities: 1 = deciduous woodland (*dec*-1, 9 ha); 2 = deciduous woodland (*dec*-2, 16 ha); 3 = mixed-coniferous woodland (*mix*, 35 ha). Scale bar = 1000 m. Forest stands are marked in grey, meadows in white, checked patterns indicate study sites.

genetic drift in a highly fragmented environment (Verheyen *et al.* 1997). Differentiation within local populations driven by density regulated differences in habitat quality can even be detected over small spatial scales as has been reported recently in a study of *Parus major* (Garant *et al.* 2005). Significant local differentiation has also been found in a population of citril finches (*Serinus citronella*) that were separated by only a few kilometres, as a result of fine scale habitat choice when individuals disperse non-randomly and gene flow is inhomogeneous (Senar *et al.* 2006). Furthermore, non-random dispersal of great tit individuals into different habitat types can be a prerequisite under which local conditions strongly influence nestling body mass (Garant *et al.* 2005), i.e. we can observe a diversifying effect.

The present study on sympatric populations of great tits (*Parus major*) and blue tits (*P. caeruleus*) tests the hypothesis that the more habitat-specialised blue tits (Stauss *et al.* 2005a, 2005b) show more pronounced genetic differentiation among different habitat types than sympatrically living great tits. If local adaptation to different habitat types occurs we expect genetic differences between local blue tit populations but not in great tits. However, our genetic data do not support the hypothesis that source-sink dynamics are realized in either of both species despite the lower breeding performance of blue tits in the low quality habitat.

Materials and methods

Populations of great tits (*Parus major*) and blue tits (*P. caeruleus*) were investigated in three different forest areas near Tübingen (48°33´N, 9°00´E) in southwestern Germany (Fig. 1). Two study plots (*dec*-1 and *dec*-2) that were geographically separated within the same forest area by approximately 1 km were deciduous woodland (9 ha and 16 ha, respectively). They mainly consisted of beech *Fagus sylvatica* (67%), oak *Quercus robur* (21%) and other deciduous trees (3%) but coniferous trees such as pine *Pinus sylvestris*, spruce *Picea abies* and larch *Larix decidua* also occurred in very low numbers (9%). Our third study area (*mix*) was a mixed-coniferous forest of approximately 35 ha dominated by coniferous trees and separated by approximately 1 km from the other two study plots. It consists of Scots pine (39%), spruce (18%), beech (41%) and a small number of other trees (2%). Within the study area no breeding pairs in natural holes could be observed. Deciduous study plots are considered high quality habitats, whereas the mixed-coniferous study plot is considered a low quality habitat.

All nests were checked weekly during the breeding season from 1990 to 2002 and the occupancy of the nest boxes, the number of hatchlings and fledglings, respectively, were recorded. The nestlings were weighed between the 14th–17th day after hatching (data not shown). For blood sampling individuals were captured in the nest box while feeding their nestlings and were classified as yearlings or birds older than one year according to Svensson (1992). Adults were caught and individually marked with numbered aluminium rings. The average success rate of trapping adults (number of captured birds in relation to the number of birds breeding in the nest boxes) was 0.84 for female and 0.80 for male blue tits, and 0.78 for female and 0.71 for male great tits, respectively. The survival rates of adults were estimated by the frequency of recaptured individuals in the breeding season of succeeding years. The proportions of recaptured individuals and immigrants with respect to habitat type, sex and age were analysed using χ^2-test.

In total 342 adult great tits and 337 adult blue tits were studied genetically during the breeding seasons in 1994 to 1996, 1999 and 2000 (for description of sample sizes *see* Table 1). Blood samples (about 50 μl) were obtained through tapping the *vena ulnaris*. Blood was stored in 250 μl EDTA buffer (10% EDTA, 1% NaF, and thymol, adjusted to pH 8, Arctander 1988) in plastic vials and frozen until further processing. About 150 μl of the blood sample was used for DNA isolation using the Qiagen DNA Blood Mini Kit (Qiagen, Hilden, Germany). To study genetic variation of nine microsatellite loci, PCR reactions were set with the following primers:

*Gf*04 (Petren 1998): forward primer CCT TTG CAA AAC CGG GTC TG, reverse primer TTT TCT TAT ATC TAT TGA GAG ATG GT.

*Gf*06 (Petren 1998): forward primer GCT ATT GAG CTA ACT AAA TAA ACA ACT, reverse primer CAC AAA TAG TAA TTA AAA GGA AGT ACC.

Mcyμ-4 (Double *et al.* 1997): forward primer ATA AGA TGA CTA AGG TCT CTG GTG, reverse primer TAG CAA TTG TCT ATC ATG GTT TG:

*Pat*14 (Galbusera *et al.* 2000): forward primer GAA CAG ATA AAG CCA AAT TAC, reverse primer TAG TGA ATG CTT GAT TTC TTT G.

*Pat*43 (Galbusera *et al.* 2000): forward primer ACA GGT AGT CAG AAA TGG AAA G, reverse primer GTA TCC AGA GTC TTT GCT GAT G.

*Pca*9 (Dawson *et al.* 2000): forward primer ACC CAC TGT CCA GAG CAG GG, reverse primer ACC ACT GCA GCA GTT TGT GGG.

*Pk*12 (GenBank accession number AF041466): forward primer CCT CCT GCA GTT GCC TCC CG, reverse primer CGT GGC CAT GTT TAT AGC CTG G.

*POCC*6 (Bensch *et al.* 1997): forward primer TCA CCC TCA AAA ACA CAC ACA, reverse primer ACT TCT CTC TGA AAA GGG GAG C:

*POCC*8 (Bensch *et al.* 1997): forward primer GCA TGT CTC TTC AGA CAT CTG C, reverse primer ATG TAG AGC TCC CAT GGT GG.

Our reaction profiles were 94 °C for 3 min, X °C for 1 min (X = 54 °C for *Gf*04 and *POCC*8; 55 °C for *Gf*06, *Pat*43, *Mcyμ*4 and *POCC*6; 56 °C for *Pk12*; 57 °C for *Pat*14 and *Pca*9), then 65 °C for 1 min, followed by 94 °C for 30 s, Y °C for 30 s (Y = 48 °C for *Gf*04; 53 °C for *POCC*8; 54 °C for *Gf*06 and *Mcyμ*-4; 55 °C for *Pat*43 and *POCC*6; 56 °C for *Pat*14 and *Pk*12; 57 °C for *Pca*9) and 65 °C for Z s (Z = 30 s for *Gf*04, *Gf*06, *Pat*14 and *Pat*43; 60 s for *Mcyμ*-4, *Pca*9, *Pk*12, *POCC*6 and *POCC*8,) for N cycles (N = 25 for *Pca*9; 26 for *POCC*6, 29 for *Mcyμ*-4 and *Pat*14; 30 for *Gf*04 and *Pk*12; 32 for *Pat*43; 36 for *Gf*06, 39 for *POCC*8), then 65 °C for 30 s. DNA amplifications were performed with a Peltier thermal cycler PTC-200 and PCR products were subsequently processed for fragment length polymorphism. At the beginning of our study, microsatellite variation was studied by polyacrylamide gel electrophoresis and silver staining and later on analyses were done on an automatic sequencer (ABI 310, Applied Biosystems, Forster City, USA).

The remaining blood of individuals sampled in 1999 and 2000 was electrophoresed for three esterase loci (*EST*-1, *EST*-2 and *EST*-3) and the

Table 1. Sample sizes of genetically characterized adult individuals of great tits (*P. major*) and blue tits (*P. caeruleus*) in three southwestern German woodlands (one mixed-coniferous forest: *mix*, two deciduous forests: *dec-1* and *dec-2*). Individuals were sampled during the breeding seasons from 1994 to 2000. The numbers left to the slash refer to great tits and to the right to blue tits.

	Habitat type (great tit/blue tit)			
Year	*mix*	*dec-1*	*dec-2*	Total
1994	20/17	15/19	–/–	35/36
1995	23/14	18/29	–/–	41/43
1996	28/25	24/36	–/–	52/61
1999	36/27	33/34	34/29	103/90
2000	52/36	30/36	29/35	111/107
Total	159/119	120/154	63/64	342/337

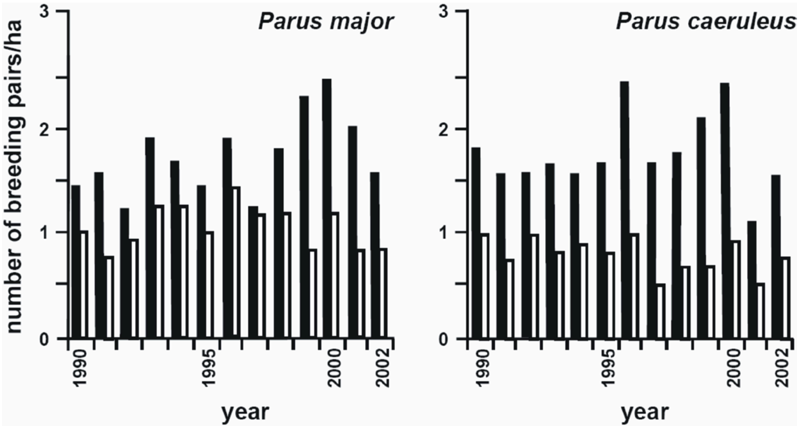

Fig. 2. Number of breeding pairs per ha of great tits (*P. major*) and blue tits (*P. caeruleus*) in the breeding seasons from 1990 to 2002. Black columns represent the deciduous habitat and white columns the mixed-coniferous habitat.

phosphoglucose isomerase locus (*PGI*) (Driesel *et al*. 2004).

The computer programs GENEPOP 3.4 (Raymond & Rousset 1995), FSTAT 2.93 (Goudet 2001), ARLEQUIN 3.0 (Excoffier *et al*. 2005), CERVUS 2.0 (Marshall *et al*. 1998) and SAS 9.1 (SAS Institute 2003) were used to analyse genetic and demographic population structures. For the analysis of molecular variance (AMOVA) (Excoffier *et al*. 1992) only microsatellite data were analysed to compare populations among all years. In the case of multiple testing a sequential Bonferroni procedure was applied in order to compensate for an inflating type 1 error (Jaccard & Wan 1996).

Results

Number of breeding pairs

Breeding density was estimated through the mean number of breeding pairs per ha on the basis of surveys from 1990 to 2002 (Fig. 2). In great tits as well as in blue tits the number of breeding pairs in deciduous forests was almost twice as large as that observed in mixed-coniferous forests (deciduous habitat: 1.73 ± 0.38 for great tits and 1.76 ± 0.37 for blue tits; mixed-coniferous habitat: 1.04 ± 0.21 for great tits and 0.79 ± 0.18 for blue tits). In both species the number of breeding pairs differed significantly between habitat types ($t > 5.67$, df < 19, $p < 0.001$). Nevertheless, the number of breeding pairs did not differ significantly between habitat types across years ($r_s < 0.27$, $p > 0.30$, $n = 13$).

Reproductive success of breeding pairs

The mean number of great tit fledglings per nest box and their average body mass was similar in both habitat types ($F < 1.64$, $df_1 = 1$, $df_2 > 282$, $p > 0.20$). Fledgling mass and fledgling number, however, changed significantly across years ($F > 6.93$, $df_1 = 10$, $df_2 > 282$, $p < 0.001$) and there was a significant combined habitat-year effect on the fledgling mass ($F = 4.36$, $df_1 = 10$, $df_2 = 283$, $p < 0.001$).

The mean number of blue tit fledglings per nest box and their average body mass was significantly higher in the deciduous than in the mixed habitat ($F > 16.62$, $df_1 = 1$, $df_2 > 291$, $p < 0.001$). Again both reproductive parameters varied significantly across years ($F > 8.14$, $df_1 = 10$, $df_2 > 291$, $p < 0.001$) but there was no significant interactive effect of habitat type and year ($F < 1.82$, $df_1 = 9$, $df_2 > 291$, $p > 0.07$).

Survival

We estimated survival between succeeding years and compared the demographic parameters from two different habitat types; deciduous (*dec-1* and *dec-2*) and mixed-coniferous (*mix*) forests (Table 2). The breeding tit population consisted of residents and immigrants from outside the study area. The frequency of recaptured individuals and immigrants is homogeneous in the different habitat types for both species and even across species (within species: $\chi^2 < 1.141$, df = 1, $p > 0.423$; across species: $\chi^2 = 2.806$, df = 3, $p = 0.423$). The average rate of recaptured individuals is about 35% in succeeding years. Similarly, the frequency of immigrating yearlings and adults is homogeneous in both habitats and species (within species: $\chi^2 < 0.645$, df = 1, $p > 0.422$; across species: $\chi^2 = 0.712$, df = 3, $p = 0.870$). The group of immigrants consisted of 65% yearlings, and was independent of habitat type, species and sex.

The comparison of the distribution of cohorts within the group of recaptured individuals revealed differences between the species. There was a homogeneous distribution of yearlings (~20%) and adults older than one year in both habitats in great tits ($\chi^2 = 0.014$, df = 1, $p = 0.906$) but a significantly lower number of blue tit yearlings in the mixed-coniferous forest (~8%) than in the deciduous forest ($\chi^2 = 0.014$, df = 1, $p = 0.018$).

Considering the group of recaptured individuals in both species, only 1%–2% of the adult birds migrated to a different habitat type from one year to the next but 15%–35% of the yearlings bred in habitat types that differed to that of their natal site (Table 2). About 45% of yearlings found breeding populations in both species. Accepting that 20% of individuals change habitat type, we conclude that roughly 10% of yearlings originate from another habitat type.

Population genetics

Great tit

From 1994 to 1996 and 1999 to 2000 individuals breeding in two and three woodlands, respectively, (12 temporally and spatially different breeding populations) were genotyped at 12

Table 2. Recaptured individuals and immigrants of great tits and blue tits in two deciduous and one mixed-coniferous forests during the breeding seasons from 1994 to 2002. Two groups of individuals are considered: 1. Adults older than one year and 2. one year old adults (yearlings). Recaptured individuals are separated into individuals observed in the same habitat type in consecutive years (no change) and those observed in a different habitat type in preceding years (change).

	Recaptured				Immigrants	
	No change		Change			
	♂	♀	♂	♀	♂	♀
Great tit						
Deciduous forest						
Older than one year	33	41	1	–	33	14
Yearlings	8	3	4	1	33	54
Total	85		6		134	
Mixed-coniferous forest						
Older than one year	29	33	–	2	22	11
Yearlings	7	3	2	1	21	37
Total	72		5		91	
Blue tit						
Older than one year	54	41	–	–	32	26
Yearlings	9	13	3	1	42	76
Total	117		4		176	
Mixed-coniferous forest						
Older than one year	35	23	–	1	15	26
Yearlings	4	–	1	–	27	41
Total	62		2		109	

polymorphic loci (enzymes: *PGI*, *EST*-1, *EST*-2 and *EST*-3; microsatellites: *Gf*04, *Gf*06, *Mcyμ*-4, *Pat*14, *Pat*43, *Pk*12, *POCC*6 and *POCC*8). The number of alleles ($3 \leq n \leq 18$) and the observed degree of genetic heterozygosity ($0.089 \leq H_{obs} \leq 0.940$) showed large variation among loci (*see* Appendix 1). The Hardy-Weinberg equilibrium could be tested in 102 individual data sets, each defined by locus, habitat type and the year of sampling — for the remaining 18 combinations, loci were monomorphic within the respective population. In only four cases we observed a significant deficiency of heterozygotes (locus/year/habitat: *Gf*04/1999/*mix*, *Gf*04/2000/*mix*, *Gf*06/2000/*mix, Pk*12/1999/*dec*-1). The remaining genotype distributions of loci found within different years and habitats are close to the Hardy-Weinberg distribution. Significant genotypic linkage disequilibrium was not detected for most pairs of loci. Only *EST*-2 and *EST*-3 showed consistently significant genotypic linkage disequilibrium in the populations from 1999 and 2000 (*see* Appendix 2). Thus the results of our population studies are consistent with those of linkage analyses in families that disclosed linkage among esterase loci (Stauss *et al.* 2003). Nevertheless, genotypic linkage disequilibrium between all esterase loci, e.g. between *EST*-1/*EST*-2 and *EST*-1/*EST*-3, might have disappeared by recombination during many generations.

Twelve tests of homogeneity revealed genotypic differences between populations across all years for three loci *Gf*06, *Mcyμ*-4 and *Pk*12. The genetic differentiation considering all populations was low with $F_{ST} = 0.010$ where the 95% confidence interval of F_{ST} ranges from 0.001 to 0.020.

Blue tit

Individuals that lived within the same woodlands, as the great tits studied here, were genotyped at 11 polymorphic loci (enzymes: *PGI*, *EST*-1, *EST*-2 and *EST*-3; microsatellites: *Gf*04, *Gf*06, *Mcyμ*-4, Pat43, *Pca*9, *Pk*12 and POCC6). The number of alleles ($2 \leq n \leq 13$) and the observed degree of genetic heterozygosity ($0.072 \leq H_{obs} \leq 0.806$) show large variation among loci (*see* Appendix 1). The Hardy-Weinberg equilibrium could be tested in 105 individual data sets, each defined by locus, habitat type and the year of sampling – for the remaining 15 combinations, loci were monomorphic within the respective population or only few genotypes were analysed in populations. Using the sequential Bonferroni procedure genotypic distribution of only two samples indicated significant deviations from Hardy-Weinberg proportions (in 2000: *EST*-2 in the deciduous forest *dec-1*, and locus *Pk*12 in the mixed-coniferous forest). Significant genotypic linkage disequilibrium was only found for the combination *Mcyμ*-4/Pca 9 in one from five years (1994 and 1995: $p = 1.000$; 1996: $p = 0.742$; 1999: $p = 0.045$, 2000: $p < 0.001$; *see* Appendix 3). Eleven tests for genetic homogeneity revealed differences between populations across habitats and years for only two loci (*Gf*06 and *POCC*6). The genetic differentiation considering all populations was about five times higher than in great tits with $F_{ST} = 0.055$ where the 95% confidence interval of F_{ST} ranges from 0.009 to 0.144.

For both species the analysis of molecular variance revealed that more than 90% of the total genetic variation occurred within populations ($p < 0.001$, Tables 3 and 4). Relatively high temporal variation (2%–8%) across years was also found in populations from different woodlands (Table 3) and from different habitat types (2%–7%, Table 4) but genetic differences between habitat types within years account for less than 1% to the total variation ($p > 0.151$, Table 4).

Discussion

Theory predicts the following genetic and demographic characteristics for source and sink populations (Watkinson & Sutherland 1995, Dias *et al.* 1996, Gaggiotti 1996, Hanski & Gilpin 1997, Diffendorfer 1998, Rousset 1999): (1) reproductive rates differ significantly between source and sink populations, (2) gene flow between populations of the same habitat type is low, (3) if migration from source to sink populations is large, homogeneous genotypic or allelic structures are expected, (4) if migration from source to sink populations is low, selection may produce genetic differences between source and sink populations, (5) individuals migrate from different source populations into sink populations. Such mixed populations can show genotypic distributions that deviate from the Hardy-Weinberg equilibrium due to heterozygote deficiency (Wahlund 1928). However, the observed significant deviations of genotype frequencies from the Hardy-Weinberg proportions at some loci as well as the significant genotypic differences between some pairs of habitats observed in our study can most likely be attributed to a random sampling bias because the significant deviations do not show consistent spatial and temporal patterns across all loci.

Great tits and blue tits that live in central Europe are well adapted to deciduous woodland. In this type of habitat the tits showed higher breeding performance compared to other habitats like mixed-coniferous woodlands due to higher food availability (Dhondt *et al.* 1984, Blondel *et al.* 1987, Cowie & Hinsley 1987, van Balen & Potting 1990). Furthermore, survival related to juvenile condition can be assumed to be lower in mixed-coniferous than in deciduous forests (e.g. Verboven & Visser 1998, Both *et al.* 1999, Naef-Daenzer *et al.* 2001, Monros *et al.* 2002). Therefore, in the context of source-sink processes, deciduous forest populations may act

Table 3. Genetic differences among populations of two *Parus* species from three different woodlands (*dec*-1, *dec*-2 and *mix*, for further details *see* Material and methods) analysed with hierarchical AMOVA that is based on nine microsatellites. The estimates of tiers of genetic diversity — (1) among three woodlands, (2) among populations from different years (1994–1996, 1999 and 2000) within woodlands and (3) within populations — and their relative contribution to the total variance are given. The *F* values resulting from *F*-statistics and the corresponding type-1 error *p* (H_0: $F = 0$) are listed.

Source of variation	Variance component	Variance component (%)	*F*-statistic	*p*
Great tit				
Among three woodlands	0.004	0.21	0.002	0.308
Among populations from different years within woodlands	0.040	2.08	0.021	<0.001
Within populations	1.887	97.71	0.023	< 0.001
Blue tit				
Among three woodlands	< 0.001	0.01	< 0.001	0.432
Among populations from different years within woodlands	0.184	7.35	0.074	<0.001
Within populations	2.314	92.63	0.074	<0.001

Table 4. Genetic differences between populations of two *Parus* species from two different habitat types (deciduous and mixed-coniferous forests) analysed with hierarchical AMOVA that is based on nine microsatellites. The estimates of tiers of genetic diversity — (1) between two habitat types, (2) among populations from different years within habitat types and (3) within populations — and their relative contribution to the total variance are given. The *F* values resulting from *F*-statistics and the corresponding type-1-error *p* (H_0: $F = 0$) are listed.

Source of variation	Variance component	Variance component (%)	*F*-statistic	*p*
Great tit				
Between habitat types	0.004	0.19	0.002	0.151
Among populations from different years within habitat type	0.034	1.60	0.016	< 0.001
Within populations	2.062	98.21	0.018	< 0.001
Blue tit				
Between habitat types	< 0.001	< 0.001	< 0.001	0.275
Among populations from different years within habitat type	0.184	7.93	0.079	< 0.001
Within populations	2.135	92.07	0.078	< 0.001

as source populations and sink populations can be found in mixed-coniferous forest in southwestern Germany. However, despite the higher number of breeding pairs in the deciduous than mixed-coniferous habitat, the genetic structure of southwestern German populations do not disclose the predicted genetic differences within and among populations from deciduous (source) and mixed-coniferous (sink) habitats in both species. High genetic homogeneity among different habitats and even between succeeding years is more likely to be the result of large bidirectional migration between different habitat types and large dispersal rates over long distances. In both tit species immigration is considerably high in all local populations. 50%–100% of all adult birds hatch outside the patch where they reproduce and more than 90% of immigrants disperse more than 2 km from their natal site (Winkel & Frantzen 1991, Verhulst *et al.* 1997, Matthysen *et al.* 2001). However, in this study the interaction between habitat type and reproductive performance is different in both species. Compared to blue tits, great tits are resource generalists: (1) the reproductive potential of great tits is not higher in deciduous woodlands than in the mixed-coniferous ones, (2) the number and the quality of their offspring do not differ between habitat types, (3) the proportion of recaptured yearlings (local recruits) among all recaptured individuals is also similar for deciduous and mixed-coniferous habitats, and (4) in great tits, the quality of adult males and females (body mass, tarsus length, condition calculated as body mass divided by tarsus length) do not differ significantly between habitat types (Stauss *et al.* unpubl. data) but the number of breeding pairs per ha was higher in the deciduous than in the mixed-coniferous habitat. Differently, blue tits appear to be better adapted to deciduous forests: (1) they have higher breeding success in high quality habitats than in low quality ones, (2) the number of fledglings is higher and offspring are in better condition in the deciduous than in the mixed-coniferous habitat, and (3) during the period of nestling provisioning, parental foraging effort is considerably lower in the deciduous habitat due to shorter distances between the nest and the foraging sites. Considering the number and quality of offspring and the parental investment, the benefit-to-cost ratio is two to three times higher in the deciduous habitat than in the mixed-coniferous woodland (Stauss *et al.* 2005b). Summarizing, the more resource generalist *P. major* seems to be well-adapted to deciduous as well as mixed-coniferous forests and, therefore, our data support random dispersal of individuals for this species.

Considering demographic and genetic data in both species the argument that high migration and dispersal rates create homogeneous genetic structures in different habitat types is strongly supported. Results of the AMOVA did not reveal any genetic differences between different habitat types (deciduous versus mixed-coniferous) nor among the three different woodlands. But we found that a considerable proportion of genetic variation can be explained by differences among years. Obviously this suggests that future studies of fine scale genetic structure should be based rather on long term data series than on observations from only one year.

In both species we observed a large proportion of immigrating individuals not recaptured in habitats during two succeeding years and recaptured yearlings had a high tendency to change habitat types. The relatively high number of fledglings that change habitat type can therefore explain the temporally and spatially homogeneous genetic structures of our populations. Local environmental conditions, e.g., the availability and quality of food resources determine differently, but without a lasting adaptational effect, the reproduction of the species. The evolutionary scenario, however, might be different in geographically different populations. Habitat colonization strategies of blue tit populations in the Mediterranean area were explained by local adaptation where a mosaic of habitats with different qualities acts as a source-sink system (Blondel *et al.* 1992). Laying date, clutch size and breeding success may be adapted in relation to food supply in different habitats. Blondel *et al.* (1992) assumed that birds can genetically be programmed to breed in the respective habitat by temporarily adjusting to food availability and abundance. Thus migrating individuals experience a fitness loss through mistiming when changing habitat type. Such adaptive processes to habitat quality are widely known in birds and those adaptive preferences are often correlated with survival and reproductive success. Accordingly, Postma and Noordwijk (2005) observed that differences in dispersal rates can have a strong influence on the evolution of local adaptation and genetic population structures in Dutch island populations of great tits.

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Appendix 1. Parameters of genetic diversity of four enzyme loci and nine microsatellites estimated from southwestern German populations of *P. major* and *P. caeruleus*. Adult individuals were sampled during the breeding seasons from 1994 to 2000. The number of alleles, the expected and observed degree of genetic heterozygosity H_{exp} *and* H_{obs}, the allele frequencies and an estimate of the frequency of null alleles f_{n0} (Marshall *et al*. 1998) are listed. The order of allele frequencies do not correspond to identity between species but only to their magnitude. The number left of the slash refer to *P. major* and the right one to *P. caeruleus*.

Locus	n_a	H_{exp}	H_{obs}	Allele frequency	f_{n0}	m
*PGI**	4/5	0.192/0.072	0.191/0.074	0.895/0.964 0.086/0.013 0.012/0.013 0.007/0.008 /0.002	0 048/–0.010	209/190
EST-1*	6/2	0.528/0.460	0.548/0.577	0.656/0.643 0.273/0.357 0.082/– 0.072/– 0.012/– 0.005/–	-0.024/-0.114	208/189
EST-2*	6/4	0.62/0.680	0.634/0.735	0.536/0.467 0.286/0.235 0.087/0.198 0.046/0.100 0.036/– 0.010/–	-0.006/-0.054	208/189
EST-3*	6/5	0.571/0.741	0.573/0.730	0.600/0.347 0.247/0.254 0.082/0.196 0.051/0.196	-0.006/0.005	206/189

continued

Appendix 1. Continued.

				0.015/0.008		
				0.005/–		
*Gf*04	9/6	0.605/0.4576	0.438/0.442	0.446/0.716	0.109/-0.003	242/292
				0.440/0.151		
				0.043/0.086		
				0.023/0.041		
				0.019/0.005		
				0.012/0.002		
				0.012/–		
				0.002/–		
				0.002/–		
*Gf*06	18/6	0.811/0.691	0.741/0.626	0.354/0.488	0.043/0.054	332/326
				0.176/0.206		
				0.114/0.123		
				0.095/0.097		
				0.066/0.077		
				0.053/0.011		
				0.041/–		
				0.039/–		
				0.027/–		
				0.015/–		
				0.006/–		
				0.003/–		
				0.003/–		
				0.002/–		
				0.002/–		
				0.001/–		
				0.001/–		
				0.001/–		
*Mcy*µ-4	12/11	0.626/0.853	0.549/0.815	0.519/0.309	0.029/0.007	259/319
				0.315/0.186		
				0.041/0.131		
				0.040/0.102		
				0.037/0.088		
				0.033/0.063		
				0.004/0.040		
				0.003/0.032		
				0.003/0.023		
				0.001/0.015		
				0.001/0.011		
				0.001/–		
*Pat*14	4/-	0.084/–	0.086/–	0.957/–	0.008/–	336/–
				0.025/–		
				0.016/–		
				0.002/–		
*Pat*43	5/7	0.116/0.703	0.115/0.411	0.940/0.414	0.037/0.276	339/328
				0.028/0.321		
				0.018/0.120		
				0.010/0.073		
				0.004/0.055		
				-/0.014		
				-/0.002		
*Pca*9	-/13	-/0.862	-/0.824	-/0.275	-/0.023	-/324
				-/0.133		
				-/0.128		
				-/0.091		
				-/0.079		

continued

Appendix 1. Continued.

				-/0.076		
				-/0.060		
				-/0.048		
				-/0.042		
				-/0.026		
				-/0.026		
				-/0.012		
				-/0.005		
*Pk*12	8/9	0.719/0.755	0.557/0.652	0.358/0.422	0.125/0.082	334/328
				0.320/0.181		
				0.219/0.120		
				0.057/0.107		
				0.021/0.079		
				0.009/0.034		
				0.009/0.021		
				0.007/0.018		
				-/0.015		
				-/0.002		
*POCC*6	14/13	0.862/0.849	0.932/0.809	0.222/0.255	0.003/0.025	263/320
				0.218/0.209		
				0.114/0.148		
				0.106/0.094		
				0.084/0.063		
				0.076/0.048		
				0.042/0.042		
				0.028/0.040		
				0.026/0.031		
				0.024/0.014		
				0.020/0.011		
				0.020/0.011		
				0.014/0.002		
				0.006/–		
*POCC*8	3/1	0.502/0.000	0.605/0.000	0.536/1.000	–0.086/–	337/36
				0.449/–		
				0.015/–		

* frequency data from Driesel *et al.* (2004)

Appendix 2. Genotypic linkage disequilibrium between pairs of 11 loci studied in great tits. The type-1 errors are listed for five samples from 1994–1996, 1999 and 2000 (boldface: significant at the 5% significance level; italics: significant after Bonferroni correction).

Locus	*EST*-1	*EST*-2	*EST*-3	*Gf*04	*Gf*06	*Mcyμ*-4	*PAT*14	*PAT*43	*Pk*12	*POCC*6	*POCC*8
PGI											
1999	0.947	0.989	0.745	0.813	0.707	0.701	0.868	0.620	**0.020**	0.136	0.472
2000	0.667	0.968	0.997	0.770	0.801	**0.015**	0.238	0.824	0.121	0.073	0.416
EST-1											
1999		0.445	**0.044**	0.642	0.502	0.841	0.351	0.351	0.599	0.382	0.943
2000		***< 0.001***	**0.045**	0.300	0.967	0.706	0.829	0.486	0.369	0.650	0.287
EST-2											
1999			***< 0.001***	0.335	0.589	0.172	0.462	0.183	0.138	0.630	0.231
2000			***< 0.001***	0.125	0.365	0.304	0.362	0.646	0.178	**0.036**	0.775
EST-3											
1999				0.863	0.857	0.143	0.910	0.088	0.052	**0.048**	0.672
2000				**0.028**	0.084	0.420	0.952	0.368	0.710	0.811	0.979

continued

Appendix 2. Continued.

*Gf*04							
1994	0.659	**0.007**	0.066	0.177	0.753	0.805	**0.039**
1995	0.279	0.623	0.651	0.496	0.526	0.500	0.111
1996	0.514	0.677	0.446	0.569	0.894	0.383	0.751
1999	0.564	**0.017**	0.478	0.275	0.568	0.392	0.117
2000	0.337	0.450	0.797	0.612	0.334	0.527	0.487
*Gf*06							
1994		0.720	0.164	0.494	0.610	0.573	0.979
1995		0.516	0.078	0.824	0.184	0.683	0.493
1996		**0.006**	0.567	0.398	0.138	0.514	0.223
1999		0.957	0.067	0.124	0.335	0.680	0.093
2000		0.653	0.211	0.280	0.634	**0.008**	0.471
Mcyμ-4							
1994			1.000	0.749	0.970	0.521	0.280
1995			0.874	0.147	0.985	0.789	0.391
1996			0.369	0.720	0.853	0.491	0.208
1999			0.590	0.847	0.068	0.647	0.178
2000			0.805	0.349	0.414	0.842	0.509
*PAT*14							
1994				1.000	0.219	0.570	1.000
1995				1.000	0.561	1.000	0.404
1996				1.000	0.976	0.607	**0.008**
1999				0.306	0.071	0.534	0.348
2000				0.767	0.095	0.274	0.627
*PAT*43							
1994					0.516	0.789	0.360
1995					0.652	0.067	0.055
1996					0.500	0.354	0.982
1999					0.840	0.817	0.455
2000					0.523	0.473	0.085
*Pk*12							
1994						**0.018**	0.584
1995						0.408	0.406
1996						0.350	0.276
1999						0.646	0.341
2000						0.418	0.252
*POCC*6							
1994							0.694
1995							0.190
1996							0.287
1999							0.663
2000							0.437

Appendix 3. Genotypic linkage disequilibrium between pairs of 10 loci studied in blue tits. The type-1-errors are listed for five samples from 1994-1996, 1999 and 2000 (boldface: significant at the 5% significance level; italics: significant after Bonferroni-correction).

Locus	*EST*-1	*EST*-2	*EST*-3	*Gf*04	*Gf*06	*Mcyμ*-4	*PAT*43	*Pca*9	*Pk*12	*POCC*6
PGI										
1999	1.000	0.661	0.294	0.379	0.203	0.552	0.148	0.633	0.750	0.517
2000	1.000	0.832	0.441	0.168	0.169	0.920	0.653	0.277	0.340	0.357
EST-1										
1999		0.472	0.194	0.669	0.688	0.703	0.115	0.303	0.600	0.366
2000		0.084	0.211	0.892	0.713	0.885	0.410	0.768	0.624	0.545

continued

Appendix 3. Continued

EST-2								
1999	**0.037**	0.338	0.384	0.052	0.237	0.209	0.750	**0.045**
2000	0.104	0.295	0.779	0.326	0.846	0.454	0.341	0.332
EST-3								
1999		0.904	0.967	0.821	0.510	0.912	0.777	0.398
2000		0.225	0.711	0.808	0.057	0.584	0.208	0.349
*Gf*04								
1994			0.302	0.713	0.488	0.928	0.966	0.820
1995			0.363	0.661	0.274	0.632	0.163	0.426
1996			0.686	0.802	0.897	0.498	0.804	0.373
1999			0.855	0.151	0.288	0.998	0.457	0.714
2000			0.347	0.655	0.357	0.463	0.946	0.563
*Gf*06								
1994				0.230	0.410	0.138	0.929	0.805
1995				0.297	0.472	0.967	0.821	0.819
1996				0.872	0.156	0.794	0.649	0.854
1999				0.562	0.770	0.939	0.080	0.665
2000				0.355	0.838	0.702	0.819	0.622
Mcyμ-4								
1994					0.154	1.000	**0.003**	1.000
1995					0.866	1.000	0.563	1.000
1996					0.493	0.742	0.224	0.890
1999					0.205	**0.045**	0.445	0.172
2000					0.404	***< 0.001***	0.930	0.903
*PAT*43								
1994						0.066	0.512	0.881
1995						0.511	0.685	0.907
1996						0.736	0.052	0.236
1999						0.384	0.945	0.139
2000						0.233	0.112	0.251
*Pca*9								
1994							0.915	0.195
1995							0.522	1.000
1996							0.844	1.000
1999							0.876	0.255
2000							0.730	0.223
*Pk*12								
1994								0.771
1995								0.279
1996								0.322
1999								0.940
2000								0.548