

GENETIC VARIATION AND DIFFERENTIATION IN THE SPOTTED OWL (*STRIX OCCIDENTALIS*)

GEORGE F. BARROWCLOUGH¹ AND R. J. GUTIÉRREZ²

¹Department of Ornithology, American Museum of Natural History,
Central Park West at 79th Street, New York, New York 10024 USA, and

²Department of Wildlife, Humboldt State University, Arcata, California 95521 USA

ABSTRACT.—We used starch-gel electrophoresis to investigate genetic variability at 23 loci in 107 individuals from seven populations of the Spotted Owl (*Strix occidentalis*). These populations sample all three currently recognized subspecies. No genetic variation was found in six populations from Oregon and California. Average heterozygosity in owls from New Mexico was 0.022. The low level of genetic variability will make it more difficult to monitor the genetics of this threatened species; the paucity of variation is possibly due to a small overall effective population size or bottlenecks in the past. At one locus there was a major allelic frequency difference between the Pacific Coast populations (*S. o. caurina* and *S. o. occidentalis*) and the allopatric taxon (*S. o. lucida*) found in New Mexico; our estimate of F_{ST} is 0.55. We believe the two allopatric populations have long been isolated, and it is probable that they represent two species. The data do not help elucidate the subspecific status of *S. o. caurina*. Received 11 December 1989, accepted 11 May 1990.

OVER the last two decades, evolutionary and systematic biologists have devoted a major effort to assessing the extent of genetic variation within, and differentiation among, populations (Lewontin 1985, 1986). This has been true of many disciplines, including ornithology (for recent reviews, see Avise and Aquadro 1982, Avise 1983, Barrowclough 1983, Corbin 1983, Barrowclough et al. 1985, Barrowclough and Johnson 1988). These results have allowed biologists to make inferences about the evolutionary history of a species and, perhaps, to generalize about evolutionary processes (e.g. Lewontin 1974). In addition, these studies provide systematists with data, independent of classical morphology and phenotypes, to assess biogeographic and taxonomic patterns (e.g. Barrowclough 1985).

Conservation biologists now realize that such data permit monitoring threatened populations for evidence of genetic deterioration (Lande and Barrowclough 1987) as well as identifying evolutionary and taxonomic units for conservation (e.g. Ryder 1986a, b). The recent report on genetic variation in the endangered New Zealand Kakapo (*Strigops habroptilus*; Triggs et al. 1989) is an example. One North American species that is the subject of much concern among conservationists, foresters, and wildlife biologists is the Spotted Owl, *Strix occidentalis* (e.g. Gutiérrez and Carey 1985, Dawson et al. 1987, U.S. Dep. Agric. For. Serv. 1988).

We report on an electrophoretic examination of genetic variation within and among populations of the Spotted Owl over much of its range. This study adds to the small, but growing, body of reports on the extent of genetic variation and differentiation in natural populations of birds; it also provides a survey of the population genetics of a species of particular environmental concern.

METHODS

Study areas and populations.—We sampled Spotted Owls from eight populations representing all three currently recognized subspecies (AOU 1957) (Fig. 1; Table 1). Populations differed greatly in their habitat characteristics and their population size. The Oregon and northwest California populations were large, continuous populations within a mix of old growth and younger age conifer forests. The Sierra Nevada and New Mexico populations were large and continuous. The habitat was characterized by a mixture of old growth, mixed age, and young forests. The southern California owls existed in small isolated populations located primarily in mixed age conifer stands.

We probably sampled <5% of the birds from the large continuous populations. Because we sampled only two birds from the Black Range, we combined the New Mexico samples for the purpose of analysis. We sampled approximately 20% of the San Bernardino range population. The two smallest populations were sampled most intensively: approximately 90% of the San Jacinto mountain population and approximately 50% of the Palomar Mountain birds. All estimates of

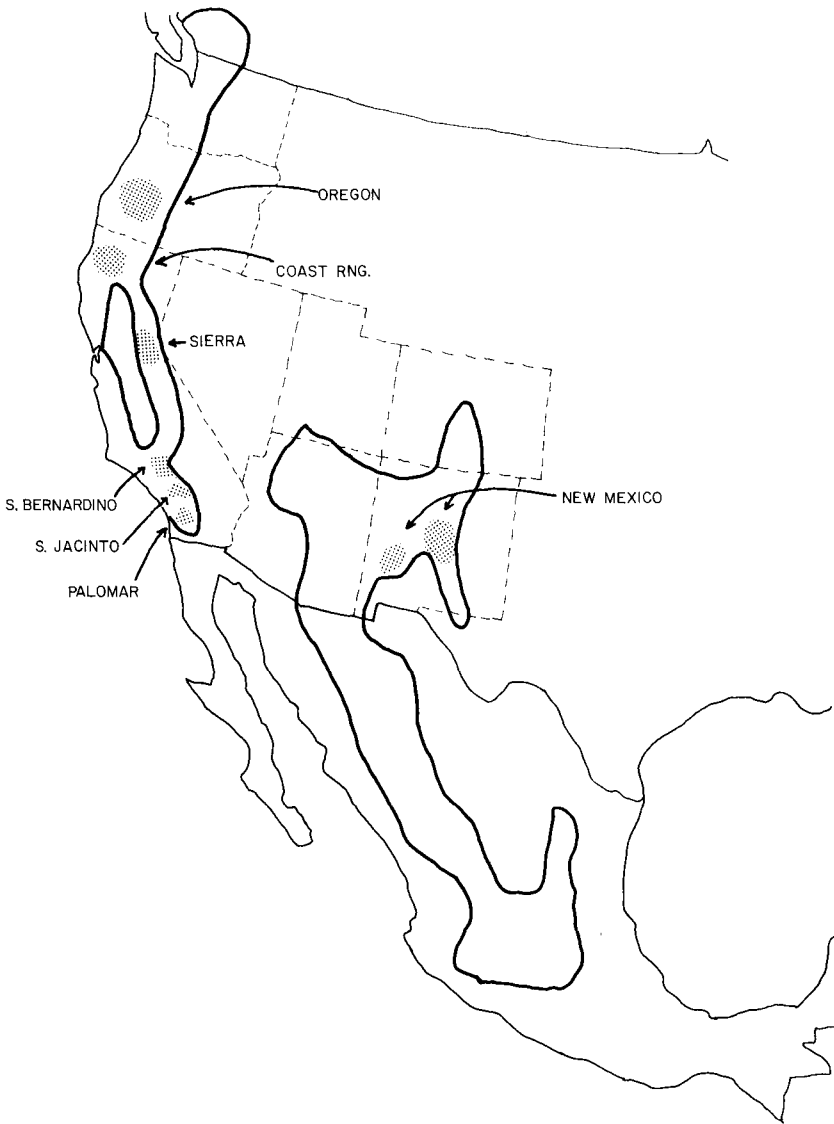


Fig. 1. Approximate distribution of the Spotted Owl. Localities from which genetic samples were obtained are indicated.

the proportion of the population sampled were derived from extensive census and banding studies (Franklin et al. 1990).

Capture methods.—Spotted Owls were located by imitating their calls to elicit a response (Forsman 1983, Franklin et al. 1990). Responding birds were captured with noose poles, mist nets, or pan traps (Forsman 1983, Bull 1987). Blood was taken from a brachial vein with a 23-ga needle and 1-cc syringe washed with EDTA as an anticoagulant. Each blood sample was immediately transferred to a cryogenic vial and frozen in liquid nitrogen within one hour of extraction.

A few (<6) blood samples were not frozen for several hours because the sampling location was remote. Samples were maintained in liquid nitrogen, dry ice, and ultracold freezers until used for electrophoresis.

With a few exceptions (accounted for in the analyses), only unrelated individuals were sampled. Although we did not know the exact lineage of each bird, we inferred from previous banding and population studies that most of the birds were probably not closely related. However, the small isolated populations must have a substantial background level of relatedness.

TABLE 1. Taxa, localities, sample sizes, and estimates of genetic heterozygosity in Spotted Owl (*Strix occidentalis*) samples.

| Population | n^a | \hat{H}^b | Subspecies |
|---|-------|-------------|---------------------------|
| Oregon; Cascade & Coast ranges; Douglas County | 20 | 0.0 | <i>S. o. caurina</i> |
| California; Coast Ranges; Humboldt & Mendocino counties | 19 | 0.0 | <i>S. o. caurina</i> |
| California; Sierra Nevada; Placer & El Dorado counties | 11 | 0.0 | <i>S. o. occidentalis</i> |
| California; San Bernardino Mtns.; San Bernardino County | 20 | 0.0 | <i>S. o. occidentalis</i> |
| California; San Jacinto Mtns.; Riverside County | 19 | 0.0 | <i>S. o. occidentalis</i> |
| California; Mt. Palomar; San Diego County | 9 | 0.0 | <i>S. o. occidentalis</i> |
| New Mexico; Sacramento & Black ranges; Grant & Lincoln counties | 9 | 0.022 | <i>S. o. lucida</i> |

^a Number of individuals sampled from population.

^b Averaged over 23 genetic loci.

Analysis.—Thawed blood was diluted with deionized water to lyse cells, then centrifuged at 6,000 rpm for 20 min. The supernatant was used for standard starch-gel electrophoresis. The electrophoretic conditions, buffer systems, and staining methods have been described previously (Harris and Hopkinson 1976, Barrowclough and Corbin 1978, Richardson et al. 1986). Nomenclature of enzymes, multiple isozymes, and alleles follows standard conventions.

Genotypic and allelic frequencies were computed from the starch-gel results. These data were analyzed using Hardy-Weinberg tests (Crow and Kimura 1970), contingency Chi-square tests (Workman and Niswander 1970), Slatkin's (1985) rare allele procedure, Rogers' (1972) genetic distance, Wright's (1978) F_{ST} statistics, and Nei's (1978) standard genetic distance and heterozygosity. The latter three statistics all included corrections for sampling error due to finite numbers of individuals.

RESULTS

We scored 23 loci that yielded consistently strong staining patterns. Additional enzymes that are typically scored using liver and skeletal muscle in birds were found to be weak, inconsistent, or absent in these red cell lysates.

Genetic variation.—Of the 23 loci examined, 22 were monomorphic in all populations (EC numbers in parentheses): ACP (3.1.3.2), ADA (3.5.4.4), CK (2.7.3.2), EST-2 (3.1.1.1), G6PDH (1.1.1.49), GPT (2.6.1.2), HGB (hemoglobin), IDH (1.1.1.42), LDH-1 and LDH-2 (1.1.1.27), MDH-1 and MDH-2 (1.1.1.37), NP (2.4.2.1), PEP-A, PEP-B, and PEP-C (3.4.11), 6PGDH (1.1.1.44), PGI (5.3.1.9), PGM (2.7.5.1), PT-1 and PT-2 (plasma proteins), and SOD (1.15.1.1). One locus, EST-D (3.1.1.1; by the methylumbelliferyl fluorescent method), was variable in the New Mexico sample. It was, however, monomorphic in all the Pacific Coast populations. The allele fixed in the coastal populations had a frequency of

0.389 in the New Mexico sample; a single alternate allele in that population had a frequency of 0.611.

Sample sizes and estimates of overall heterozygosity are in Table 1. Esterase-D is dimeric (Harris and Hopkinson 1976); the heterozygotes for this locus all had the characteristic three-banded pattern of such proteins. The observed three genotypic frequencies do not differ from Hardy-Weinberg proportions ($\chi^2 = 0.255$, $df = 1$, $P > 0.05$) in the New Mexico sample.

Genetic differentiation.—At the Esterase-D locus, the differences in allelic frequencies among the populations are statistically significant ($\chi^2 = 1273.4$, $df = 6$, $P < 0.005$; Workman and Niswander 1970). For this single locus, the estimate of F_{ST} among the seven populations is 0.55. Of course, this is actually an estimate of F_{ST} between the New Mexico population and those of California and Oregon. Similarly, our estimate of Nei's distance between New Mexico and the west coast is 0.016; for Rogers' distance, the estimate is 0.027.

Slatkin (1985) provided a method of estimating the extent of gene flow among populations from the frequency of private polymorphisms. The Esterase-D allele found segregating in the New Mexico population is an example of such a situation. From Slatkin's formula, we obtain an estimate of $Nm = 0.021$. This is equivalent to an average of one individual exchanged among populations every 50 generations.

DISCUSSION

Genetic variation.—This is the first published report on genetic variability in a natural population of owls. The estimated level of genetic variation (heterozygosity) in the samples of *S. o. occidentalis* and *S. o. caurina* (0.0) is remarkably

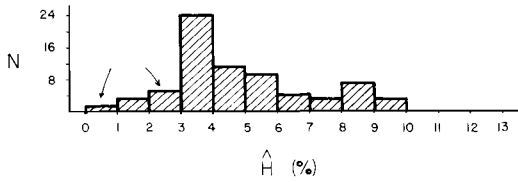


Fig. 2. Distribution of reports of genetic heterozygosity in birds based on 20 or more loci and 10 or more individuals. (Based on summaries of Barrowclough et al. 1985, Corbin 1987; and additional reports of Braun and Robbins 1986, Baker and Moeed 1987, Haig and Oring 1988, Seutin and Simon 1988).

low. This is one of very few cases of an apparent lack of detected variation in a population of birds for which a relatively large number of individuals and loci have been examined. For example, in recent reviews of genetic heterozygosity in birds (Barrowclough 1983; Corbin 1983, 1987; and Barrowclough et al. 1985), only one species with such an estimate is tabulated. This is the Lesser Prairie-Chicken (*Tympanuchus pallidicinctus*), based on 13 individuals and 23 loci (Gutiérrez et al. 1983). There are other nominal estimates of H of 0.0 (e.g. Patton and Avise 1986), but these are based on samples of one or a few individuals. We summarized (Fig. 2) estimates of H reported for birds calculated from at least 20 loci and 10 individuals. The west coast Spotted Owl populations lie at the extreme of the distribution. Outside of birds, estimates of H of zero are not common, but are known from a variety of taxa (e.g. see Nevo 1978).

The population of *S. o. lucida* has an estimate of H more typical of birds. It may seem odd that within the same species there should be populations with such disparate values, but the variability in *S. o. lucida* is the result of one polymorphic locus, and we will argue that these taxa probably have been isolated from each other for a long time.

We emphasize that these results do not mean that there is no genetic variability in the coastal populations of Spotted Owls. They indicate only that at some of the loci routinely screened by electrophoresis, the birds are invariant. Other structural genetic or regulatory loci, or the loci coding for quantitative traits, may be variable.

There are several possible reasons for the lack of genetic variability reported for the Pacific coast populations. First, the 23 loci we examined may be a particularly poor choice in that they

are relatively monomorphic compared with "average" loci. This is equivalent to arguing that some genetic loci show more variability than others, across all taxa, and that members of the subset we examined are relatively invariant. Consequently, examination of another—complementary—set of loci may reveal substantial variation. We believe this is not a likely explanation. Some of the loci we examined (e.g. ADA, EST, NP, PEP, 6PGDH, and PGM) are among the most variable in birds (Evans 1987).

Second, it is possible that *Strix* owls, or large predators in general, because of their elevated position on the food chain, inherently have lower population sizes than do, for example, insectivores and granivores. Hence, they might have lower heterozygosity if H scales positively with effective population size (N_e), as neutralist theory suggests (Barrowclough et al. 1985). This possibility cannot be ruled out easily. Unfortunately there have been few studies of genetic variability in raptors. Barrowclough and Coats (1985) suggest that the genetic population structure of *S. occidentalis* in the Pacific Northwest consists of a series of overlapping demes of approximately 220 individuals each. If there are ten or so such demes in the range of the owls, then the total effective size of the coastal population might be only a couple of thousand (i.e. much smaller than the overall population size of taxa such as thrushes, warblers, and sparrows, etc., that include hundreds, if not thousands, of interconnected demes in their ranges). A way to check this hypothesis would be to compute correlations of heterozygosity on trophic level. However, this is not a strong test and is subject to many difficulties (Schnell and Selander 1981).

Third, the low variability observed could be due to extended population bottlenecks or founder effects in the past few thousands of years. Nei et al. (1975), for example, demonstrate that the heterozygosity of a population, following a long period of reduced numbers, will require 10^5 to 10^6 generations to recover to equilibrium levels. Likewise, a founder effect could also produce low levels of genic variation, but only if the founder population remained small for a very long period of time. This class of explanations can neither be ruled out easily, nor tested readily.

A fourth possibility is that the low heterozygosity in these owls is the result of habitat

destruction caused by timber cutting. This might have led to a reduction in the numbers of owls and hence to a loss of variability. This is an unlikely explanation, and is equivalent to arguing that the owls are now in a bottleneck because of logging. However, Spotted Owls are not yet rare (e.g. Gould 1977, Forsman et al. 1987, Franklin et al. 1990), even though this activity probably has reduced the population substantially in the past 100 years. It takes tens to hundreds of generations of reduced population size to reduce genetic variability (Nei et al. 1975).

Finally, it is possible that genic heterozygosity is less affected by population processes and demography than by internal genetic processes, physiology, and the efficacy of DNA repair mechanisms. From the point of view of whole organism biologists, heterozygosity might be a stochastic variable and Spotted Owls just happen to have variability of zero for these loci at this time. If so, then genetic data are uninformative about any aspect of the population biology of a species. This explanation is basically a null hypothesis best adopted as an alternative to specific causative models. It is not tested easily.

Clearly, at present we are unable to discriminate among the competing explanations of the reduced heterozygosity in the Spotted Owl. Nevertheless, only three of the possible explanations are particularly plausible, and these allow us to draw inferences of interest.

None of the most probable causes for the low variability lead to the conclusion that the owls are currently at risk because of genetic difficulties such as inbreeding. This may seem counterintuitive, but observed variation in an electrophoretic study has to be viewed simply as a genetic marker for monitoring the genetic makeup of a population (Lande and Barrowclough 1987). Variation in the loci studied should not be interpreted as being critical per se.

A second conclusion we can draw is that, unfortunately, it will be difficult to monitor the genetics of Pacific Coast populations of these owls for conservation purposes. If further testing of structural genes does not yield easily observed variation, then more difficult and expensive techniques, such as mtDNA restriction mapping or heritability of quantitative traits, will have to be used to monitor the genetic structure of this species. In the populations of

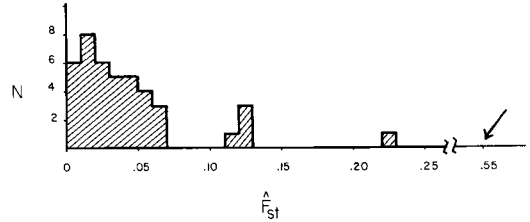


Fig. 3. Distribution of reports of Wright's F_{ST} in birds. (Based on summaries of Barrowclough 1983, Barrowclough and Johnson 1988; and additional reports of Van Wagner and Baker 1986, Baker and Moed 1987, Grudzien et al. 1987, Haig and Oring 1988, Seutin and Simon 1988).

S. o. lucida, EST-D is readily available, easy to monitor with blood samples, and polymorphic.

Genetic differentiation.—No variation was observed in the samples of *S. o. occidentalis* and *S. o. caurina*. Thus it was not possible to observe any differentiation or to estimate its magnitude. Therefore, our data were neutral with respect to the taxonomy of these two currently recognized subspecies. Our results cannot be used to argue either for or against lumping of the taxa. Other molecular or morphological studies will be required to address this issue.

The extent of differentiation observed between the New Mexico sample of *S. o. lucida* and the California and Oregon samples is large by avian standards (see Fig. 3 for estimates of F_{ST} among conspecific populations of birds from the literature). The observed value for this species (0.55) is the largest reported to date. Of course, this estimate is based on a single locus and has an unknown, but probably quite large, standard error.

The estimates of Nei's and Rogers' genetic distances are also large in comparison with reports of the same statistics from other conspecific avian populations (e.g. see summary in Barrowclough 1980). The estimate lies in the range of overlap between the average differentiation among subspecies and that among species. Some subspecies with larger genetic distances are known (e.g. Baker and Strauch 1988, Capparella 1988). Likewise, some species show smaller distances (e.g. Hackett 1989).

The geographical extent of the EST-D polymorphism found in the New Mexico birds requires analysis. We presume the polymorphism characterizes the entire *S. o. lucida* taxon, but that should be established through further sam-

pling. If the polymorphism is widespread, then we find this pattern of variation interesting because of its implications for the past evolutionary history and taxonomic status of the owls.

The most frequent allele (at the EST-D locus) in our sample of *S. o. lucida* was absent in the relatively large sample of the Pacific Coast birds, which represents six populations including those geographically nearest to the range of the interior taxon. We suggest that there is currently no gene flow between the taxa, nor has there been any for an evolutionarily long period of time. We draw this inference because the period of time required for an allele to go from rare to common, or from common to lost (either may be the case in this situation because we do not know the ancestral state), is on the order of the inverse of the effective population size. Certainly, in this case, this is more than a thousand generations. Barrowclough and Coats (1985) estimated that generation time for Spotted Owls is approximately seven years. The private polymorphism analysis using Slatkin's technique reinforces this conclusion. The magnitude of estimated gene flow (one individual per 50 generations) is an upper bound that may seriously overestimate current gene flow (Larson et al. 1984, Rockwell and Barrowclough 1987).

Species status.—Allopatric populations of birds, with their own evolutionary histories and a lack of gene flow for thousands of years, present taxonomic problems. In the worldview of the Biological Species Concept (e.g. Mayr 1970), these populations are not in contact. Whether they are behaviorally or genetically reproductively isolated is unknown. One might suppose, given our knowledge of the propensity for hybridization among birds in general (e.g. Rising 1983), that they would interbreed if given a chance. However, treating the owls as conspecific based on that line of reasoning does not reflect the status quo. It is essentially a prediction about what the owls might do at some indefinite time in the future if environmental changes facilitate contact.

In the framework of the Evolutionary Species Concept (*sensu* Wiley 1978), the populations apparently have had their own separate evolutionary histories indicated by the major allelic frequency difference. Consequently, they would be considered separate species.

Within the Phylogenetic Species Concept (e.g. Cracraft 1983), these owls are not quite diag-

nosable. The frequency of the EST-D allele in the New Mexico sample is such that 90% of those birds can be unambiguously distinguished from the *S. o. occidentalis* and *S. o. caurina* populations. This is a more extreme case of the pattern in the *Empidonax difficilis* complex (Johnson and Marten 1988), a situation in which the biogeography is concordant with that of these owls. However, based on our interpretation of the available data, we suggest that the past period of isolation has been sufficiently long that further examination will reveal fixed differences when more genetic loci are assayed. Thus, there exists the distinct possibility that these are two species of birds.

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LITERATURE CITED

- AMERICAN ORNITHOLOGISTS' UNION. 1957. Check-list of North American birds. Fifth ed. Washington, D.C., Am. Ornithol. Union.
- AVISE, J. C. 1983. Commentary. Pp. 262-270 in *Perspectives in ornithology* (A. H. Brush and G. A. Clark Jr., Eds.). New York, Cambridge Univ. Press.
- , & C. F. AQUADRO. 1982. A comparative summary of genetic distances in the vertebrates. *Evol. Biol.* 15: 151-185.
- BAKER, A. J., & A. MOEED. 1987. Rapid genetic differentiation and founder effect in colonizing populations of Common Mynas (*Acridotheres tristis*). *Evolution* 41: 525-538.
- , & J. G. STRAUCH JR. 1988. Genetic variation and differentiation in shorebirds. Pp. 1639-1645 in *Acta XIX Congressus Internationalis Ornithologici* (H. Ouellet, Ed.). Ottawa, Ontario, Natl. Museum Nat. Sci.

- BARROWCLOUGH, G. F. 1980. Genetic and phenotypic differentiation in a wood warbler (genus *Dendroica*) hybrid zone. *Auk* 97: 655-668.
- . 1983. Biochemical studies of microevolutionary processes. Pp. 223-261 in *Perspectives in ornithology* (A. H. Brush and G. A. Clark Jr., Eds.). New York, Cambridge Univ. Press.
- . 1985. Museum collections and molecular systematics. Pp. 43-54 in *Museum collections: their roles and future in biological research* (E. H. Miller, Ed.). Victoria, British Columbia Prov. Museum.
- , & S. L. COATS. 1985. The demography and population genetics of owls, with special reference to the conservation of the Spotted Owl (*Strix occidentalis*). Pp. 74-85 in *Ecology and management of the Spotted Owl in the Pacific Northwest* (R. J. Gutiérrez and A. B. Carey, Eds.). Portland, Oregon, U.S. Dep. Agric. For. Serv.
- , & K. W. CORBIN. 1978. Genetic variation and differentiation in the Parulidae. *Auk* 95: 691-702.
- , & N. K. JOHNSON. 1988. Genetic structure of North American birds. Pp. 1630-1638 in *Acta XIX Congressus Internationalis Ornithologici* (H. Ouellet, Ed.). Ottawa, Ontario, Natl. Museum Nat. Sci.
- , & R. M. ZINK. 1985. On the nature of genic variation in birds. Pp. 135-154 in *Current ornithology*, vol. 2 (R. F. Johnston, Ed.). New York, Plenum Press.
- BRAUN, M. J., & M. B. ROBBINS. 1986. Extensive protein similarity of the hybridizing chickadees *Parus atricapillus* and *P. carolinensis*. *Auk* 103: 667-675.
- BULL, E. L. 1987. Capture techniques for owls. Pp. 291-293 in *Biology and conservation of northern forest owls* (R. W. Nero, R. J. Clark, R. J. Knapton, & R. H. Hamre, Eds.). Ft. Collins, Colorado, U.S. Dep. Agric. For. Serv.
- CAPPARELLA, A. P. 1988. Genetic variation in Neotropical birds: implications for the speciation process. Pp. 1658-1664 in *Acta XIX Congressus Internationalis Ornithologici* (H. Ouellet, Ed.). Ottawa, Ontario, Natl. Museum Nat. Sci.
- CORBIN, K. W. 1983. Genetic structure and avian systematics. Pp. 211-244 in *Current ornithology*, vol. 1 (R. F. Johnston, Ed.). New York, Plenum Press.
- . 1987. Geographic variation and speciation. Pp. 321-353 in *Avian genetics* (F. Cooke & P. A. Buckley, Eds.). London, Acad. Press.
- CRACRAFT, J. 1983. Species concepts and speciation analysis. Pp. 159-187 in *Current ornithology*, vol. 1 (R. F. Johnston, Ed.). New York, Plenum Press.
- CROW, J. F., & M. KIMURA. 1970. *An introduction to population genetics theory*. New York, Harper and Row.
- DAWSON, W. R., J. D. LIGON, J. R. MURPHY, J. P. MYERS, D. SIMBERLOFF, & J. VERNER. 1987. Report of the scientific advisory panel on the Spotted Owl. *Condor* 89: 205-229.
- EVANS, P. G. H. 1987. Electrophoretic variability of gene products. Pp. 105-162 in *Avian genetics* (F. Cooke and P. A. Buckley, Eds.). London, Acad. Press.
- FORSMAN, E. D. 1983. Methods and materials for locating and studying Spotted Owls. U.S. Dep. Agric. For. Serv. Gen. Tech. Rep. PNW-162: 1-8.
- , C. R. BRUCE, M. A. WALTER, & E. C. MESLOW. 1987. A current assessment of the Spotted Owl population in Oregon. *Murrelet* 68: 51-54.
- FRANKLIN, A. B., J. P. WARD, R. J. GUTIÉRREZ, & G. I. GOULD JR. 1990. Density of Northern Spotted Owls in northwest California. *J. Wildl. Manage.* 54: 1-10.
- GOULD, G. I., JR. 1977. Distribution of the Spotted Owl in California. *Western Birds* 8: 131-146.
- GRUDZIEN, T. A., W. S. MOORE, J. R. COOK, & D. TAGLE. 1987. Genic population structure and gene flow in the Northern Flicker (*Colaptes auratus*) hybrid zone. *Auk* 104: 654-664.
- GUTIÉRREZ, R. J., & A. B. CAREY. 1985. Ecology and management of the Spotted Owl in the Pacific Northwest. Portland, Oregon, U.S. Dep. Agric. For. Serv.
- , R. M. ZINK, & S. Y. YANG. 1983. Genic variation, systematic, and biogeographic relationships of some Galliform birds. *Auk* 100: 33-47.
- HACKETT, S. J. 1989. Effects of varied electrophoretic conditions on detection of evolutionary patterns in the Laridae. *Condor* 91: 73-90.
- HAIG, S. M., & L. W. ORING. 1988. Genetic differentiation of Piping Plovers across North America. *Auk* 105: 260-267.
- HARRIS, H., & D. A. HOPKINSON. 1976. *Handbook of enzyme electrophoresis in human genetics*. Amsterdam, North Holland Publ. Co.
- JOHNSON, N. K., & J. A. MARTEN. 1988. Evolutionary genetics of flycatchers. II. Differentiation in the *Empidonax difficilis* complex. *Auk* 105: 177-191.
- 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, Cambridge Univ. Press.
- LARSON, A., D. B. WAKE, & K. P. YANEV. 1984. Measuring gene flow among populations having high levels of genetic fragmentation. *Genetics* 106: 293-308.
- LEWONTIN, R. C. 1974. *The genetic basis of evolutionary change*. New York, Columbia Univ. Press.
- . 1985. Population genetics. *Annu. Rev. Genet.* 19: 81-102.
- . 1986. How important is genetics for an understanding of evolution? *Am. Zool.* 26: 811-820.
- MAYR, E. 1970. Populations, species, and evolution.

- Cambridge, Massachusetts, Belknap/Harvard Univ. Press.
- NEI, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* 89: 583-590.
- , T. MARUYAMA, & R. CHAKRABORTY. 1975. The bottleneck effect and genetic variability in populations. *Evolution* 29: 1-10.
- NEVO, E. 1978. Genetic variation in natural populations: patterns and theory. *Theor. Popul. Biol.* 13: 121-177.
- PATTON, J. C., & J. C. AVISE. 1986. Evolutionary genetics of birds. IV. Rates of protein divergence in waterfowl (Anatidae). *Genetica* 68: 129-143.
- RICHARDSON, B. J., P. R. BAVERSTOCK, & M. ADAMS. 1986. Allozyme electrophoresis. Sydney, Australia, Acad. Press.
- RISING, J. D. 1983. The Great Plains hybrid zones. Pp. 131-157 in *Current ornithology*, vol. 1 (R. F. Johnston, Ed.). New York, Plenum Press.
- ROCKWELL, R. F., & G. F. BARROWCLOUGH. 1987. Gene flow and the genetic structure of populations. Pp. 223-255 in *Avian genetics* (F. Cooke and P. A. Buckley, Eds.). London, Acad. Press.
- ROGERS, J. S. 1972. Measures of genetic similarity and genetic distance. *Stud. Genet.* 7: 145-153.
- RYDER, O. A. 1986a. Genetic investigations: tools for supporting breeding programme goals. *Int. Zoo Yearbook* 24/25: 157-162.
- . 1986b. Species conservation and systematics: the dilemma of subspecies. *Trends Ecol. Evol.* 1: 9-10.
- SCHNELL, G. D., & R. K. SELANDER. 1981. Environmental and morphological correlates of genetic variation in mammals. Pp. 60-99 in *Mammalian population genetics* (M. H. Smith and J. Joule, Eds.). Athens, Univ. Georgia Press.
- SEUTIN, G., & J.-P. SIMON. 1988. Genetic variation in sympatric Willow Flycatchers (*Empidonax traillii*) and Alder Flycatchers (*E. alnorum*). *Auk* 105: 235-243.
- SLATKIN, M. 1985. Rare alleles as indicators of gene flow. *Evolution* 39: 53-65.
- TRIGGS, S. J., R. G. POWLESLAND, & C. H. DAUGHERTY. 1989. Genetic variation and conservation of Kakapo (*Strigops habroptilus*: Psittaciformes). *Conserv. Biol.* 3: 92-96.
- U.S. DEP. AGRIC. FOR. SERV. 1988. Final supplement to the environmental impact statement for an amendment to the Pacific Northwest regional guide: Spotted Owl guidelines, vols. 1 and 2. Portland, Oregon, U.S. Dep. Agric. For. Serv., Pacific Northwest Region.
- VAN WAGNER, C. E., & A. J. BAKER. 1986. Genetic differentiation in populations of Canada Geese (*Branta canadensis*). *Can. J. Zool.* 64: 940-947.
- WILEY, E. O. 1978. The evolutionary species concept reconsidered. *Syst. Zool.* 27: 17-26.
- WORKMAN, P. L., & J. D. NISWANDER. 1970. Population studies on Southwestern Indian tribes. II. Local genetic differentiation in the Papago. *Am. J. Human Genet.* 22: 24-49.
- WRIGHT, S. 1978. *Evolution and the genetics of populations*, vol. 4. Chicago, Univ. Chicago Press.