GENETIC DIFFERENTIATION AMONG STEAMER-DUCKS (ANATIDAE: *TACHYERES*): AN ELECTROPHORETIC ANALYSIS[1]

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Abstract. Electrophoretic and isoelectric focusing analyses of liver proteins of the steamer-ducks, *Tachyeres patachonicus, T. pteneres, T. brachypterus,* and *T. leucocephalus,* show these species to be distinct genetically, with the latter three species being more closely related to one another than any one of them is to *T. patachonicus.* There is also significant differentiation among populations of *T. patachonicus.* Estimates of the average calculated heterozygosity per species are high: 0.185, 0.160, 0.165, and 0.084, respectively, and observed heterozygosities are 0.115 ± 0.090, 0.202 ± 0.118, 0.201 ± 0.085, and 0.080 ± 0.069, respectively. The genetic distances of Cavalli-Sforza and Edwards, Nei, and Rogers were estimated and used with a Wagner tree algorithm to prepare a consensus tree based on 1,000 subsets of allelic frequency data prepared by bootstrapping over loci. Estimates of F_{st} for comparisons among populations of *T. patachonicus* and estimates of Nm based on the distribution of private alleles indicate that gene flow between some pairs of populations is significantly reduced. Values of F_{st} are much higher than those previously found for other pairs of avian populations. Genotypic distributions that deviate significantly from equilibrium expectations are found for several of the polymorphic loci of one or more species.

Key words: Tachyeres; steamer-ducks; subspecies; population genetic structure; electrophoresis; protein polymorphism; gene flow; phylogenetic relationships.

INTRODUCTION

As recently as 1981, a new species of steamer-duck of the genus *Tachyeres* was recognized (Humphrey and Thompson 1981), which underscores the fact that this genus is not yet well-known and continues to offer systematists and evolutionary biologists a number of interesting problems. The evolutionary relationship of *Tachyeres* to other anatid genera was examined previously using morphological and behavioral traits (Delacour 1954; Moynihan 1958; Johnsgard 1965, 1978; Weller 1976; Livezey 1986a), and most authors agree that the steamer-ducks are allied with the shelducks; but see Woolfenden (1961).

Intrageneric relationships within *Tachyeres* have been studied only recently (Livezey 1986b, Livezey et al. 1986), although it has been assumed that the Flying Steamer-Duck (*T. pata-*

chonicus) is the most similar to the ancestor of the genus (Murphy 1936, Weller 1976). This hypothesis is supported by the cladistic analysis of Livezey (1986b) who showed that the three flightless species, *T. pteneres, T. brachypterus,* and *T. leucocephalus,* are monophyletic, with the latter two being closest relatives. These relationships are consistent with the morphometric differences in the genus, most of which are related to flightlessness (Livezey and Humphrey 1986). However, Livezey et al. (1986) compared the composition of uropygial secretions of *Tachyeres,* and found that by this criterion *T. leucocephalus* was most distinct from the other three species.

We have used the techniques of starch-gel electrophoresis and isoelectric focusing in agarose gel to examine the allozyme variation in each of the four currently recognized species of *Tachyeres* and in four geographically isolated populations of the Flying Steamer-Duck, *T. patachonicus.* Here we present these data and the results of phylogenetic and population genetic analyses based on them.

[773]

TABLE 1. Allelic frequencies of four populations of *Tachyeres patachonicus* in Argentina and Chile, South America. *n* is the number of genomes assayed for a given locality and locus.

Locus allele	Ushuaia Argentina	Pto. Deseado Argentina	Argentine Andean Lakes	Chile
AcP				
a	—	—	0.275	—
b	0.222	0.292	0.100	—
c	0.778	0.708	0.625	1.00
n	18	24	40	22
Es-1				
a	—	0.024	0.023	0.273
b	—	—	0.432	0.318
c	0.115	0.190	0.432	0.227
d	0.846	0.738	0.114	0.182
e	0.038	—	—	—
f	—	0.048	—	—
n	26	42	44	22
GOT-1				
a	—	0.095	0.659	0.545
b	0.808	0.762	0.318	0.455
c	0.192	0.143	0.023	—
n	26	42	44	22
MPI				
a	0.115	0.167	0.114	0.091
b	0.885	0.762	0.886	0.909
c	—	0.071	—	—
n	26	42	44	22
PGI-2				
a	—	0.071	—	0.091
b	—	—	—	0.682
c	0.769	0.786	0.975	0.227
d	0.231	0.071	0.025	—
e	—	0.071	—	—
n	26	42	40	22

MATERIALS AND METHODS

Between December 1980 and May 1984 PSH and BCL collected 103 specimens of the genus *Tachyeres* from Argentina and Chile for biochemical analysis. Of these specimens, six are from two populations of *T. pteneres* (Puerto Montt, Region X, Chile, and Ushuaia, Tierra del Fuego, Argentina), nine are from a single population of *T. leucocephalus* (Puerto Melo, Provincia de Chubut, Argentina), 21 are from a single population of *T. brachypterus* (Lively Island, Falkland Islands), and 67 represent 12 collecting localities of *T. patachonicus*. These latter 12 localities can be grouped into three geographic regions, one of which is coastal Argentina (Co) represented by Puerto Melo, Provincia de Chu-

but (two specimens), Puerto Deseado, Provincia de Santa Cruz (21 specimens), and Ushuaia, Tierra del Fuego (13 specimens). The second region consists of Argentine Andean lakes (AL) represented by Lago Futalaufquen, Provincia de Chubut (four specimens), Lago Fontana, Provincia de Chubut (six specimens), Lago Krugger, Provincia de Chubut (one specimen), Parque Nacional Francisco Perito Moreno, Provincia de Santa Cruz (four specimens), and Lago Roca, Provincia de Santa Cruz (five specimens). The third region lies near Puerto Montt, Chile (Ch) and includes Lago Rupanco, Provincia de Osorno (five specimens), Lago Llanquihue, Provincia de Llanquihue (one specimen), Ensenada Huito, Provincia de Llanquihue (three specimens), and Ensenada Codihue, Provincia de Llanquihue (two specimens). A map showing these localities is given in Livezey (1986c). The populations of coastal Argentina span a distance of about 1,150 km, whereas they are 300 to 600 km from the Argentine Andean lakes region, and from 600 to 1,400 km from the Chilean population.

For the purpose of the statistical and genetic analyses the specimens of *T. patachonicus* have been dealt with in two ways. For interpopulation comparisons the samples from Ushuaia and Puerto Deseado, Argentina were not combined, whereas specimens from the other localities were grouped as described in the previous paragraph. For the interspecific comparisons, specimens of *T. patachonicus* are represented by the three groups indicated above, whereas the separate populations of each of the other species have been treated as a single sample for the species. These various groupings, identified either by taxon or collecting locality, and the number of genomes assayed for each locus are given in Tables 1 and 2.

Within 3 hr of collection, samples of liver tissue were frozen in liquid nitrogen. Extracts of these tissues were later prepared either according to the procedure of Corbin et al. (1974) and Barrowclough and Corbin (1978) for analysis by starch-gel electrophoresis or in distilled water for isoelectric focusing studies. During the early stages of our study we examined all 15 of the loci we normally assay in liver tissue by horizontal starch-gel electrophoresis. These early analyses did not include tissue from *T. brachypterus,* however. Thus, the allozymic variation at only 10 loci (detailed below) of all populations was analyzed by isoelectric focusing (IEF) in thin layers (0.5 mm

thick) of agarose, using the LKB Multiphor electrophoretic system following procedures of Dykes et al. (1982). Technical problems prevented us from examining more loci by IEF.

The polymorphic loci assayed and their Enzyme Commission numbers are as follows: acid phosphatase (*Acp*, 3.1.3.2), esterase (*Es*, 3.1.1.1), aspartate aminotransferase-1 (*Got-1*, 2.6.1.1), glucose-6-phosphate isomerase-2 (*Gpi-2*, 5.3.1.9), α-glycerol phosphate dehydrogenase (α-*gpdh*, 1.1.1.8), malic enzyme (*Me*, 1.1.1.40), mannose-6-phosphate isomerase (*Mpi*, 5.3.1.8), valyl-leucine dipeptidase (*Pep-A*, 3.4.11.11), leucyl-glycyl-glycine dipeptidase (*Pep-B*, 3.4.11.4), and leucyl-alanine dipeptidase (*Pep-C*, 3.4.11). The monomorphic loci are: aspartate amino-transferase-2 (*Got-2*, 2.6.1.1), glucose phosphomutase-1 and -2 (*Pgm-1* and *Pgm-2*, 5.4.2.2), isocitrate dehydrogenase (*Idh*, 1.1.1.42), and glucose-6-phosphate isomerase-1 (*Gpi-1*, 5.3.1.9). The loci we were unable to score following IEF included the three dipeptidases, α-glycerol phosphate dehydrogenase, and malic enzyme.

For the IEF gels, the agarose was prepared by adding 1.10 ml of pH 3–10 carrier ampholyte (Sigma Pharmalyte No. P-1522) to a 70°C solution containing 0.19 g agarose, 2.3 g sucrose, and 17.4 ml distilled water (Agarose IEF, Pharmacia; IsoGel, FMC; and Agarose No. A-4905, Sigma, were used). Gels were allowed to cure for a minimum of 12 hr at 4°C prior to use. The anodal electrode wick contained 1 M H_3PO_4 and the cathodal wick contained 0.2 N NaOH. Two lambda of each of 40 extracts were then applied directly to the agarose gel within 0.5- × 2- × 7-mm wells made by placing a plastic template on the gel surface. IEF was begun only after the extracts had soaked into the gel. During IEF the limiting settings were 1,800 v and 25 ma, with the power being increased from 5 w to 7, 9, and 11 w at 15, 30, and 45 min into the run. Thereafter, IEF was continued until the wattage dropped back to 3, which took from 1.5 to 2 hr. Following IEF, the gels were assayed for the above named enzymes using agar overlays, based on the recipes of Shaw and Prasad (1970) and Barrowclough and Corbin (1978).

The allelic frequencies at each locus were estimated from the observed genotypic (electromorph) distributions, and these estimates were then used in the other genetic and statistical analyses. For the estimation of heterozygosities we used the data set based on 15 loci for *T. pata-*

TABLE 2. Allelic frequencies of *Tachyeres* species. *Tachyeres patachonicus* is represented by the coastal populations of Puerto Deseado and Ushuaia, Argentina, whose genotypic distribution data were combined for the estimation of allelic frequencies. *n* is the number of genomes sampled for a locus and taxon.

Locus allele	patachonicus (coastal)	leucocephalus	brachypterus	pteneres
AcP				
a	—	—	0.191	—
b	0.262	—	0.071	—
c	0.738	1.00	0.738	1.00
n	42	4	42	8
Es-1				
a	0.015	—	0.306	0.167
b	—	0.222	0.306	0.167
c	0.162	0.667	0.194	0.250
d	0.779	0.111	0.194	0.417
e	0.015	—	—	—
f	0.029	—	—	—
n	68	18	36	12
GOT-1				
a	0.059	—	0.526	0.417
b	0.779	0.889	0.474	0.583
c	0.162	0.111	—	—
n	68	18	38	12
MPI				
a	0.147	0.083	—	—
b	0.809	—	—	—
c	0.044	0.917	1.00	1.00
n	68	12	42	4
PGI-2				
a	0.044	—	—	—
b	—	—	—	0.167
c	0.779	1.00	1.00	0.750
d	0.132	—	—	0.083
e	0.044	—	—	—
n	68	18	28	12

chonicus, T. pteneres, and *T. leucocephalus,* and that based on 10 loci for *T. brachypterus.* Mean calculated heterozygosities are estimated as the average over all loci of $1 - \Sigma x_i^2$, where x_i is the frequency of the *i*th allele at a locus. Mean observed heterozygosities are calculated as the average over individuals of the ratio of heterozygous loci to the total number of loci assayed for the individual.

The restricted data set was used in calculating the fixation indices F_{st} and F_{is}, estimates of gene flow, *Nm*, and four measures of genetic distance. The estimation of coancestry among populations of *T. patachonicus* by means of F_{st} analysis used the method of Wright (1965, 1978). These values

of F_{st} are corrected for sampling error due to the small number of individuals sampled, and their deviation from zero is tested as χ^2, where χ^2 is the sum over loci of $2NF_{st}$. See Corbin and Wilkie (1988) for an assessment of this test.

Values of F_{is} were estimated as 1.0 minus the ratio of heterozygotes observed to the number expected. Rather than use the Hardy-Weinberg equilibrium values for the number of heterozygotes expected, we have estimated the heterozygosity per locus, h, according to the method of Nei (1978), which corrects for sampling error bias due to the size of the sample population. These values of heterozygosity were then used to calculate the expected number of heterozygotes per locus.

Gene flow between localities of the Flying Steamer-Duck (*T. patachonicus*) was estimated as Nm, the number of migrants per generation (Slatkin 1985), which is based on the linear relationship between gene flow and the conditional average frequency of alleles found in only one deme or population. In using the equation $\ln[p(1)] = a \ln(Nm) + b$ (Slatkin 1985), where $a = -0.505$ and $b = -2.44$, estimates of Nm have been adjusted for sampling error by dividing the initial values of Nm by a factor of 0.65 (i.e., $N/25$), as suggested by Slatkin (1985), where N for our study = 16.25 individuals.

For comparison to other studies, we provide four different estimates of genetic distance among taxa. These are the unbiased genetic distance of Nei (1978), the genetic distance of Rogers (1972), and the chord and arc distances of Cavalli-Sforza and Edwards (1967). Following the practice of Wright (1978), the chord distances are not multiplied by a factor equal to $(2\sqrt{2})/Pi$, and thus chord values range between zero and 1.0, as for arc and Rogers' distances.

The dendrogram depicting the evolutionary relationships of taxa (Fig. 1) was constructed as a consensus Wagner tree based on the minimum spanning network algorithms of Farris (1972, 1973). This tree is the consensus of 1,000 trees generated from randomly selected subsets of the allelic frequencies in Table 1, but bootstrapped over loci (Felsenstein 1985). Each of the 1,000 trees was based on genetic distances estimated from a random sample of the data of five polymorphic loci characterized by IEF plus the five monomorphic loci. Nodes and branch assignments were established by majority rule rather than by the 95% rule suggested by Felsenstein

(1985). Thus, the nodes chosen occurred either in a majority of the 1,000 trees or were the most frequent alternative compatible with the majority-rule branch and node assignments.

RESULTS

The percent of polymorphic loci in the different species was as follows: 66.7% in *T. patachonicus* (10 of 15 loci), 26.7% in *T. pteneres* (4 of 15 loci), 46.7% in *T. leucocephalus* (7 of 15 loci), and 30% in *T. brachypterus* (3 of 10 loci). Allelic frequencies of the five polymorphic loci examined in all samples are given in Tables 1 and 2, and Table 1 contains the data used in the interpopulation comparisons of F-statistics. The data in Table 2, along with those of columns three and four of Table 1 are used in the interspecific comparisons of heterozygosities, deviations from equilibrium expectations, and phylogenetic relationships.

HETEROZYGOSITY AND F_{is}

The mean calculated heterozygosity, $\bar{H}$, per taxon is 0.185 for *T. patachonicus* (for the subpopulations Co, AL, and Ch the values are 0.181, 0.185, and 0.188), 0.160 for *T. pteneres*, 0.165 for *T. brachypterus*, and 0.084 for *T. leucocephalus*. Observed heterozygosities are 0.115 ± 0.090, 0.202 ± 0.118, 0.201 ± 0.085, and 0.080 ± 0.069 respectively for the species and for the subpopulations Co, AL, and Ch of *T. patachonicus* they are 0.091 ± 0.072, 0.092 ± 0.079, 0.236 ± 0.064. The weighted mean values of F_{is} averaged over loci are: 0.3636 for *T. patachonicus* (Co), 0.3570 for *T. patachonicus* (AL), 0.0725 for *T. patachonicus* (Ch), 0.1975 for *T. pteneres*, 0.2814 for *T. brachypterus*, and 0.6059 for *T. leucocephalus*.

INTERPOPULATION ESTIMATES OF F_{st} AND GENE FLOW

Table 3 presents the F_{st} values and their associated Chi-squared values for the paired comparisons between populations of *T. patachonicus*, as defined by the subdivisions indicated in Table 1 and in Methods. All Chi-squared values have five degrees of freedom. The comparison between Ushuaia and Puerto Deseado yields an F_{st} value of 0.0, which indicates that these two populations are not genetically differentiated, whereas each of the other values is significantly greater than zero ($P \ll 0.005$).

The distribution of private alleles was used to

estimate gene flow, i.e., the number of migrants per generation, among the four subpopulations of *T. patachonicus* (Slatkin 1985). Ushuaia has one private allele at *Es-1* giving values of $p(i) = 0.038$ and $Nm = 7.963$. Puerto Deseado has private alleles at *Es-1, Mpi,* and *Pgi-2,* with $p(i) = 0.063$ and $Nm = 2.926$. The Andean lakes region of Argentina has one private allele at *Acp,* with $p(i) = 0.275$ and $Nm = 0.158$, or one migrant per 6.3 generations. The Chilean population has one private allele at *Pgi-2,* with $p(i) = 0.682$ and $Nm = 0.026$, or one migrant per 38.5 generations.

PHYLOGENETIC INFERENCES

The genetic distance indices are presented in Tables 4 and 5. Table 4 gives Nei's (1978) unbiased genetic distance below the diagonal and Rogers' (1972) distance above the diagonal. Table 5 gives the arc and chord distances of Cavalli-Sforza and Edwards (1967).

Relationships of the four species of *Tachyeres,* including the three major subdivisions of *T. patachonicus,* are represented by a consensus Wagner tree (Fig. 1) in which the branch lengths are the fitted arc distances of Cavalli-Sforza and Edwards (1967). This tree had the following majority percentages for the branch/node assignments: *T. brachypterus* joined node 7, which connects it to *T. leucocephalus,* in 60.6% of the trees. *Tachyeres leucocephalus* connected to node 7 in 58.5% of the trees. *Tachyeres pteneres* joins node 8, which connects it via node 7 to *T. brachypterus* and *T. leucocephalus,* in 40.2% of all trees. *Tachyeres patachonicus* (Co) connects via node 9 to node 8 in 99.8% of the trees, *T. patachonicus* (AL) joins via node 9 in 75.5% of all trees, and *T. patachonicus* (Ch) connects via node 9 in 83.2% of the trees. For the among-populations relationships within *T. patachonicus,* the Atlantic coastal population (Co) joins the tree at node 10, which connects it to the Andean lakes
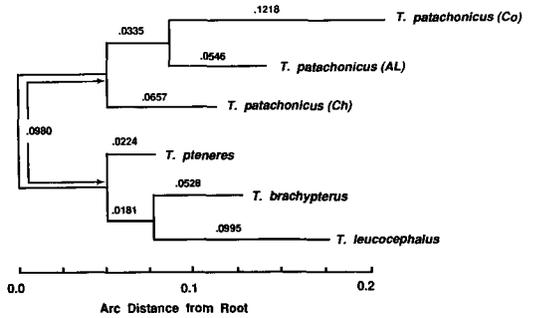

FIGURE 1. Consensus of 1,000 Wagner trees showing the phylogenetic relationships within the steamerducks, genus *Tachyeres.* Genetic distances among taxa are estimated as arc distances, calculated by the method of Cavalli-Sforza and Edwards (1967). See text for information on the localities included under each of the subdivisions of *T. patachonicus* designated as Coastal Argentina (Co), Argentine Andean lakes (AL), and Chile (Ch).

population (AL), in 83.1% of the trees, the Andean lakes populations connects at node 10 in 36% of the trees, and the Chilean population (Ch) connects at node 9 in 53.7% of the trees. The total homoplasy or the f value of Farris (1972) is 0.495, the F value of Prager and Wilson (1976) is 14.64%, and the percent standard deviation of Fitch and Margoliash (1967) is 18.99%.

DISCUSSION

PHYLOGENETIC RELATIONSHIPS

In an analysis of the relationships among the members of the avian order Anseriformes, Livezey (1986a) utilized 120 osteological characters that varied qualitatively, each having two or more discrete character states. In a subsequent cladistic analysis, Livezey (1986b) provided an hypothesis for the evolutionary relationships among the four species of *Tachyeres.*

We have compared the evolutionary relationships among the species of steamer-ducks (Fig.

TABLE 3. Wright's F_{st} values (1978) for comparisons between pairs of populations of *Tachyeres patachonicus* are given below the diagonal. These values have been corrected for sampling error. Above the diagonal are the Chi-squared values associated with the respective values of F_{st}. Each has five degrees of freedom and all except for the comparison between Ushuaia and Puerto Deseado are significantly different from zero ($P \ll 0.005$).

		(1)	(2)	(3)	(4)
Ushuaia	(1)	—	0.0	47.05	36.43
Puerto Deseado	(2)	0.0	—	42.12	41.82
Argentine Lakes	(3)	0.1361	0.0997	—	39.80
Chile	(4)	0.1543	0.1383	0.1283	—

TABLE 4. Estimates of Nei's (1978) unbiased genetic distance, D, between pairs of taxa, corrected for bias due to sample size, are given below the diagonal. Above the diagonal are estimates of Rogers' (1972) genetic distance. The taxa being compared include four species of *Tachyeres*, and *Chloephaga picta* as an outgroup comparison. The sample of *T. patachonicus* is split into three subdivisions: (Co) includes specimens from Ushuaia and Puerto Deseado, Argentina, (AL) includes specimens from the Andean lakes region of Argentina, and (Ch) includes specimens from Chile.

		(1)	(2)	(3)	(4)	(5)	(6)	(7)
T. brachypterus	(1)	—	0.1205	0.0778	0.2191	0.1256	0.2225	0.2193
T. leucocephalus	(2)	0.0470	—	0.1064	0.2001	0.2259	0.2367	0.1410
T. pteneres	(3)	0.0080	0.0274	—	0.1905	0.1953	0.1941	0.1888
T. patachonicus (Co)	(4)	0.1659	0.1424	0.1278	—	0.1529	0.1999	0.1874
T. patachonicus (AL)	(5)	0.1252	0.1637	0.1485	0.0898	—	0.1452	0.2399
T. patachonicus (Ch)	(6)	0.1941	0.2173	0.1504	0.1123	0.0827	—	0.2647
C. picta	(7)	0.1548	0.0893	0.1173	0.0984	0.1719	0.2109	—

1) to those deduced in these earlier studies of Livezey. In our phylogenetic analysis we have assumed that electromorphs are selectively neutral and hence that evolutionary rates are equivalent among lineages (see Barrowclough et al. [1985] and Hillis [1985] for discussions of this issue). With respect to the phylogenetic relationships among the four species of *Tachyeres*, the consensus tree (Fig. 1) is identical to that derived by Livezey (1986b) and differs in two major ways from a nonconsensus Wagner tree based on a single sampling of the genetic distance data of Table 5. The latter results in a tree in which the positions of *T. brachypterus* and *T. pteneres* are reversed, as are the positions of *T. patachonicus* (Ch) and *T. patachonicus* (AL). (We note that it was the nonconsensus tree to which Livezey referred in 1986b.) In addition, the nonconsensus tree has a homoplasy of 1.094, more than twice that of the consensus tree and a percent standard deviation that is 22% greater. Both Figure 1 and a cladogram based on morphological traits (Livezey 1986b) indicate that the three flightless species of *Tachyeres* (*T. brachypterus*, *T. pteneres*, and

T. leucocephalus) are monophyletic and comprise the sister group of *T. patachonicus*.

On the basis of Livezey's (1986b) morphological analysis, it appears that *Tachyeres* is a highly derived genus of shelduck, tribe Tadornini, which also includes the genus *Chloephaga*. Since we had electrophoretic data from one specimen of *Chloephaga picta*, this species was used as an outgroup in a separate comparison using Nei's unbiased genetic distance (Table 4) to carry out an UPGMA analysis. Based on these results, the branch leading to *Chloephaga* would join the tree shown in Figure 1 between the cluster of species including *T. brachypterus*, *T. pteneres*, and *T. leucocephalus* on the one hand, and the populations of *T. patachonicus* on the other. This pattern also is consistent both with the cladogram of Livezey (1986b) and with the hypothesis that *T. patachonicus* is the most primitive member of *Tachyeres*.

Livezey (1986b) provided a biogeographic hypothesis for the evolution of *Tachyeres* (Fig. 1) that takes account of the major glacial vicariance events in southern South America over the latter

TABLE 5. Estimates of Cavalli-Sforza and Edwards' (1967) arc distance between pairs of taxa of *Tachyeres* and *Chloephaga picta* are given below the diagonal. Above the diagonal are Cavalli-Sforza and Edwards' chord distances. The designation of the populations of *T. patachonicus* is the same as in Table 4.

		(1)	(2)	(3)	(4)	(5)	(6)	(7)
T. brachypterus	(1)	—	0.1656	0.1027	0.2656	0.1389	0.2451	0.2548
T. leucocephalus	(2)	0.1523	—	0.1530	0.2346	0.2617	0.2485	0.1671
T. pteneres	(3)	0.0933	0.1400	—	0.2460	0.2191	0.1941	0.2288
T. patachonicus (Co)	(4)	0.2490	0.2191	0.2300	—	0.1920	0.2379	0.2363
T. patachonicus (AL)	(5)	0.1350	0.2478	0.2084	0.1763	—	0.1660	0.2831
T. patachonicus (Ch)	(6)	0.2346	0.2375	0.1861	0.2209	0.1537	—	0.2839
C. picta	(7)	0.2431	0.1612	0.2179	0.2239	0.2698	0.2717	—

part of the Quaternary Period. In summary, he proposed that the ancestor common to the four species inhabited much of Argentina and Chile during the interglacial period about 100,000 years ago. With the advance of the major glacial mass 60,000 years ago, from Tierra del Fuego and Patagonia northward throughout southern Chile and well into the Andes of Bolivia, two populations of *Tachyeres* might have been isolated from one another. These isolates would have been a smaller population along the northern coast of Chile and a much larger population to the east of the Andes, extending all the way from their base to the Atlantic coast of Argentina. The larger isolate subsequently would have evolved into *T. patachonicus,* whereas the smaller isolate is postulated as an ancestral lineage that eventually divided, during or subsequent to the Llanquihue glaciation at about 19,000 years ago, into *T. pteneres* and a form ancestral to *T. leucocephalus* and *T. brachypterus.* The final stage involved the division of the population ancestral to *T. leucocephalus* and *T. brachypterus* into a coastal Argentine isolate and a Falkland Islands isolate. These then differentiated in situ into these two species.

This hypothesis is parsimonious in that the nonflying species evolved from the more widely distributed flier, *T. patachonicus,* and it seems plausible on the basis of the current geographic distributions of the species. Furthermore, it offers an evolutionary sequence in which a monotonic evolutionary trend toward increasing body mass and wing loading culminates in the most derived member of the genus, *T. pteneres.*

We now take the date of 60,000 years ago as an estimate of the time of the vicariance event that separated the lineage leading to *T. patachonicus* from that leading to the other three species, and use it to calibrate an evolutionary clock. For this clock we fit Nei's unbiased genetic distances (Table 4) to our consensus tree topology to provide branch lengths that are proportional to evolutionary time. This then allows a comparison to be made between the time of the Llanquihue glaciation at about 19,000 years ago and the time of divergence based on the molecular clock. The latter estimate falls between 14,300 and 25,000 years ago depending upon whether the time since divergence is proportional to a Nei's distance of 0.0258 (the distance from the common ancestor along the lineage leading to *T. brachypterus*) or 0.0452 (the distance leading to *T. leucocephalus*). The mean of these values, 19,650 years ago, thus closely approximates the time of the vicariance event. Likewise, by this same reasoning, the divergence of lineages leading to *T. brachypterus* and *T. leucocephalus* would have occurred about 13,000 years ago.

HETEROZYGOSITY AND INBREEDING

The genic diversity of a taxon may be estimated as the average number of loci that are heterozygous per individual, whereas the deviation from the expected number of heterozygous individuals per locus is estimated by F_{is} values, which take values greater than 0.0, but less than 1.0, when there is a deficiency of heterozygotes. Among our samples of *Tachyeres*, values of 1.0 primarily reflect the absence of heterozygotes within relatively small samples, but there are significant deficiencies of heterozygotes at some loci, as indicated by the high average F_{is} values for each species. For example, for the *Acp* locus of *T. brachypterus*, only five heterozygotes from the three heterozygote classes *Acp a/b, Acp a/c,* and *Acp b/c* were observed but 8.91 were expected, giving an F_{is} value of 0.4389. There is, however, a high degree of variance in F_{is} values among loci, which may be attributed to relatively small sample sizes.

A significant number of avian species has now been examined electrophoretically, and the heterozygosity data of these studies have been summarized by Corbin (1983, 1987) and Barrowclough (1983). The average $\bar{H}$ for 21 species of nonpasserines is 0.076 ± 0.072, $\bar{H}$ for 55 species of passerines is 0.062 ± 0.035, and for all species and studies combined, $\bar{H}$ is 0.066 ± 0.046 (Corbin 1987). In a subset of these studies, which accounts for 30 avian species whose breeding populations were well analyzed in terms of numbers of individuals and number of loci analyzed, the average value of $\bar{H}$ is 0.053 ± 0.028 (Barrowclough 1983). Bearing in mind that we have examined a limited number of loci, the heterozygosity values of *T. patachonicus, T. pteneres,* and *T. brachypterus* apparently are higher than are the heterozygosities for birds in general. The value of $\bar{H}$ for *T. leucocephalus,* however, is only slightly higher than the average for nonpasserine species.

INTERPOPULATION GENE FLOW

Slatkin (1985) has provided a method for the estimation of gene flow, or number of migrants

per generation, among demes that uses the distribution of private alleles, i.e., those alleles found in only one deme. When this method is applied to the subpopulations of *T. patachonicus* the results reveal major differences in the patterns of gene flow among populations. Gene flow involving Ushuaia, Argentina is high, with an *Nm* value of 7.963, or roughly eight migrants per generation. The situation is similar for Puerto Deseado with 2.926 migrants per generation. The picture changes markedly, however, for both the Andean lakes region of Argentina and for the Chilean population. The former population has an *Nm* value of 0.158 or one migrant per 6.3 generations, and the latter has an *Nm* = 0.026, or one migrant per 38.5 generations.

These results confirm the inferences drawn from an analysis of interpopulation F_{st} values (Table 3). The fixation index F_{st} (Wright 1978) is, among other things, a measure of the amount of gene flow between demes. Among closely related demes one expects the value of F_{st} to be nearly zero and as gene flow is reduced, either by distance or by physical restrictions to dispersal, then values of F_{st} increase. Distributions of interpopulation values of F_{st} have been summarized for several groups of organisms (Barrowclough 1983; Corbin 1983, 1987); for vertebrates, interdemic F_{st} values range between 0.0 and 0.91, with the largest of these values being found among salamander populations. Among avian populations within subspecies, the largest interdemic values previously have been in the range of 0.029 to 0.039. The values we present for the interpopulation comparisons of *T. patachonicus* are, therefore, much higher than those previously found within any species of bird. The maximum value obtained (0.154) is for the comparison between the population of Ushuaia, Argentina, and that in the vicinity of Puerto Montt, Chile (Table 3).

The estimates of *Nm* and F_{st} may help to explain the relatively large morphometric differences among populations of *T. patachonicus* (Livezey 1986c). Although it is not surprising that gene flow between populations on opposite sides of the Andean Cordillera might be greatly reduced, we did not expect the values of *Nm* and F_{st} to be so high for some of the other comparisons. Based on the populations we have examined, gene flow is extensive only among populations of *T. patachonicus* in southern, coastal Argentina. These findings, and the morphomet-

ric patterns described by Livezey (1986c), support the hypothesis that *T. patachonicus* may be a superspecies comprised of several, as yet poorly defined, allospecies.

ACKNOWLEDGMENTS

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