VARIATION IN PIED FLYCATCHER (*FICEDULA HYPOLEUCA*) MITOCHONDRIAL DNA

HÅKAN TEGELSTRÖM, HANS P. GELTER, AND MAARIT JAAROLA
Department of Genetics, Uppsala University, Box 7003, S-750 07 Uppsala, Sweden

ABSTRACT.—Pied Flycatchers (*Ficedula hypoleuca*) in Sweden have low levels of variation in nuclear genes relative to most other bird species. This lack of variation has been attributed to population bottlenecks caused by Pleistocene glaciations. We studied mitochondrial DNA (mtDNA) variation in 20 Pied Flycatchers from four Swedish localities. Eight restriction endonucleases yielded a total of 207–212 DNA fragments (approximately 5% of the mtDNA genome). The mean pairwise divergence between the individuals was 0.35 ± 0.16% (range 0.00–0.82%), which suggests that the 18 identified mtDNA clones diverged within the past million years, and that the majority of clones evolved within the last 100,000 years.

If genetic variation was reduced by prolonged bottlenecking during the last glacial period, low protein heterozygosity and high variability in mtDNA can be explained by a difference in rates of recovery of nuclear and mtDNA variation. The Pied Flycatcher in northern Europe may have only recently begun to regain variation in nuclear genes, whereas considerable variation in mtDNA has already accumulated through mutation. *Received 7 December 1989, accepted 11 May 1990.*

GENETIC variability and relationships among clones of mitochondrial DNA (mtDNA) can provide background data to hypotheses on biogeographical history and population structure. As a clonally transmitted marker, the distribution of the maternally inherited mtDNA will reflect founder or rare immigration events more directly than nuclear DNA (Wilson et al. 1985). The study of mtDNA variability is highly relevant for avian species because of the relative lack of observed differentiation in proteins as assessed by electrophoresis (Barrowclough 1983).

The last glacial period probably played a major role in determining the amount of diversity and the distribution of mtDNA clones in North American and Eurasian species. During the Pleistocene glacial period, populations were isolated in refugia and subjected to severe reductions in effective population size, which decreased the number of mtDNA clones within populations (Wilson et al. 1985, Gyllensten and Wilson 1987). Postglacial recolonization involved founder events and population bottlenecks, and further reduced mtDNA variation. Multiple range expansions from one or a few refugia have led to a geographic structuring of mtDNA clones, especially in small mammals (Avise et al. 1983, Avise et al. 1987, Tegelström 1987a) where female dispersal is restricted compared with that in most avian species.

Most studies of avian mtDNA have concentrated on genetic differences between species or subspecies (Kessler and Avise 1985a; Mack et al. 1986; Ovenden et al. 1987; Shields and Wilson 1987a, b; Avise and Nelson 1989; Zink and Avise 1990). Studies of mtDNA variation within bird populations (Shields and Wilson 1987a, Tegelström 1987b, Ball et al. 1988, Avise and Nelson 1989, Zink and Avise 1990) indicate small genetic distances between mtDNA clones and, with one exception (Avise and Nelson 1989), an absence of structure in mtDNA variation. The most thorough study of mtDNA variation within an avian species was a continent-wide survey of the Red-winged Blackbird (*Agelaius phoeniceus*). The morphological subspecies exhibited little allozyme divergence (Ball et al. 1988). Red-winged Blackbirds also had only small genetic distances between mtDNA clones, and the different mtDNA clones showed widespread geographic distributions.

We studied mtDNA in the Pied Flycatcher (*Ficedula hypoleuca*), a species whose main breeding area is in rich deciduous woodlands in northwestern Europe. The Fennoscandian populations are probably descended from populations isolated in refugia during the Pleistocene period (von Haartman 1949). Protein electrophoresis of 35 loci revealed a low amount of genetic variation compared with other avian species (Gelter et al. 1989). The proportion of observed polymorphic loci was 11.4 ± 0.3% (mean of 24.0% in other avian species; Evans

TABLE 1. Eighteen mtDNA clones in the Pied Flycatcher (*Ficedula hypoleuca*) from 4 localities in Sweden. Letters in the composite mtDNA genotypes refer (left to right) to restriction morphs for enzymes *Hae*III, *Dde*I, *Rsa*I, *Mbo*I, *Hin*fI, *Hpa*II, *Sau*96 AI, and *Taq*I, respectively.

Clone no.	Locality	Composite mtDNA genotype	No. of individuals
1	A	BBAFBCEA	1
2	A, B	BBADBCBA	2
3	A	BCABBCDA	1
4	A	BGADFCBA	1
5	A	BFAFEAFC	1
6	A	BCABCCEA	1
7	B	BBAEBCBA	1
8	B	BFAGECBA	1
9	B	BFAHBCGA	1
10	B	ABBAABCA	1
11	B	BCAFEAFC	1
12	C	BCAABCEA	1
13	C	BBAADCBA	1
14	C	BAACBAAC	1
15	D	BAAFBCEA	1
16	D	BDAEBCBB	1
17	D	BAAFEABC	2
18	D	CEAIECBA	1

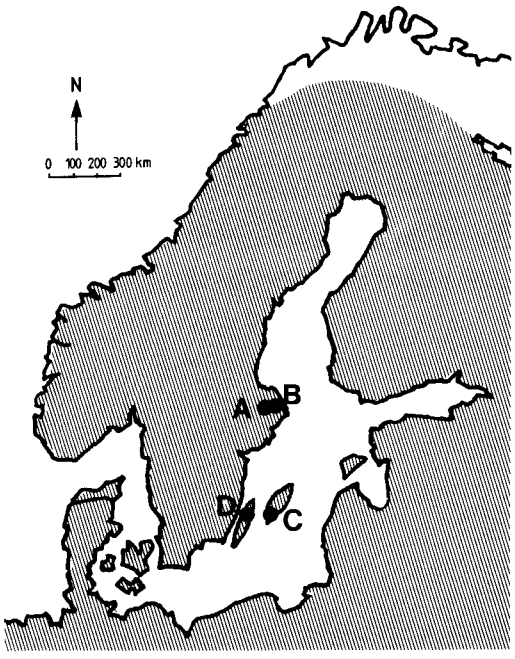


Fig. 1. Sampling localities (A–D) for individuals of the Pied Flycatcher (*Ficedula hypoleuca*). The shaded area indicates the geographic distribution of the species.

1987), and the observed mean heterozygosity was 0.9% (compared with a mean of 4.4% in other avian species; Evans 1987). Our objectives were to investigate whether mtDNA in *F. hypoleuca* exhibits low genetic variation and whether mtDNA clones are geographically structured.

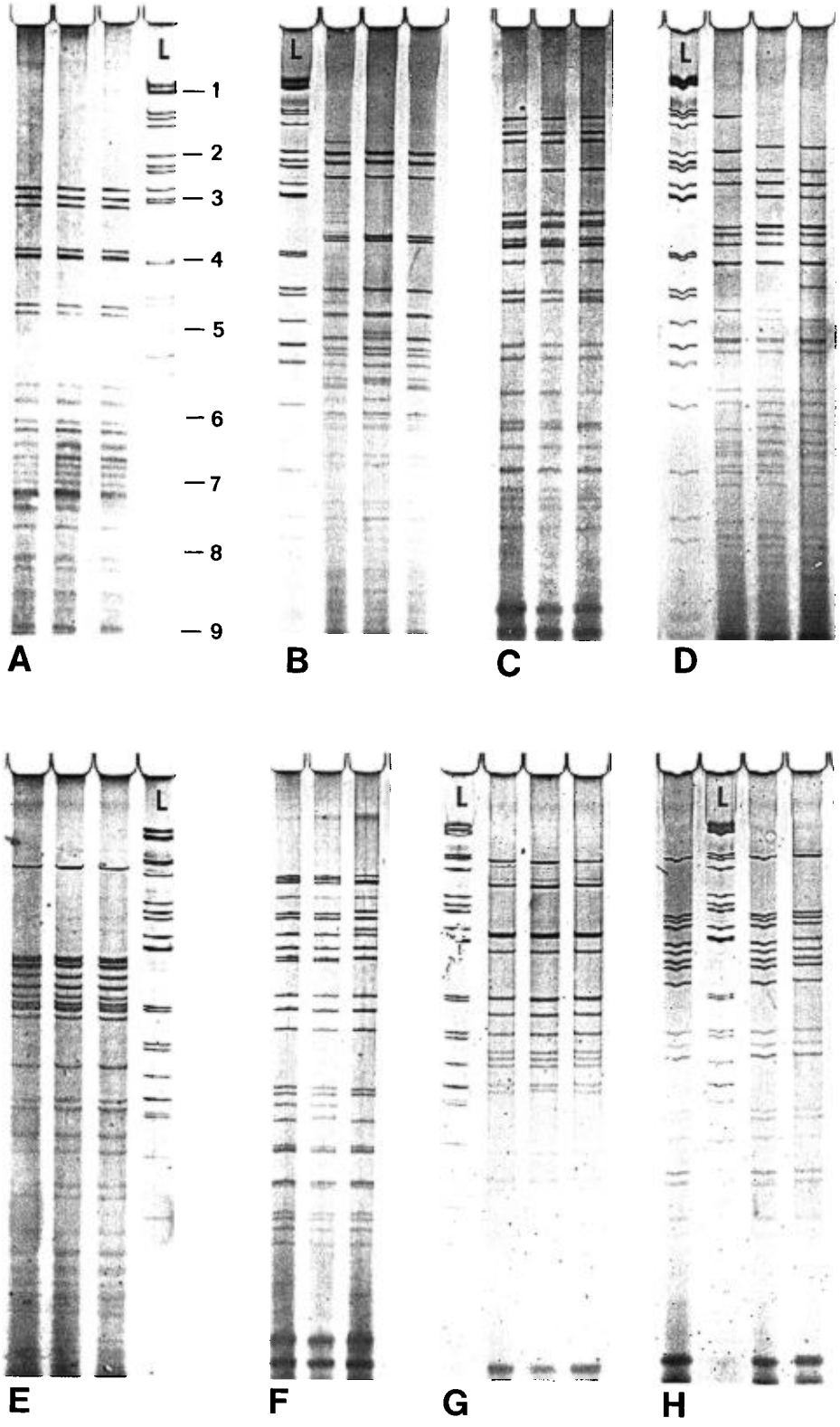
METHODS

Twenty *Ficedula hypoleuca* were live-trapped at their nest boxes (Fig. 1, Table 1). We isolated mitochondria from fresh or frozen liver and heart according to Lansman et al. (1981). We isolated mtDNA by ultracentrifugation in CsCl density gradients (Beckman SW 50.1 swing out rotor) for 48 h at 35,000 rpm. After collection, 10–40 ng of mtDNA was digested according to Tegelström (1986). Eight type II tetranucleotide restriction endonucleases (Boehringer Mannheim or Pharmacia P-L Biochemicals) were used to characterize mtDNA (recognition sequences in parenthesis): (1) *Hae*III (GGCC); (2) *Dde*I (CTNAG); (3) *Rsa*I (GTAC); (4) *Mbo*I (GATC); (5) *Hin*fI (GANTC); (6) *Hpa*II (CCGG); (7) *Sau*96 AI (GGNCC), and (8) *Taq*I (TCGA). These eight enzymes have a GC:AT ratio of 2.2, which is similar to that of other studies of avian mtDNA (mean 1.9, range 1.3–3.3; Tegelström and Gelter 1990). Fragments of mtDNA were separated in 5% polyacrylamide-gels according to Tegelström (1986) and Tegelström and Wyöni (1986), and visualized by silver staining (Guillemette and Lewis 1983). Each of the distinctive mtDNA restriction fragment patterns produced by a given restriction endonuclease was designated by a letter. Every specimen was assigned a composite mtDNA phenotype of eight letters to characterize the restriction fragment patterns given by the eight endonucleases used in the study. Individuals that shared a common composite phenotype were regarded as belonging to the same mtDNA matrilineal clone.

We calculated the total proportion of shared fragments between two individuals as

$$F = 2Nxy/(Nx + Ny),$$

where Nx and Ny are the numbers of fragments in individuals X and Y, respectively, and Nxy is the number of fragments shared by X and Y. Values of F were converted to estimates of nucleotide sequence divergence, p, by eq. 20 (Nei and Li 1979). Phenograms were constructed from matrices of p-values by the unweighted pair-group method (UPGMA; Sneath and Sokal 1973). Fragment data are not presented in their entirety, but are available on request from Tegelström.


L
1
2
3
4
5
6
7
8
9
A
B
C
D
E
F
G
H

Results

The eight restriction endonucleases yielded a total of 207–212 fragments per mtDNA clone, corresponding to approximately 850 nucleotides per individual (ca. 5% of the mtDNA genome). Altogether 40 different fragment patterns were identified and representative examples are shown (Fig. 2). Each of the enzymes detected more than one clone. *Rsa*I was the least discriminating and yielded 2 phenotypes. *Mbo*I detected 9 phenotypes. Among the 20 individuals, 18 different mtDNA clones were identified (Table 1). The estimated total number of base pairs (bp) in the *Ficedula hypoleuca* mtDNA molecule varied between 12,900 bp (*Hae*III) and 16,980 bp (*Taq*I). Excluding *Hae*III (which often gives a high number of smaller fragments not detectable on the gels), the remaining seven endonucleases give a mean molecular size of 16,225 bp, which is comparable to values obtained for 40 other bird species (16.3–17.3 kb; Shields and Helm-Bychowski 1988). No mtDNA size variants were identified, and each bird was homoplasmic for the identified genotype.

The mean pairwise-divergence among the 20 individuals (including identical clones) was 0.35% (SD = ±0.16%) and the range of pairwise genetic distances between clones was 0.00–0.82%. The majority of clones had low pairwise genetic distances (Fig. 3), which indicates a recent divergence. Assuming a rate of 2% sequence divergence per million years (Shields and Wilson 1987b), all the clones identified in *F. hypoleuca* have diverged within the last million years (largest *p*-value: 0.82%). The majority of clones evolved within the last 100,000 years ($p < 0.2\%$).

The phenogram (Fig. 3) implies little geographic structuring of the clonal branches. Clones in neighboring localities in Uppsala (locality A and B) and clones from the islands of Öland and Gotland are mixed randomly. For example, the four clones from Öland (locality C; clones 15, 16, 17, and 18) appear in different parts of the phenogram and always have a clone from the mainland as the closest relative.

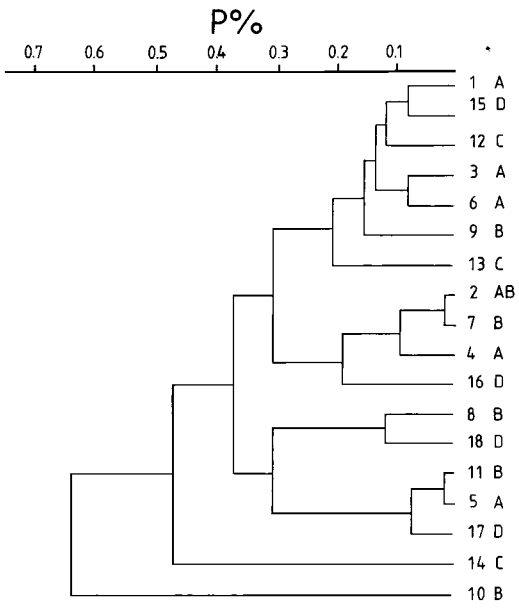


Fig. 3. Phenogram derived from an UPGMA cluster analysis (Sneath and Sokal 1973) of 18 mtDNA clones in the Pied Flycatcher (*Ficedula hypoleuca*). Clones are numbered 1–18 (Table 1). Letters refer to sampling localities (Fig. 1).

Discussion

We identified a large number of mtDNA clones in the Swedish Pied Flycatcher populations. The 20 individuals studied yielded 18 different maternal lineages. The different clones showed no obvious geographic structuring and were closely related. However, the small sample sizes meant that population subdivision cannot be ruled out. Larger population sample sizes would allow estimates of within vs. between population variation (cf. Takahata and Palumbi 1985). The presence of closely related mtDNA clones at different geographic locations could have two explanations. Either an ances-

←

Fig. 2. Representative examples of fragment patterns after restriction endonuclease digestion of mtDNA from individuals of the Pied Flycatcher (*Ficedula hypoleuca*): (A) *Hae*III, (B) *Dde*I, (C) *Rsa*I, (D) *Mbo*I, (E) *Hin*fI, (F) *Hpa*II, (G) *Sau*96, and (H) *Taq*I. Lanes marked L contain λ DNA digested with restriction endonuclease *Bgl*I to produce fragment size markers: (1) 9,649 base pairs, (2) 1,650 bp, (3) 1,138 bp, (4) 790 bp, (5) 562 bp, (6) 366 bp, (7) 267 bp, (8) 186 bp, and (9) 115 bp.

tral retention of clones (Neigel and Avise 1986) after the colonization of the European continent and Scandinavia from one or several refugial areas (von Haartman 1949) or recent interconnections through gene flow, which would prevent mtDNA differentiation of populations by stochastic lineage sorting (Avise et al. 1987). A more extensive sampling of populations is necessary to distinguish these explanations.

Even though electrophoresis of proteins in the Pied Flycatcher reveals a low level of heterozygosity, mtDNA shows relatively high levels of variation—comparable to the "DNA-fingerprinting" level of the mtDNA variation found by Avise et al. (1989). Other studies of mtDNA variation have revealed lower levels of variation (Spolsky and Uzzell 1984, Kessler and Avise 1985b, Saunders et al. 1986, Shields and Wilson 1987a, Avise and Zink 1988, Ball et al. 1988, Avise and Nelson 1989, Lamb et al. 1989, Mulligan and Chapman 1989). Explanations for the difference in amounts of nuclear and mtDNA variation may be found in demographic factors such as differences in dispersal between the sexes. Alternatively, populations may have incurred bottlenecks during the colonization of the European continent and Scandinavia from previously glaciated regions.

A demographic situation that affected the distribution of diversity in mtDNA and nuclear genes has been described in Canada Geese (*Branta canadensis;* Shields and Wilson 1987a), where males disperse more widely than females. Founding of new breeding populations by a low number of closely related females and many males from diverse lineages has led to the fixation of different mtDNA-variants in different populations. Nuclear diversity, however, is high, and there is little differentiation even between subspecies, because of nuclear gene flow between populations through male dispersal. Demographic factors that characterize the Pied Flycatcher, such as female-biased dispersal (which would increase the effective population size for both mtDNA and nuclear genes) or polygynic males (which would decrease the effective population size for nuclear genes) cannot explain low levels of nuclear variation accompanied by substantial levels of variation in mtDNA.

Low levels of protein variation (Sage and Wolff 1986) as well as mtDNA (Wallis and Arntzen 1989, Gyllensten and Wilson 1987) occur in species from previously glaciated regions and imply past genetic bottlenecks. In diploid organisms, the effective population size for mtDNA will always be less than that for nuclear genes, which makes variation in mtDNA more sensitive to population bottlenecks (Wilson et al. 1985). If genetic variation in both nuclear and mtDNA genes was reduced during the last glacial period, the current genetic variation in the Pied Flycatcher will be the sum of the variation that survived the bottleneck and mutations that have been incorporated subsequently.

We suggest that differences in recovery time for nuclear and mtDNA variation after a population bottleneck explain the pattern of mtDNA and nuclear genetic variation in the Pied Flycatcher. The majority of mtDNA clones in the Pied Flycatcher have diverged less than 0.2% (cf. Shields and Wilson 1987b). This implies that most mtDNA variation has been accumulated during the last 100,000 years, which supports the suggestion that the species may have been exposed to population bottlenecks during the alterations of the last glacial period. In contrast to the situation with mtDNA, the regeneration of genetic variation in nuclear DNA is a comparatively slow process. Assuming a large population size, the recovery time for reaching equilibrium allele frequencies for neutral alleles at one nuclear locus is in the range of 100,000 to 10 million generations (Lande and Barrowclough 1987). Thus, the Pied Flycatcher in northern Europe may be at the beginning of the process of regaining genetic variation in nuclear genes whereas considerable mtDNA variation already may have accumulated through mutation.

ACKNOWLEDGMENTS

We thank Arne Lundberg and Dag Eriksson for help in collecting birds and permission to sample birds in their study area. We thank Karl Fredga for his unfailing support throughout the course of this work, and Honor Prentice for valuable discussions and for reading previous drafts. Allan C. Wilson provided valuable discussions. Bob Zink and an anonymous reviewer improved a previous draft. The staff at the Ölands Skogsby Ecological Research Station of Uppsala University provided a base for fieldwork and writing. This study was supported by grants from the Swedish Natural Science Research Council, the Erik Philip-Sörensen Foundation, the Nilsson-Ehle Foundation, and the Uddenberg-Nordingska Foundation.

Literature Cited

Avise, J. C., J. F. Shapira, S. W. Daniel, C. F. Aquadro, & R. A. Lansman. 1983. Mitochondrial DNA differentiation during the speciation process in *Peromyscus*. Mol. Biol. Evol. 1: 38–56.

———, J. Arnold, R. M. Ball, E. Bermingham, T. Lamb, J. E. Neigel, C. A. Reeb, & N. C. Saunders. 1987. Intraspecific phylogeography: the mitochondrial DNA bridge between population genetics and systematics. Annu. Rev. Syst. 18: 489–522.

———, B. W. Bowen, & T. Lamb. 1989. DNA fingerprints from hypervariable mitochondrial genotypes. Mol. Biol. Evol. 6: 258–269.

———, & W. S. Nelson. 1989. Molecular genetic relationships of the extinct Dusky Seaside Sparrow. Science 243: 646–648.

———, & R. M. Zink. 1988. Molecular genetic divergence between avian sibling species: King and Clapper rails, Long-billed and Short-billed dowitchers, Boat-tailed and Great-tailed grackles, and Tufted and Black-crested titmice. Auk 105: 516–528.

Ball, R. M., S. Freeman, F. C. James, E. Bermingham, & J. C. Avise. 1988. Phylogeographic population structure of Red-winged Blackbirds assessed by mitochondrial DNA. Proc. Natl. Acad. Sci. USA 85: 1558–1562.

Barrowclough, G. F. 1983. Biochemical studies of microevolutionary processes. Pp. 223–261 *in* Perspectives in ornithology (A. H. Brush and G. A. Clark, Eds.). New York, Cambridge Univ. Press.

Evans, P. G. H. 1987. Electrophoretic variability of gene products. Pp. 105–162 *in* Avian genetics (F. Cooke and P. A. Buckley, Eds.). New York, Academic Press.

Gelter, H. P., H. Tegelström, & G. Ståhl. 1989. Allozyme similarity between the Pied and the Collared Flycatchers (Aves: *Ficedula albicollis* and *F. hypoleuca*). Hereditas 111: 65–72.

Guillemette, J. G., & P. N. L. Lewis. 1983. Detection of subnanogram quantities of DNA and RNA on native and denaturing polyacrylamide and agarose gels by silver staining. Electrophoresis 4: 92–94.

Gyllensten, U., & A. C. Wilson. 1987. Interspecific mitochondrial DNA transfer and the colonization of Scandinavia by mice. Genet. Res. Camb. 49: 25–29.

Kessler, L. G., & J. C. Avise. 1985a. A comparative description of mitochondrial DNA differentiation in selected avian and other vertebrate genera. Mol. Biol. Evol. 2: 109–125.

———, & J. C. Avise. 1985b. Microgeographic lineage analysis by mitochondrial genotype: variation in the cotton rat (*Sigmodon hispidis*). Evolution 39: 831–837.

Lamb, T., J. C. Avise, & J. W. Gibbons. 1989. Phylogeographic patterns in mitochondrial DNA of the desert tortoise (*Xerobates agassizi*), and evolutionary relationships among the North American gopher tortoises. Evolution 43: 76–87.

Lande, R., & G. F. Barrowclough. 1987. Effective population size, genetic variation, and their use in population management. Pp. 87–123 *in* Viable populations for conservation (M. E. Soulé, Ed.). Cambridge Univ. Press.

Lansman, R. A., R. O. Shade, J. F. Shapira, & J. C. Avise. 1981. The use of restriction endonucleases to measure mitochondrial DNA sequence relatedness in natural populations. III. Techniques and potential applications. J. Mol. Evol. 17: 214–226.

Mack, A. L., F. B. Gill, R. Colburn, & C. Spolsky. 1986. Mitochondrial DNA: a source of genetic markers for studies of similar passerine bird species. Auk 103: 676–681.

Mulligan, T. J., & R. W. Chapman. 1989. Mitochondrial DNA analysis of Chesapeake Bay White perch, *Morone americana*. Copeia 1989(3): 679–688.

Nei, M., & W-H. Li. 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc. Natl. Acad. Sci. USA 76: 5269–5273.

Neigel, J. E., & J. C. Avise. 1986. Phylogenetic relationships of mitochondrial DNA under various demographic models of speciation. Pp. 515–534 *in* Evolutionary processes and theory (S. Karlin and E. Nevo, Eds.). New York, Academic Press.

Ovenden, J. R., A. G. Mackinlay, & R. H. Crozier. 1987. Systematics and mitochondrial genome evolution of Australian rosellas (Aves: *Platycercidae*). Mol. Biol. Evol. 4: 526–543.

Sage, R. D., & J. O. Wolff. 1986. Pleistocene glaciations, fluctuating ranges and low genetic variability in a large mammal (*Ovis dalli*). Evolution 40: 1092–1095.

Saunders, N. C., L. G. Kessler, & J. C. Avise. 1986. Genetic variation and geographic differentiation in mitochondrial DNA of the horseshoe crab, *Limulus polyphemus*. Genetics 112: 613–627.

Shields, G. F., & K. M. Helm-Bychowski. 1988. Mitochondrial DNA of birds. Curr. Ornithol. 5: 273–295.

———, & A. C. Wilson. 1987a. Subspecies of the Canada Goose (*Branta canadensis*) have distinct mitochondrial DNA's. Evolution 41: 662–666.

———, & ———. 1987b. Calibration of mitochondrial DNA evolution in Geese. J. Mol. Evol. 24: 212–217.

Sneath, P. H. A., & R. R. Sokal. 1973. Numerical taxonomy. San Francisco, W. H. Freeman and Co.

Spolsky, C., & T. Uzzell. 1984. Natural interspecies transfer of mitochondrial DNA in amphibians. Proc. Natl. Acad. Sci. USA 81: 5802–5805.

TAKAHATA, N., & S. R. PALUMBI. 1985. Extranuclear differentiation and gene flow in the finite island model. Genetics 109: 441-457.

TEGELSTRÖM, H. 1986. Mitochondrial DNA in natural populations: an improved routine for the screening of genetic variation based on sensitive silver staining. Electrophoresis 7: 226-229.

———. 1987a. Transfer of mitochondrial DNA from the northern red-backed vole (*Clethrionomys rutilus*) to the bank vole (*C. glareolus*). J. Mol. Evol. 23: 218-227.

———. 1987b. Genetic variability in mitochondrial DNA in a regional population of the Great Tit (*Parus major*). Biochem. Genet. 25: 95-110.

———, & H. GELTER. 1990. Haldane's rule and sex biassed gene flow between two hybridizing flycatcher species (*Ficedula albicollis* and *F. hypoleuca*, Aves: Muscicapidae). Evolution. In press.

———, & P-I. WYÖNI. 1986. Silanization of supporting glass plates avoiding fixation of polyacrylamide gels to glass cover plates. Electrophoresis 7: 99.

VON HAARTMAN, L. 1949. Der Trauenfliegenschnäpper. I. Ortstreue und Rassenbildung. Acta Zool. Fennica 56: 6-104.

WALLIS, G. P., & J. W. ARNTZEN. 1989. Mitochondrial-DNA variation in the crested newt superspecies: limited cytoplasmic gene flow among species. Evolution 43: 88-104.

WILSON, A. C., R. C. CANN, S. M. CARR, M. GEORGE, U. B. GYLLENSTEN, K. M. HELM-BYCHOWSKI, R. G. HIGUCHI, S. R. PALUMBI, E. M. PRAGER, R. D. SAGE, & M. STONEKING. 1985. Mitochondrial DNA and two perspectives on evolutionary genetics. Biol. J. Linn. Soc. (London) 26: 375-400.

ZINK, R. M., & J. C. AVISE. 1990. Phylogenetic analysis of mitochondrial DNA and allozyme divergence in the avian genus *Ammodramus*. Syst. Zool. In press.

(*Continued from p. 677*)

warblers; Lynn M. Gordon, Roles of endogenous and exogenous factors in settlement patterns of juvenile Western Sandpipers; Gary R. Graves, Avifauna of Volcan Sumaco: evidence for long-distance dispersal of Andean forest birds? Michael T. Green, Constraints on singing passerines in open habitats: a test of the degradation hypothesis; Jeffrey G. Groth, Biosystematics of Red Crossbills in the American Southwest; Thomas C. Grubb Jr., Ptilochronology and age-specific reproductive success; Thomas C. Gumbart, Determinants of clutch size in the Yellow-eyed Junco; Geoffrey E. Hill, Female mate preference in relation to male plumage coloration in the House Finch; Anne M. Houtman, Mate choice and sexual selection in the Zebra Finch (*Poephila guttata*); Lawrence D. Igl, Dickcissel (*Spiza americana*) site tenacity and mate fidelity in response to habitat alteration; Eduardo E. Inigo-Elias, Effects of forest fragmentation on a tropical raptor community in the Selva Lacandona region of Chiapas, Mexico; Brenda S. Johnson, Molecular analysis of the viability of Burrowing Owl populations; Michael Kaspari, Are there sedentary gleaners and mobile skimmers in the Sonoran Desert? Gustavo H. Kattan, Prenatal development of a brood parasite, the Shiny Cowbird; Julie A. Kelley, Energetic relationship of spring migration and breeding biology in Yellow Warblers; Nancy Langston, Evolution of ornamentation in the monomorphic Turquoise-browed Motmot (*Eumomota superciliosa*); Stephen C. Lougheed, Molecular variation in *Zonotrichia capensis*; Gerard Malan, Behavior ecology of the Pale Chanting Goshawk; Vicky J. Meretsky, Foraging behavior of Egyptian Vultures in the Negev Desert, Israel; David C. Moyer, Community structure of forest birds in the Uzungwa Mts., Tanzania; Christopher J. Norment, Breeding biology of the Harris' Sparrow; Kevin Omland, Study of parental investment in yearling vs. adult male American Redstarts; Linda D. Partridge, The original drawings and paintings of John James Audubon; Scott M. Pearson, Winter bird communities in Georgia piedmont landscapes; Lisa J. Petit, Factors governing habitat selection by Prothonotary Warblers; Jonathan H. Plissner, Social dominance as a proximate cause of natal dispersal in Eastern Bluebirds; Donald R. Powers Jr., Field metabolic rate in two sympatric hummingbird species; David R. C. Prescott, Differential migration in the Evening Grosbeak: a test of hypotheses; Carla Restrepo and Marta L. Mondragon, Social organization and habitat utilization by Toucan Barbets (Aves: Capitonidae); Loreta Rosselli Ingrid Ayub, and Pilar Vasquez, The courtship displays and social system of *Corapipo leucorrhoa altera*; Peter G. Ryan, The taxonomy, ecology and evolutionary history of *Nesospiza* buntings; Steve Schwelling, Macrogeographic variation in songs of the Painted Bunting; Gilles Seutin, Genetic relationships and species recognition in the redpolls; Dave Shutler, Floater Red-winged Blackbirds; Julia I. Smith, An analysis of genetic and environmental factors influencing phenotypic variation in the Song Sparrow

(*Continued on p. 771*)