Belg. J. Zool. - Volume 127 (1997) - issue 2 - pages 179-189 - Brussels 1997

Received : 14 August 1997

DIFFERENTIATION AMONG BLUE TIT (*PARUS CAERULEUS*) POPULATIONS MEASURED WITH FIVE MINISATELLITE SINGLE LOCUS PROBES

GEERT R. VERHEYEN([1+2]), ERIK MATTHYSEN([1]), FRANK ADRIAENSEN([1]), CHRISTINE VAN BROECKHOVEN([2]) AND ANDRE A. DHONDT([1+3])

([1])Laboratory of Animal Ecology, Department of Biology, University of Antwerp (UIA), Universiteitsplein 1, B-2610 Antwerpen, Belgium.
([2])Laboratory of Neurogenetics, University of Antwerp (UIA), Department of Biochemistry, Universiteitsplein 1, B-2610 Antwerpen, Belgium.
([3])Laboratory of Ornithology, Cornell University, Ithaca, New York 14850, USA.
e-mail: vheyen@hgins.uia.ac.be

Abstract. Five hypervariable minisatellite loci were scored by Southern hybridisation to study the genetic differentiation among eight blue tit (*Parus caeruleus*) populations. All loci display extreme levels of polymorphism in each of the populations. Deviations from Hardy-Weinberg equilibrium are detected for loci in some populations. There does not seem to be an influence of the current degree of habitat fragmentation on genetic variation. However, despite high levels of gene flow (low Fst-values), significant differentiation is found among some populations at these loci. The populations differentiate according to random drift patterns, but, as suggested by cluster analysis, there might still be a small influence of gene flow.

Keywords: minisatellites, single locus probe, genetic structure, random drift

INTRODUCTION

The application of DNA fingerprinting (GILBERT *et al.*, 1990), RAPDs (HADRYS *et al.*, 1993) and single locus profiling with minisatellite (HANOTTE *et al.*, 1991) or microsatellite (ESTOUP *et al.*, 1995) markers in population genetic studies is increasing. However, to date, most population genetic studies in birds employ allozyme polymorphisms (JOHNSON & MARTEN, 1989) or mitochondrial DNA (WENINK *et al.*, 1993), and rarely use the more recently developed techniques (HAIG *et al.*, 1993).

As part of our study on the reproductive success of the blue tit (*Parus caeruleus*, KEMPENAERS *et al.*, 1992), several hypervariable minisatellite single locus probes (SLPs) were developed (VERHEYEN *et al.*, 1994). These markers were subsequently applied in a preliminary study to describe the genetic population structure of blue tits in three populations around Antwerp, North Belgium (VERHEYEN *et al.*, 1995). Here, using 5 minisatellite loci, we study the genetic structure of 8 blue tit populations over a wider geographic

range in more depth. Our goal is to determine if the genetic variation and population differentiation follow specific geographical patterns and if habitat fragmentation has an effect on genetic variation within blue tit populations. In this respect, this study is part of a larger program in which the effects of habitat fragmentation on several animal species (birds, mammals and insects) are evaluated (MATTHYSEN *et al.*, 1995).

MATERIAL AND METHODS

Isolation and characterisation of minisatellite markers

Five minisatellite loci detected by three single locus probes (SLPs; cPcaMS1, cPcaMS3 and cPcaMS14) and one double locus probe (DLP; cPcaMS8, the two detected loci have well separated allele sizes), were used. The alleles of these markers range from 1.8 kb up to 14 kb. The isolation and initial characterisation of these blue tit specific markers is described in VERHEYEN *et al.* (1994). Details concerning the collection of blood samples and the techniques used during the processing of blood samples to single locus profiles can be found in VERHEYEN *et al.* (1994 and 1995). In short: high molecular weight DNA was digested with *Hinf*I restriction enzyme. Fragments were separated overnight by electrophoresis in 0.6% agarose gels. The DNA was subsequently transferred onto nylon membranes by Southern blotting. SLPs are [α–^{32}P]dCTP labelled and hybridised overnight to the nylon membranes at 68°C, followed by several high stringency washes. After autoradiography (overnight at -70°C) the patterns were analysed.

Defining alleles

The limited resolution of the agarose gel electrophoresis technique, combined with the hypervariability of the minisatellite loci, make it impossible to score the alleles discretely, resulting in quasi-continuous allele distributions (BUDOWLE *et al.*, 1991). Therefore, allele classes have to be constructed before any analysis can take place. In a previous analysis (VERHEYEN *et al.*, 1995), we estimated the allele sizes by comparing the migration distance of the fragments to the migration distances of length markers (DUGGLEBY *et al.*, 1981). The alleles were then grouped in 100 bp classes. The drawback of this method is that the 100 bp classes are too wide for the low molecular weight alleles (<5 kb) and too narrow for the larger molecular weight alleles. Therefore, the resulting distributions do not accurately reflect the genetic variation observed on the gels. In this study another approach to classify the alleles was used. By comparing the genotypes of individuals that were analysed more than once on separate gels, we observed that alleles differed by an average of 0.75% from their mean size. Allele size is not correlated with the procentual deviation ($r=-0.0003$, details not shown). For each of the markers, all alleles from all populations were sorted by size. Starting from the smallest allele, the next significantly differing allele was determined and "tagged". Significance was determined using a t-test and by determining if the difference in size of the two alleles was larger than zero (5% significance level; the standard deviation was based on the mean size of both alleles). If not, the smallest allele was compared to the next (larger) allele, until a significant difference was found.

Starting from the larger of the 2 significantly differing alleles, the process was repeated until the whole allele range of the locus was covered. Subsequently, all alleles of a locus were grouped to the closest "tagged" allele. The mean size of each of the resulting groups was determined and was considered the midpoint of that allele class. For each separate population all alleles were grouped according to these classes and the resulting allele distributions were used in the analyses.

Populations sampled

During spring 1994, blue tits from 8 populations were sampled. Five populations are situated near Antwerp: plot PB (plot B in DHONDT 1989, 12.5 ha) is part of the large "mainland" population Peerdsbos (total > 200 ha); Calixbergen (hereafter called CX, plot C in DHONDT 1989, 17 ha) is an isolated estate situated 2 km south of PB; the Boshoek populations (three plots were sampled: ZZ, KB and LL, all 7 to 11 ha) are a set of small and isolated woodlots to the south of Antwerp, 17 km from the northern populations CX and PB. One population near Ghent (Hutsepot, approximately 50 km west of Antwerp, hereafter called GE) and two populations near Kortrijk (called KA and SA, separated by 2 km; situated 90 km west of Antwerp) were also sampled. The number of individuals sampled from each population is shown in table 1. All study sites are optimal habitat for blue tits with perhaps the exception of the Ghent population (DHONDT & EYCKERMAN, 1980).

TABLE 1

Characteristics of the genetic variation displayed by the minisatellite markers in the populations surveyed. Nind= number of individuals sampled. Nall= observed number of different alleles in the population. Hexp= (unbiased) heterozygosity expected under Hardy-Weinberg (HW) equilibrium. Hobs= observed heterozygosity. P= exact probability with standard error (SE) for detection of deviations from HW-equilibrium. Fis= inbreeding coefficient. M= mean for all markers. Significant deviations (P<0.05) from HW-equilibrium are indicated in bold.

Marker	*Nind*	*Nall*	*Hexp*	*Hobs*	*P*	*SE*	*Fis*
			KA	Kortrijk			
MS1	18	21	0.961	0.889	0.332	0.004	0.076
MS3	15	16	0.911	0.800	0.216	0.004	**0.156**
MS8a	17	21	0.966	0.941	0.489	0.001	0.027
MS8b	17	21	0.972	0.941	0.650	0.006	0.032
MS14	16	16	0.935	0.875	0.125	0.004	0.067
M			0.955	0.889			
			SA	Kortrijk			
MS1	43	21	0.945	0.860	**0.024**	0.004	**0.085**
MS3	41	31	0.966	0.902	**0.036**	0.003	**0.067***
MS8a	42	37	0.976	0.976	0.417	0.012	-0.003

MS8b	43	35	0.975	0.977	0.960	0.002	-0.001
MS14	44	28	0.935	0.864	**0.009**	0.001	**0.097**
M			0.964	0.916			
			GE	Ghent			
MS1	28	20	0.937	0.821	**0.005**	0.001	0.125
MS3	27	24	0.958	0.815	**0.0004@**	0.000	**0.151***
MS8a	28	30	0.974	1.000	0.588	0.015	-0.026
MS8b	26	30	0.972	0.846	**0.037**	0.008	**0.132***
MS14	28	24	0.962	0.893	**0.0004@**	0.000	0.073
M			0.961	0.875			
			PB	Antwerp			
MS1	34	20	0.947	0.824	**0.017**	0.003	**0.132**
MS3	33	29	0.959	0.788	**0.002**	0.000	**0.180***
MS8a	33	31	0.966	1.000	0.707	0.011	-0.036
MS8b	33	31	0.974	0.909	0.305	0.006	0.067
MS14	34	27	0.963	0.882	**0.032**	0.004	**0.089**
M			0.962	0.881			
			ZZ	Antwerp			
MS1	47	25	0.943	0.851	**0.002**	0.001	**0.098**
MS3	44	34	0.961	0.932	0.516	0.008	0.034
MS8a	48	35	0.962	0.979	0.658	0.012	-0.017
MS8b	49	37	0.966	0.898	**0.021**	0.003	0.071
MS14	50	32	0.968	0.960	0.615	0.017	0.008
M			0.960	0.924			
			KB	Antwerp			
MS1	23	19	0.944	0.913	0,688	0.003	0.033
MS3	24	23	0.960	0.875	0.246	0.005	**0.090***
MS8a	23	25	0.964	0.870	0.118	0.007	**0.105**
MS8b	23	26	0.968	0.957	0.642	0.006	0.012
MS14	23	23	0.957	1.000	0.772	0.008	-0.052
M			0.959	0.923			
			LL	Antwerp			
MS1	30	24	0.956	0.933	0.442	0.004	0.023
MS3	29	29	0.954	0.793	**0.020**	0.005	**0.178***
MS8a	29	27	0.956	0.931	0.181	0.009	0.027

MS8b	29	28	0.970	0.862	**0.007**	0.001	**0.113**
MS14	30	29	0.966	0.933	0.541	0.010	0.034
M			0.960	0.891			
			CX	Antwerp			
MS1	49	21	0.945	0.980	0.970	0.001	-0.038
MS3	48	35	0.971	0.960	0.587	0.006	0.012
MS8a	50	34	0.962	0.878	**0.011**	0.002	**0.088***
MS8b	49	35	0.964	0.938	0.703	0.004	0.028
MS14	50	33	0.968	0.941	0.360	0.014	0.027
M			0.962	0.939			

* $P<0.005$; @ significant after correcting for multiple testing

Analysis

Exact tests on Hardy-Weinberg (HW)-equilibrium and population differentiation were performed using the Genepop version 2 software (RAYMOND ROUSSET, 1995). Wright's Fst was also estimated using Genepop version 2. Fst is a measure of the amount of genetic differentiation between subpopulations. Significance of Fst-values was estimated by resampling over genotypes (RAYMOND & ROUSSET, 1995). The inbreeding coefficient Fis is a measure of the deviation from HW-proportions within populations and was also estimated with Genepop. PHYLIP 3.5c (FELSENSTEIN, 1989) was used to perform clusteranalyses (using the CONTML option). CONTML assumes that the loci evolve under the influence of random genetic drift. Significance of the nodes in the dendrograms was tested by bootstrapping over loci.

RESULTS

Genetic variation, heterozygosity and Hardy-Weinberg equilibrium

The main characteristics of the minisatellite loci in each of the populations are given in table 1. All loci displayed extreme polymorphism in each of the populations, which was illustrated by the large number of alleles and resulting high observed and expected heterozygosities. The mean number of alleles (observed over all populations) per locus equalled 50. This number might even be an underestimate because of the limited resolution of the agarose gel electrophoresis technique and the possible variation within the repeat sequence of the minisatellites (JEFFREYS *et al.*, 1991).

The mean observed heterozygosities were always lower than their expected values, and some of the Fis-values were significantly larger than zero. These values might at first appear to suggest that inbreeding occurs within the populations. However, there was no consistent deficit of heterozygotes for all loci within a specific population as could be expected with inbreeding. More probably, given the technical limitations in the resolution

of the alleles, the deviations were mainly caused by pseudohomozygosity (DEVLIN *et al.*, 1990) and the occurrence of null-alleles (alleles that only have a limited amount of repeats and therefore fail to hybridise efficiently; CHAKRABORTY *et al.*, 1992).

We tested the differences between mean heterozygosities among pairs of populations as described by NEI (1987; details not shown). No significant differences were found. However, single locus heterozygosities (especially the observed values) fluctuated more between populations and several significant differences ($P<0.05$, details not shown) were found.

According to the exact test, in 15 (out of 40) cases, loci had genotype frequencies that did not conform to their expected HW-proportions. However, when Bonferroni-corrections were applied, only 2 significant deviations remained ($P<0.00125$).

Genetic differentiation between populations

An exact test over all populations indicated that there were significant differences among the populations (table 2). Four of the 5 loci showed significant differentiation, the fifth was just off significance at the 5% level.

TABLE 2

Exact test (Raymond and Rousset 1995) of differentiation over all 8 populations. Significant P-values (S.E. = standard error) are indicated in bold.

Locus	P	S.E.
MS1	0.0645	0.0025
MS3	**0.0006**	0.0002
MS8a	**0.0031**	0.0005
MS8b	**0.0003**	0.0001
MS14	**0.0320**	0.0035
Total	**<0.0001**	

Table 3 gives the Fst-values and the results of the exact test of population differentiation between all combinations of 2 populations. Significant differentiation was found among many of the populations. Because 2 loci showed significant deviations from HW-equilibrium, resampling procedures over genotypes were also performed (Fst-based test). The level of significance is indicated by a (not significant), b ($0.05>P>0.01$), c ($0.005>P>0.002$) or d ($P<0.002$). Most Fst-values are relatively low, indicating high levels of gene flow between the populations. The Fst-values are not correlated with sample size ($r=0.007$; $df=26$; ns) and were used to search for correlations with geographic distance. The expected positive correlation (under the hypothesis of isolation-by-distance) was not found (Mantel test; $r=-0.22$; $df=26$; ns), suggesting random differentiation between the populations. Finally, a cluster analysis was performed on the data. CONTML assumes that the loci evolve under the influence of pure random drift and might therefore provide the most appropriate model. The results are shown in figs. 1a and 1b. The first

dendrogram shows that nearby populations are preferentially clustered together. The result of the bootstrap option is shown as well but indicates that several nodes are not significant.

TABLE 3

Exact P-values for differentiation among pairs of populations (below the diagonal) and Fst-values above the diagonal. a, b, c, d: levels of exact P-values among the populations according to the (genotype based) exact test of Raymond and Rousset (1995). (a: P> 0.05; b: 0.05> P> 0.01; c: 0.005> P> 0.002; d: P< 0.002).

	KA	SA	GE	PB	CX	ZZ	KB	LL
KA	/	0.000a	0.003a	0.004a	0.003a	0.010d	0.004a	0.008b
SA	0.413	/	0.001a	0.001a	0.001a	0.005d	0.003a	0.002b
GE	0.010	0.117	/	0.002a	0.003a	0.005b	0.004a	0.004b
PB	0.025	0.052	0.009	/	0.001a	0.005d	0.003a	0.006c
CX	0.107	0.005	0.030	0.099	/	0.005d	0.004b	0.007d
ZZ	0.00005	0.00003	0.002	0.0002	0.00002	/	0.004b	0.006d
KB	0.036	0.039	0.016	0.025	0.003	0.027	/	0.001a
LL	0.012	0.004	0.006	0.0005	0.000005	0.00008	0.256	/

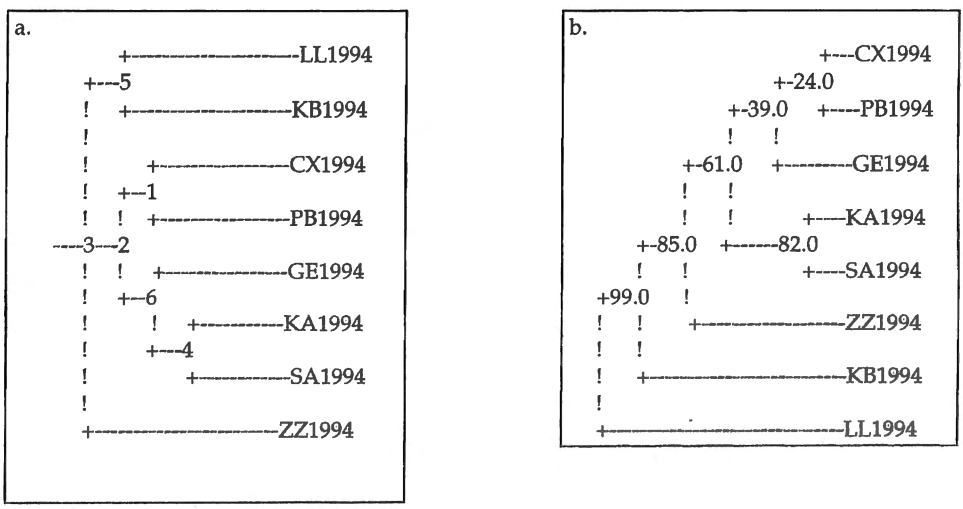

Fig. 1. – Dendrograms clustering the 8 populations. Figure 1.a. results of the CONTML-option; figure 1.b. results of the bootstrap option. Numbers at the nodes indicate the number of times this node was formed in 100 dendrograms.

DISCUSSION

Apart from its frequent use in paternity studies (KEMPENAERS *et al.*, 1992), multilocus DNA fingerprinting has only been used in some population genetic studies in natural populations, for example to find the phylogenetic relationships between small, isolated populations (GILBERT *et al.*, 1990). WAUTERS *et al.*, (1994) have also shown that genetic variation is reduced in isolated populations of the red squirrel (*Sciurus vulgaris*) due to reduced immigration. However, multilocus profiles are not easy to score and cannot be used in studies where the characterisation of single loci is desirable. Most population genetic studies that use minisatellite SLPs are performed in human populations (FLINT *et al.*, 1989; DEKA *et al.*, 1991). This is due to the species-specificity of the SLPs. However, the difficulties in the characterisation of the alleles at these loci is probably also an important reason, because studies using species-specific microsatellite markers are increasing (ESTOUP *et al.*, 1995). The allele scoring method used here is specific for this study, making direct comparisons among different studies impossible (even if they make use of the same markers). New studies, making use of the same markers used in this study, should preferentially reanalyse all data in order to construct allele classes that can be used to compare directly the allele distributions of new populations with the populations studied here. However, this should not be necessary if one wishes to compare the general results obtained over studies. The inability to score all alleles discretely is a serious drawback of minisatellites, which limits the use of these markers. However, the markers are capable of detecting subtle differences among populations (as shown in this study) and are therefore very helpful in testing specific hypotheses (as has been documented in Dias *et al.*, 1996). Microsatellite markers might also be useful in these kinds of studies, because they are highly polymorphic (although they are less polymorphic than the minisatellite markers described here). The alleles of microsatellites can be scored without error, making direct comparisons among different studies possible. Analysing microsatellite markers is much faster and requires less DNA than Southern blot based methods. Moreover, using fluorescence techniques, several markers can be analysed simultaneously. Unfortunately, there are no microsatellite markers available for the blue tit.

In this study, all loci were extremely polymorphic in each of the populations. This high heterozygosity is found in human minisatellite loci as well (JEFFREYS *et al.*, 1988). The heterozygosities reported here are much larger than those found for minisatellite loci in the Indian peafowl *Pavo cristatus* (22-78%, HANOTTE *et al.*, 1991) or in the chicken (BRUFORD BURKE, 1991). However in those studies the birds belonged to semi-captive or inbred populations, which might explain the reduced heterozygosities. Here, the observed genetic variation of the separate loci was highly variable and fluctuated strongly between populations. However, the mean heterozygosity was comparable in each of the populations. It therefore appears that, despite the highly fragmented nature of some of these populations, there is no loss of genetic variation within the populations. This result is not surprising, considering the dispersal capacities of blue tits. Nevertheless, KEMPENAERS *et al.*, (1996) found that in population CX the proportion of bands shared between parents on multilocus fingerprints was correlated with the number of eggs which failed to hatch from their nests, providing evidence of a deleterious effect of possible inbreeding.

Apart from technical influences, the observed deviations from HW-equilibrium (table 1) can be caused by several population processes. However, because the alleles are grouped in classes, the results can only be considered as possible indications of deviations from equilibrium. Minisatellites seem to behave neutrally (CLARK, 1987; JEFFREYS *et al.*, 1988), excluding selection as a possible cause for the deviations. The high mutation rates at these loci are probably negligible compared to other processes that might influence HW-equilibrium. Rather, the deviations are influenced by the combination of dispersal (which is high in the blue tit; BERNDT & STERNBERG, 1969), variation in reproductive success (KEMPENAERS *et al.*, 1992) and random genetic drift.

As also observed in an earlier study (VERHEYEN *et al.*, 1995), significant differentiation between populations sometimes occurs, despite high gene flow levels. Interestingly and unlike in the previous study, only a proportion of the populations appear to be strongly differentiated from the other populations (if the Fst-based test is used, which is based on resampling over genotypes): ZZ, KB and LL, which all belong to the BO-population. In our previous study, data from 2 breeding seasons were pooled. Perhaps this stressed the differences among the populations due to the conservation of related alleles over breeding seasons. If this is so, the significant results of the BO subpopulations might be explained by a smaller genetic turnover in these populations, which could be due to the high level of fragmentation from these populations. However, as already mentioned, no significant effects of fragmentation on genetic diversity were seen.

Despite the use of a different type of marker, the Fst-values are comparable with estimates in other bird species (ROCKWELL BARROWCLOUGH, 1987). The previously reported isolation-by-distance effect (VERHEYEN *et al.*, 1995) was not observed in this study over a wider geographical area, and there is no correlation between Fst and geographic distance. We also estimated Nei's genetic distance, but Nei's distance appears to be negatively correlated with sample size ($r=-0.73$; $df=26$; $P<0.001$), and was therefore not suitable for further analyses (details not shown). The Fst-values rather suggest a random drift pattern of differentiation. This pattern is supported by the fluctuations in heterozygosities. Nevertheless, the results of a clusteranalysis could indicate that gene flow between close-by populations might influence the random drift effects. However, several nodes were not significant, making alternative dendrograms possible as well.

In conclusion, we can say that the present high level of habitat fragmentation does not seem to have an effect of loss of genetic variation within blue tit populations as measured with minisatellite SLPs. Differentiation between populations follows a pattern resembling random drift. Random drift and migration probably cause the observed deviations from HW-equilibrium (apart from technical reasons) and the fluctuations in heterozygosities. Gene flow and/or the resolution of the markers are not high enough to induce an isolation-by-distance effect. The correlations between the genetic distance measures (Nei) and the sample size are very troublesome and we urge caution in interpreting minisatellite data. Fortunately, Fst-values were not correlated with sample size. Nevertheless, when using VNTR markers in population genetic studies, all the drawbacks have to be kept in mind and appropriate sampling designs worked out whenever possible.

ACKNOWLEDGEMENTS

We would like to thank Mr. De Baere and his collegues from Kortrijk for collecting the blue tit bloodsamples of KA and SA populations. In this respect we also thank Dr. J. De Laet for taking bloodsamples from the population near Ghent. We wish to thank Dr. Michel Raymond for providing the Genepop software. We thank the Bracht family for allowing us to work on their estate. GRV was supported by a grant from the IWONL (Instituut voor Wetenschappelijk Onderzoek in Nijverheid en Landbouw). EM is Research Associate with the Belgian National Fund for Scientific Research.

REFERENCES

BERNDT, R. & H. STERNBERG (1969) – Terms, studies and experiments on the problems of bird dispersion. *ibis*, **110**: 256-269.

BRUFORD, M.W. & T. BURKE (1991) – Hypervariable DNA markers and their applications in the chicken. In: BURKE, T., DOLF, G., JEFFREYS, A.J. & WOLFF, R. (Eds) *DNA fingerprinting: approaches and applications.* Birkhä user Verlag, Basel, Boston, Berlin: 230-242.

BUDOWLE, B., A.M. GIUSTI, J.S. WAYE, F.S. BAECHTEL, R.M. FOURNEY, D.E. ADAMS, L.A. PRESLEY, H.A. DEADMAN & K.L. MONSON (1991) – Fixed-bin analysis for statistical evaluation of continuous distributions of allelic data from VNTR loci, for use in forensic comparisons. *Am. J. Hum. Genet.*, **48**: 841-855.

CHAKRABORTY, R., M. DE ANDRADE, S.P. DAIGER & B. BUDOWLE (1992) – Apparent heterozygote deficiencies observed in DNA typing data and their implications in forensic applications. *Ann. Hum. Genet.*, **56**: 45-57.

A.G. CLARK (1987) – Neutrality tests of highly polymorphic restriction fragment length polymorphisms. *Am. J. Hum. Genet.*, **41**: 948-956.

DEKA, R., R. CHAKRABORTY, & R.E. FERRELL (1991) – A population genetic study of six VNTR loci in three ethnically defined populations. *Genomics*, **11**: 83-92.

DEVLIN, B., N. RISCH & K. ROEDER (1990) – No Excess of Homozygosity at Loci Used for DNA Fingerprinting. *Science*, **249**: 1416-1420.

A.A. DHONDT (1989) – Blue Tit. In: NEWTON, I.A. (ed) *Lifetime Reproduction in Birds.* Academic Press, London: 15-33.

DHONDT, A.A. & R. EYCKERMAN (1980) – Competition and the regulation of numbers in Great and Blue Tit. *Ardea*, **68**: 121-132.

DIAS, P.C., G.R. VERHEYEN & M. RAYMOND (1996) – Source-Sink populations in Mediterranean blue tits: evidence using single-locus minisatellite probes. *J. Evol. Biol.*, **9**: 965-978.

DUGGLEBY, R.G., H. KINNS & J.L. ROOD (1981) – A computer Program for Determining the Size of DNA Restriction Fragments. *Analyt. Biochem.*, **110**: 723-725.

ESTOUP, A., L. GARNERY, M. SOLIGNAC & J.-M. CORNUET (1995) – Microsatellite variation in Honey Bee (*Apis Mellifera* L.) populations: hierarchical genetic structure and test of the infinite allele and stepwise mutation models. *Genetics*, **140**: 679-695.

J. FELSENSTEIN (1989) – PHYLIP—Phylogeny Inference Package (version 3.2). *Cladistics*, **5**: 164-166.

FLINT, J., A.J. BOYCE, J.J. MARTINSON & J.B. CLEGG (1989) – Population bottlenecks in Polynesia revealed by minisatellites. *Hum. Genet.*, **83**: 257-263.

GILBERT, D.A., N. LEHMAN, S.J. O'BRIEN & R.K. WAYNE (1990) – Genetic fingerprinting reflects population differentiation in the California channel island fox. *Nature*, **344**: 764-766.

HADRYS, H., B. SCHIERWATER, S.L. DELLAPORTA & L.W. BUSS (1993) –Determination of paternity in dragonflies by Random Amplified Polymorphic DNA fingerprinting. *Molec. Ecol.,* **2**: 79-87.

HAIG, S.M., J.R. BELTHOFF & D.H. ALLEN (1993) – Examination of population structure in red-cockaded woodpeckers using DNA profiles. *Evolution,* **47**: 185-194.

HANOTTE, O., T. BURKE, J.A.L. ARMOUR & A.J. JEFFREYS (1991) – Hypervariable minisatellite DNA sequences in the Indian Peafowl Pavo cristatus. *Genomics,* **9**: 587-597.

HILLIS, D.M. & C. MORITZ (eds) (1990) – *Molecular Systematics.* Sinauer Ass. Inc., ... pp.

JEFFREYS, A.J., N.J. ROYLE, V. WILSON & Z. WONG (1988) – Spontaneous mutation rates to new length alleles at tandem-repetitive hypervariable loci in human DNA. *Nature,* **332**: 278-281.

JEFFREYS, A.J., A. MACLEOD, K. TAMAKI, D.L. NEIL & D.G. MONCKTON (1991) – Minisatellite repeat coding as a digital approach to DNA typing. *Nature,* **354**: 204-209.

JOHNSON, N.K. & J.A. MARTEN (1991) – Evolutionary genetics of flycatchers. III. Variation in *Empidonax hammondii* (Aves: *Tyrannidae*). *Can. J. Zool.* **69**: 232-238.

KEMPENAERS, B., G.R. VERHEYEN, M. VAN DEN BROECK, T. BURKE, C. VAN BROECKHOVEN & A.A. DHONDT (1992) – Extra-pair paternity results from female preference for high-quality males in the blue tit. *Nature,* **357**: 494-496.

KEMPENAERS, B., F. ADRIAENSEN, A.J. VAN NOORDWIJK & A.A. DHONDT (1996) – Genetic similarity, inbreeding and hatching failure in blue tits: are unhatched eggs infertile? *Proc. R. Soc. London*, in press.

MATTHYSEN, E., L. LENS, S. VAN DONGEN, G.R. VERHEYEN, L.A. WAUTERS, F. ADRIAENSEN & A.A. DHONDT (1995) – Diverse effects of forest fragmentation on a number of animal species. *Belg. J. Zool.,* **125**: 175-183.

M. NEI (1987) – *Molecular Evolutionary Genetics.* Columbia University Press, New York, ... pp.

RAYMOND, M. & F. ROUSSET (1995) – Genepop (ver. 1.2.): a population genetics software for exact test and ecuniscism. *J. Hered.,* **95**: 248-249.

ROCKWELL, R.F. & G.F. BARROWCLOUGH (1987) – Gene flow and the genetic structure of populations. In: F. COOKE & P.A. BUCKLEY (Eds), *Avian Genetics: a population and ecological approach.* Acad. Press Inc. London: 223-255.

VERHEYEN, G.R., B. KEMPENAERS, T. BURKE, M. VAN DEN BROECK, C. VAN BROECKHOVEN & A.A. DHONDT (1994) – Identification of hypervariable single locus minisatellite DNA probes for the blue tit (*Parus caeruleus*). *Molec. Ecol.,* **3**: 137-143.

VERHEYEN, G.R., B. KEMPENAERS, F. ADRIAENSEN, M. VAN DEN BROECK, E. MATTHYSEN, C. VAN BROECKHOVEN & A.A. DHONDT (1995) The genetic structure of blue tit (*Parus caeruleus*) populations as revealed by minisatellite single locus probes. *Heredity*, **75**: 571-577.

WAUTERS, L.A., Y. HUTCHINSON, D.T. PARKIN. & A.A. DHONDT (1994) – The effects of habitat fragmentation on demography and on the loss of genetic variation in the red squirrel. *Proc. Roy. Soc. London B,* **255**: 107-111.

WENINK, P.W., A.J. BAKER & M.G.J. TILANUS (1993) – Hypervariable-control-region-sequences reveal global population structuring in a long-distance migrant shorebird, the dunlin (*Caledris alpina*). *Proc. Natl. Acad. Sci. USA,* **90**: 94-98.