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EFFECTS OF VARIED ELECTROPHORETIC CONDITIONS ON DETECTION OF EVOLUTIONARY PATTERNS IN THE LARIDAE[1]

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Abstract. The extent and effects of hidden variation on detecting evolutionary patterns in the family Laridae were examined using sequential starch-gel electrophoresis of proteins. To determine whether more alleles were hidden at higher taxonomic levels than within species, 25 individuals of *Sterna forsteri* and 20 species in the family Laridae were analyzed. Varied conditions detected two hidden alleles in the 25 *S. forsteri,* whereas 26 alleles were hidden across the 20 larid species. These alleles were hidden using buffer conditions not commonly employed in avian electrophoretic surveys and had no effect on phylogenetic reconstruction. Distance-Wagner analysis showed the following to be distinct groups: (1) Rynchopinae, (2) Stercorariinae, (3) *Anous stolidus,* (4) Larinae, and (5) Sterninae. Within the Sterninae, *S. antillarum* and *Chlidonias niger* are the most divergent. *Sterna maxima* and *S. forsteri* are sister taxa, as are *S. nilotica* and *Phaetusa simplex. Sterna hirundo* and *S. vittata* show no differences at the 23 loci examined, which does not support the hypothesis that *S. vittata* arose from a migrant population of *S. paradisaea* that remained in the Antarctic. Cladistic analysis provides evidence that *A. stolidus,* placed with the Sterninae under all classification schemes, is indeed a tern, but may be evolving faster than other terns. The results of this study agree generally with a morphometric analysis of the Laridae by Schnell (1970a, 1970b).

Key words: *Evolution; genetic variation; Laridae; phylogeny; protein electrophoresis; Sterninae.*

INTRODUCTION

Accurate estimates of phylogenetic relationships underlie comparative biological studies (Felsenstein 1985). For instance, two organisms could share the same morphological, ecological, or behavioral trait either owing to phylogeny (common ancestry) or convergence. To interpret patterns of interspecific character variation, knowledge of phylogenetic relationships is crucial for estimating the degree of similarity owing to descent.

In this paper I present a phylogenetic analysis of terns (Laridae: Sterninae) and other families in the Laridae based on starch-gel electrophoresis of proteins. The terns were selected because previous morphological (Schnell 1970a, 1970b) and behavioral (Moynihan 1959) analyses provide a framework against which to contrast patterns of genetic variation. Electrophoretic examinations of the Laridae have typically involved few species or loci (Tegelstrom et al. 1980, Zink and Winkler 1983, Karl et al. 1987). A preliminary electrophoretic analysis (unpubl. data) of 11 tern, three gull, and one skimmer species showed little interspecific genetic differentiation, which made it difficult to detect phylogenetic relationships.

To confirm the observation of low genetic divergence, I performed a sequential electrophoretic analysis, whereby the pH and composition of buffer systems were varied to reveal hidden variation, i.e., alleles not detected on all buffer conditions. Although a number of buffers are used, a typical "one-pass" electrophoretic study involves examining a locus on only one buffer type; usually, this buffer type has been determined useful for this specific locus in other species. In my study each locus was examined on nine different buffer types. Only one sequential electrophoretic study of birds has been published (Aquadro and Avise 1982); therefore, the extent of hidden variation in birds has not been established. Studies of *Drosophila* have shown that hidden variation can be important for resolving evolutionary relationships among species (Coyne et al. 1979). Application of sequential electrophoresis to 71 *Drosophila* species resulted

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TABLE 1. Classification by Morony, Bock, and Farrand (1975).

Suborder Lari
Family Stercorariidae (skuas and jaegers)
Family Laridae
Subfamily Larinae (gulls)
Subfamily Sterninae (terns)
Family Rynchopidae (skimmers)

TABLE 2. Classification by the AOU (1983).

Family Laridae
Subfamily Stercorariinae
Subfamily Larinae
Subfamily Sterninae
Subfamily Rynchopinae

in the detection of 19 electromorphs at the α-glycerophosphate dehydrogenase locus, whereas standard electrophoretic conditions had shown this locus to be invariant. These hidden alleles served to distinguish various groups of species.

SYSTEMATIC BACKGROUND

The classification and phylogenetic relationships of the gulls, terns, and allies have been addressed by several authors. The classification by Morony et al. (1975; Table 1) is the traditional one, following classifications by Peters (1934) and Wetmore (1960). The AOU (1983) departed from these classifications and gave subfamilial status to the skuas and jaegers, gulls, terns, and skimmers (Table 2).

Although changes at higher taxonomic levels have been proposed by various researchers, a detailed analysis of the Laridae was lacking. Moynihan (1959) used behavioral evidence to propose his classification of the Laridae, recognizing only two subfamilies, Stercorariinae and Larinae (including gulls, terns, and skimmers). Timmerman (1957a, 1957b) considered the Rynchopinae most closely related to the Sterninae. Schnell (1970a, 1970b) published a morphometric study of relationships within the suborder Lari, in which he analyzed 51 skeletal and 72 external characters in 93 species. Because all larid species were included in his phenograms, Schnell's result can be compared in detail to my biochemical analysis based on starch-gel electrophoresis of proteins.

METHODS

Specimens, English names, collecting localities, and sample sizes are listed in Appendix 1. Arbitrarily, I followed the classification of the Laridae by the AOU (1983). Twenty-five specimens of *Sterna forsteri* were examined to determine the extent of intraspecific hidden variation. These were collected along the Louisiana Gulf coast at various times of the year, but predominantly in winter. These specimens might represent three allopatric breeding populations, all of which are thought to winter on the Louisiana coast (R. A. Martin, pers. comm.). Thus, calculations of population genetic parameters requiring sampling a single breeding deme (Wright's inbreeding coefficient, F_{IS}, for example) are inappropriate. In addition, 30 specimens representing 21 species in the Laridae and three outgroup taxa (see Appendix 1) were compared. Resulting data were used to assess hidden variation at higher taxonomic levels and to estimate phylogenetic relationships.

Specimens were placed on dry ice (−78.5°C) within an hour of death; tissue samples taken from these specimens were stored at −70°C. Tissue extracts were prepared by grinding liver, heart, and pectoral muscle with a razor blade; 1 ml of deionized water was then added. This mixture was then spun in a Sorvall RC-5B centrifuge (Sorvall rotor SM 24) at 16,000 rpm for 30 min (Johnson et al. 1984), and the resulting supernatant was frozen (−70°C) for subsequent electrophoretic experiments.

The buffer systems, percent starch, and running conditions are listed in Table 3. These buffer systems are the same as those employed by Aquadro and Avise (1982) in their study of sequential electrophoresis in passerine birds. Fresh buffer was used in each run to minimize effects due to changes in ionic composition of a buffer. The same lot of Sigma potato starch was used throughout the study.

Nineteen enzyme systems representing 23 different genetic loci were examined. Table 4 lists the loci examined, their abbreviations, and Enzyme Commission (EC) numbers.These loci were chosen on the basis of two criteria: (1) they were resolvable on a wide variety of buffer types, and (2) they comprised a mixture of variable and nonvariable loci in birds (Hackett and Zink, unpubl. data).

Enzymes were assayed using the procedures outlined by Harris and Hopkinson (1976), with slight modifications. Specific recipes for enzyme

TABLE 3. Electrophoretic conditions used in this study.

Condition number	Gel buffer[a]	Electrode buffer	Running conditions
1	0.02 M Acetate, pH 4.6	0.2 M Acetate, pH 4.6	60 mA, 8 hr
2	Amine-citrate, pH 6.0	Amine-citrate, pH 6.1	75 mA, 5 hr
3	Tris-citrate, pH 6.3	Tris-citrate, pH 6.7	75 mA, 5 hr
4	Tris-citrate-EDTA, pH 7.2	Tris-citrate-EDTA, pH 7.4	75 mA, 6 hr
5	Tris-citrate, pH 8.0	Tris-citrate, pH 8.0	75 mA, 5 hr
6	Lithium hydroxide, pH 8.2	Lithium hydroxide, pH 8.1	350 V, 12 cm
7	Poulik, pH 8.2	0.3 M Borate, pH 8.2	250 V, 12 cm
8	Tris-HCl, pH 8.5	0.3 M Borate, pH 8.2	250 V, 4 hr
9	0.05 M Glycine-NaOH, pH 10.0	0.05 M Glycine-NaOH, pH 10.0	60 mA, 20 hr Buffer mixing

[a] All gels were composed of 11.7% starch.

assays are available on request. Alleles at a locus were coded by their mobility from the origin. The most anodal allele was designated "a," with successively slower alleles denoted as "b," "c," etc. At each locus the maximum and minimum number of alleles detected was determined for each buffer type to compare the extent of hidden variation. The maximum number of alleles for each locus was determined from the particular buffer type(s) that revealed the most variation at that particular locus. This procedure was performed for all loci, and then the maximum numbers of alleles at each locus were summed to give the maximum number of alleles detected in this study. The minimum number of alleles was determined in the same way, by summing across all loci the minimum number of alleles detected at a particular locus under the "poorest" buffer conditions.

The computer program BIOSYS-1 (Swofford and Selander 1981) was used to compute genetic distances (Rogers 1972, Nei 1978) and estimate Distance-Wagner trees (Farris 1972, 1981) and a UPGMA (Sneath and Sokal 1973) phenogram. The "multiple addition criterion," designed by Swofford (1981), was used in the Distance-Wagner procedure because it generally finds trees of better fit to the original distances (Farris 1981). The trees were rooted at the consensus of the three outgroups, and the maximum number of trees to be held at each successive step in the procedure was set at 20. Only unoptimized trees are presented. The computer program PAUP, written by Swofford (1985), was used for a cla-

TABLE 4. Loci examined.

Enzyme	Abbreviation	E. C. Number
Aconitase	ACON	4.2.1.3
Acid phosphatase	ACP	3.1.3.2
Adenosine deaminase	ADA	3.5.4.4
Esterase	EST	3.1.1.1
Glutamate-oxaloacetate transaminase	GOT1,2	2.6.1.1
Glycerol-3-phosphate dehydrogenase	GPD	1.1.1.8
Glucose phosphate isomerase	GPI	5.3.1.9
Isocitrate dehydrogenase	ICD1,2	1.1.1.42
Lactate dehydrogenase	LDH	1.1.1.27
Leucine-alanine dipeptidase	LA	3.4.*.*
Leucyl-glycine-glycine tripeptidase	LGG	3.4.*.*
Malate dehydrogenase	MDH1,2	1.1.1.37
Malic enzyme	ME1,2	1.1.1.40
Mannose phosphate isomerase	MPI	5.3.1.8
Phenylalanyl-proline dipeptidase	PP	3.4.*.*
Phosphoglucomutase	PGM1	2.7.5.1
Phosphogluconate dehydrogenase	PGD	1.1.1.44
Purine nucleoside phosphorylase	NP	2.4.2.1
Sorbitol dehydrogenase	SDH	1.1.1.14

TABLE 5. Loci variable within *Sterna forsteri.*

Locus	Number of alleles	Frequency of most common allele
LGG	2	0.62
MPI	2	0.82
NP	3	0.72

distic analysis of alleles using the parsimony criterion. The computer specifications were: ADDSEQ = CLOSEST, HOLD = 3, SWAP = GLOBAL, MULPARS, ROOT = OUTGROUP. A consensus tree summarizing equally parsimonious trees was obtained. I recognize that the use of genetic distance matrices for inferring phylogenetic relationships is controversial (Farris 1986, Felsenstein 1986), but conclude that both distance and cladistic analyses are informative.

RESULTS

LEVELS OF GENETIC VARIATION WITHIN *STERNA FORSTERI*

Across the 23 loci, 31 alleles were detected. Three loci (13%; LGG, NP, and MPI) were found to be polymorphic (Table 5). Heterozygosity in my sample of *S. forsteri* averaged 3.8%. Use of nine buffer conditions revealed only two hidden alleles. One hidden allele was found at the GPI locus using electrophoretic conditions 7 and 8. The other hidden allele was found at the MPI locus using electrophoretic condition 2. The allele at MPI was previously considered the same as one of the other two alleles present at this locus. Both hidden alleles were found in one individual and were present in a heterozygous state; therefore, the frequency of each allele is only 0.02. These rare alleles had little effect on heterozygosity, which increased to only 4.0% with inclusion of the hidden allele at the GPI locus.

LEVELS AND PATTERNS OF AMONG-TAXON GENETIC DIFFERENTIATION IN THE LARIDAE

The sequential electrophoretic survey generated allelic frequencies (Appendices 2 and 3), which can be summarized by distance matrices representing the maximum and minimum amounts of variation detected (Table 6; matrix summarizing least variation can be obtained from the author). Considering all combinations of electrophoretic conditions, the maximum number of alleles uncovered was 169; the minimum number of alleles was 143 (84.7% of maximum). The amine-citrate buffer type (condition 2) was useful for resolving "cryptic" variation. For example, alleles differing in mobility by only 1 to 2 mm at the ADA locus on some buffer types differed by 5 mm on the amine-citrate buffer type. This buffer system clarified the four-banded pattern of heterzygotes at the NP locus; on other buffer types (conditions 1, 5, and 9), the heterozygous genotypes appear as diffuse bands.

A typical "one-pass" electrophoretic survey will not uncover all variation present. Variation at the GPI locus might be hidden when a more basic gel type is used. The situation at the GPI locus is the only example of "true" hidden variation at any of the loci examined in this survey; that is, variation not detected using commonly used buffer types. Other examples of alleles that went undetected (i.e., were hidden) by a particular buffer type usually involved the tris-HCl buffer type, one not commonly used in avian electrophoretic studies. In fact, the tris-HCl buffer type resolved the lowest amount of genetic variation at 40% of the loci examined.

Nei's (1978) average interspecific genetic distance (Table 7) among congeneric species were (taxon; taxonomic reference): *Sterna* (AOU 1983), 0.17; *Sterna* (AOU 1957), 0.20; *Thalasseus* (AOU 1957), 0.09; *Stercorarius,* 0.15. At the level of the family Laridae, genetic distances among species averaged 0.33 (taxonomy following Morony et al. 1975) and 0.50 (taxonomy following the AOU 1983).

The UPGMA phenogram (Fig. 1) based on the maximum number of alleles detected (Table 6), shows Sterninae (excluding *A. stolidus*), Larinae, *A. stolidus,* Stercorariinae, and Rynchopinae to be distinct groups. Larinae and Sterninae are most similar, as are Stercorariinae and Rynchopinae. Note that *Rynchops niger* is not more closely associated with either the Larinae or the Sterninae.

Within Sterninae, *S. fuscata, S. antillarum, S. anaethetus,* and *Chlidonias niger* are the most divergent. In the remaining cluster of 10 terns, little structure exists. *Phaetusa simplex* and *S. nilotica* are each other's closest relatives, and *S. vittata* and *S. hirundo* were genetically identical. Short branch lengths and incomplete sampling of species indicate that caution should be taken in interpreting relationships within the cluster of 10 tern species.

The UPGMA phenogram (not shown) based

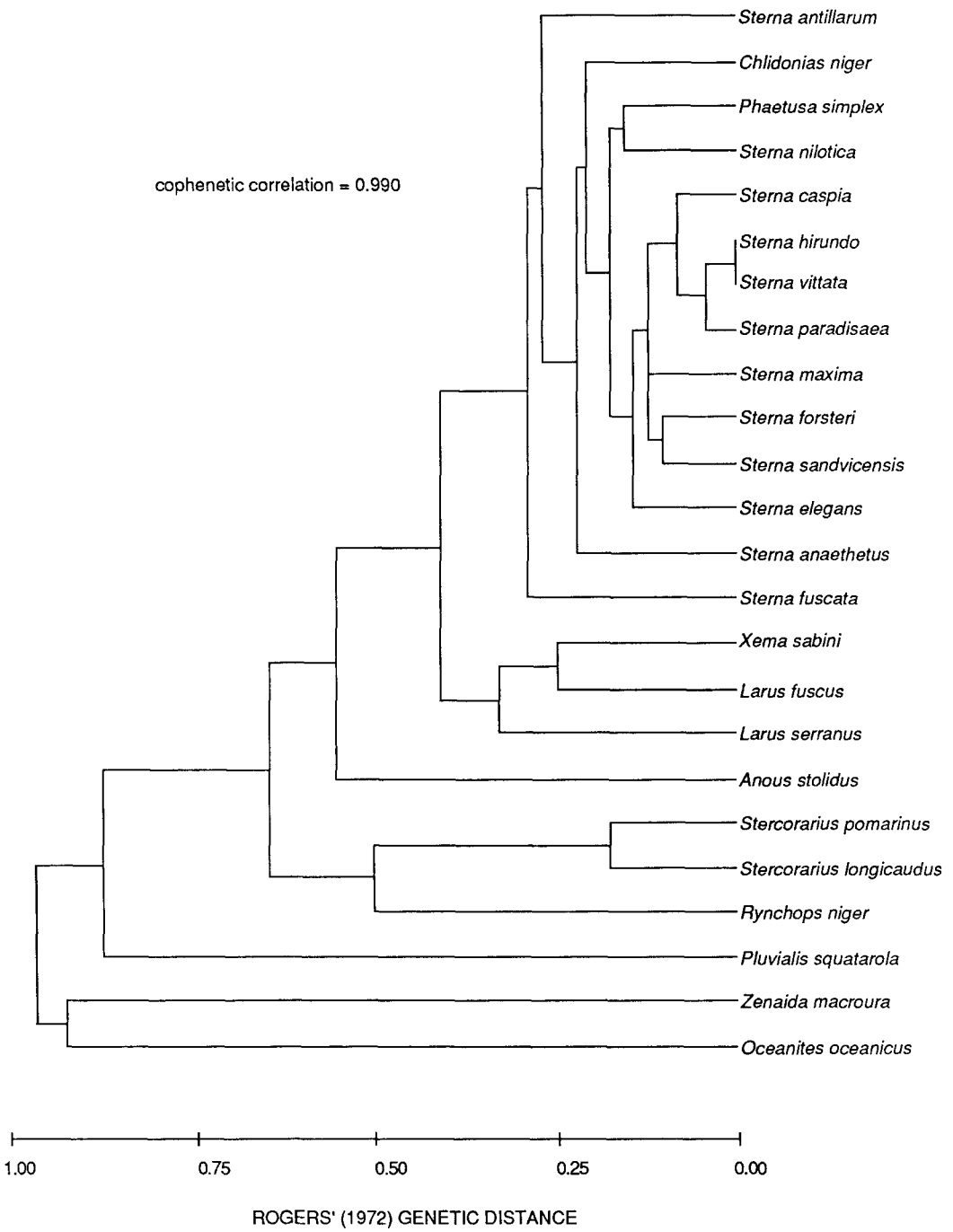

FIGURE 1. UPGMA phenogram based on Rogers' (1972) genetic distances representing the most genetic variation uncovered in this study (Table 6).

TABLE 6. Genetic distance matrix summarizing the most genetic variation uncovered in the Laridae. Above diagonal are Rogers' (1972) genetic distances; below diagonal are Nei's (1978) unbiased genetic distances. Species abbreviations are as follows: SPOM—*Stercorarius pomarinus*, SLON—*Stercorarius longicaudus*, LFUS—*Larus fuscus*, LSER—*Larus serranus*, XSAB—*Xema sabini*, SNIL—*Sterna nilotica*, SCAS—*Sterna caspia*, SMAX—*Sterna maxima*, SELE—*Sterna elegans*, SSAN—*Sterna sandvicensis*, SHIR—*Sterna hirundo*, SPAR—*Sterna paradisaea*, SVIT—*Sterna vittata*, SFOR—*Sterna forsteri*, SANT—*Sterna antillarum*, SANA—*Sterna anaethetus*, SFUS—*Sterna fuscata*, PSIM—*Phaetusa simplex*, CNIG—*Chlidonias niger*, ASTO—*Anous stolidus*, RNIG—*Rynchops niger*, OOCE—*Oceanites oceanicus*, PSQU—*Pluvialis squatarola*, ZMAC—*Zenaida macroura*. (xxxxx signifies infinite distance estimated).

Species	1	2	3	4	5	6	7	8	9	10	11	12	13
1 SPOM	—	0.174	0.565	0.478	0.591	0.587	0.570	0.603	0.619	0.609	0.565	0.565	0.565
2 SLON	0.146	—	0.641	0.581	0.694	0.619	0.629	0.662	0.650	0.668	0.625	0.625	0.625
3 LFUS	0.833	1.011	—	0.304	0.243	0.413	0.425	0.429	0.445	0.435	0.391	0.391	0.391
4 LSER	0.651	0.839	0.363	—	0.330	0.326	0.338	0.342	0.358	0.348	0.304	0.304	0.304
5 XSAB	0.816	1.096	0.180	0.305	—	0.439	0.391	0.411	0.426	0.417	0.417	0.417	0.417
6 SNIL	0.869	0.935	0.518	0.380	0.472	—	0.185	0.217	0.212	0.196	0.109	0.152	0.109
7 SCAS	0.832	0.954	0.545	0.401	0.384	0.164	—	0.157	0.179	0.163	0.076	0.092	0.076
8 SMAX	0.924	1.060	0.556	0.413	0.433	0.216	0.137	—	0.135	0.125	0.109	0.109	0.109
9 SELE	0.926	1.005	0.540	0.391	0.417	0.175	0.122	0.080	—	0.109	0.125	0.141	0.125
10 SSAN	0.938	1.075	0.571	0.427	0.448	0.203	0.152	0.125	0.045	—	0.087	0.130	0.087
11 SHIR	0.833	0.950	0.496	0.363	0.448	0.100	0.053	0.100	0.070	0.091	—	0.043	0.000
12 SPAR	0.833	0.950	0.496	0.363	0.448	0.150	0.077	0.100	0.095	0.140	0.044	—	0.043
13 SVIT	0.833	0.950	0.496	0.363	0.448	0.100	0.053	0.100	0.070	0.091	0.000	0.044	—
14 SFOR	0.908	1.045	0.541	0.398	0.380	0.146	0.122	0.095	0.065	0.085	0.061	0.110	0.061
15 SANT	0.912	1.049	0.807	0.545	0.545	0.322	0.252	0.291	0.283	0.306	0.247	0.276	0.247
16 SANA	0.916	1.053	0.628	0.548	0.465	0.295	0.211	0.181	0.181	0.223	0.169	0.169	0.169
17 SFUS	1.056	1.218	0.738	0.651	0.615	0.259	0.291	0.259	0.293	0.302	0.245	0.302	0.245
18 PSIM	0.833	0.950	0.496	0.302	0.448	0.150	0.178	0.203	0.203	0.245	0.140	0.140	0.140
19 CNIG	0.810	0.937	0.459	0.321	0.355	0.185	0.159	0.156	0.158	0.199	0.117	0.117	0.117
20 ASTO	1.190	1.385	1.190	0.938	0.873	0.769	0.735	0.723	0.714	0.738	0.738	0.738	0.738
21 RNIG	0.651	0.648	1.056	0.938	0.998	1.106	1.062	1.041	1.051	1.056	1.056	1.056	1.056
22 OOCE	3.113	3.068	2.420	3.113	2.991	3.099	3.087	3.099	3.043	3.113	3.113	3.113	3.113
23 PSQU	1.727	2.151	2.420	2.015	1.892	2.000	1.989	2.000	1.945	2.015	2.015	2.015	2.015
24 ZMAC	3.135	3.090	3.135	3.135	3.013	3.121	3.109	3.121	3.066	3.135	3.135	3.135	3.135

TABLE 6. Continued.

	Species	14	15	16	17	18	19	20	21	22	23	24
1	SPOM	0.597	0.599	0.603	0.652	0.565	0.565	0.696	0.487	0.951	0.820	0.958
2	SLON	0.656	0.657	0.662	0.712	0.625	0.624	0.755	0.494	0.939	0.880	0.945
3	LFUS	0.423	0.555	0.472	0.522	0.391	0.403	0.696	0.652	0.907	0.907	0.957
4	LSER	0.336	0.425	0.429	0.478	0.261	0.316	0.609	0.609	0.951	0.864	0.957
5	XSAB	0.390	0.472	0.423	0.504	0.417	0.391	0.607	0.651	0.920	0.835	0.927
6	SNIL	0.168	0.300	0.277	0.239	0.152	0.235	0.543	0.674	0.945	0.858	0.951
7	SCAS	0.163	0.246	0.217	0.272	0.179	0.217	0.527	0.657	0.941	0.854	0.946
8	SMAX	0.109	0.272	0.190	0.239	0.196	0.207	0.516	0.646	0.945	0.858	0.951
9	SELE	0.141	0.293	0.221	0.299	0.227	0.237	0.532	0.662	0.933	0.846	0.939
10	SSAN	0.103	0.278	0.212	0.261	0.217	0.229	0.522	0.652	0.951	0.864	0.957
11	SHIR	0.087	0.235	0.168	0.217	0.130	0.170	0.522	0.652	0.951	0.864	0.957
12	SPAR	0.130	0.251	0.168	0.261	0.130	0.170	0.522	0.652	0.951	0.864	0.957
13	SVIT	0.087	0.235	0.168	0.217	0.130	0.170	0.522	0.652	0.951	0.864	0.957
14	SFOR	—	0.222	0.196	0.261	0.190	0.191	0.510	0.641	0.939	0.852	0.945
15	SANT	0.203	—	0.250	0.409	0.235	0.279	0.496	0.642	0.941	0.854	0.946
16	SANA	0.166	0.254	—	0.299	0.212	0.229	0.516	0.646	0.945	0.858	0.951
17	SFUS	0.272	0.507	0.341	—	0.348	0.332	0.565	0.696	0.951	0.864	0.957
18	PSIM	0.188	0.247	0.223	0.427	—	0.186	0.478	0.652	0.951	0.864	0.957
19	CNIG	0.127	0.271	0.191	0.353	0.144	—	0.494	0.625	0.923	0.836	0.929
20	ASTO	0.708	0.667	0.715	0.833	0.651	0.664	—	0.739	0.994	0.907	1.000
21	RNIG	1.026	1.030	1.034	1.190	1.056	0.982	1.344	—	0.907	0.777	0.957
22	OOCE	3.083	3.087	3.091	3.113	3.113	3.039	xxxxx	2.420	—	0.945	0.907
23	PSQU	1.985	1.989	1.992	2.015	2.015	1.941	2.420	1.504	3.091	—	0.907
24	ZMAC	3.106	3.109	3.113	3.135	3.135	3.061	xxxxx	3.135	2.420	2.420	—

TABLE 7. Mean genetic distance (D; Nei 1978) as a function of taxonomic rank in Laridae.

Taxonomic level	Number of comparisons	D ± SD	Range
Between congeners			
Sterna (AOU 1983)	66	0.17 ± 0.10	0.00–0.51
Sterna (AOU 1957)	21	0.20 ± 0.12	0.00–0.34
Thalasseus (AOU 1957)	3	0.09 ± 0.03	0.05–0.13
Within a subfamily			
Sterninae	105	0.25 ± 0.20	0.00–0.83
Within a family			
Laridae (AOU 1983)	210	0.50 ± 0.35	0.00–1.119
Laridae (AOU 1957)	153	0.33 ± 0.23	0.00–1.39

on the minimum amount of genetic variation detected (i.e., the worst possible "one-pass" scenario) exhibits the same major groupings as those in Figure 1. The differences between these phenograms mostly involve minor rearrangements of taxa within the 10-species cluster of relatively undifferentiated terns. The placement of *Xema sabini* also differs in the two analyses. In both, it is located in the gull cluster, but it is a sister taxon to the *L. fuscus/L. serranus* group, rather than to *L. fuscus* (Fig. 1).

The Distance-Wagner procedure relaxes the constraint of equal rates of evolutionary change among taxa, one assumption of the UPGMA analysis. The Distance-Wagner tree (Fig. 2) based on the maximum amount of variation detected shows the same major groups as the UPGMA phenogram. Within the terns, however, patterns differ somewhat. *Chlidonias niger* and *S. antillarum* are the most divergent. *Sterna anaethetus* and *S. fuscata* are now placed within, although slightly distinct from, the large group of closely related terns. Within this cluster, *P. simplex* and *S. nilotica* are sister taxa. The Distance-Wagner tree (not shown) based on the matrix of least variation (not shown) produced a branching structure differing from those discussed above. *Phaetusa simplex* and *S. nilotica* remain sister taxa, but they are now the most divergent terns (followed by *S. fuscata, S. antillarum,* and *A. stolidus*). *Anous stolidus* is placed with the other Sterninae. Once again, there appears to be little structure in the remaining group of terns. Thus, the Distance-Wagner procedure might be more sensitive than the UPGMA procedure to the effects of hidden variation on phylogeny reconstruction.

None of the 23 loci was fixed for the same allele across all taxa. Synapomorphic alleles, those uniting subsets of taxa (Table 8), were determined by outgroup analysis (Watrous and Wheeler 1981, Maddison et al. 1984). Outgroups in this study included *Oceanites oceanicus, Pluvialis squatarola,* and *Zenaida macroura.*

A cladistic assessment (not shown) of allelic states based on the possession of shared-derived alleles and employing the parsimony criterion (PAUP; Swofford 1985) confirms the major groupings obtained in the distance analyses; Sterninae, Larinae, Stercorariinae, and Rynchopinae are distinct clades. This analysis, although not clarifying relationships among terns, did place *A.*

TABLE 8. Distribution of shared-derived alleles that unite various larid taxa at higher taxonomic levels.

Locus	Allele	Taxa united
ME2	a	Sterninae (terns)
ME2	b	Larinae (gulls)
LA	a	Laridae (gulls and terns)
SDH	a	Laridae (gulls and terns)
ICD2	a	Lari (gulls, terns, jaegers, and skimmers)
MDH1	a	Charadriiformes (Lari and plover)
MDH2	a	Charadriiformes (Lari and plover)
6PGD	a	Charadriiformes (Lari and plover)

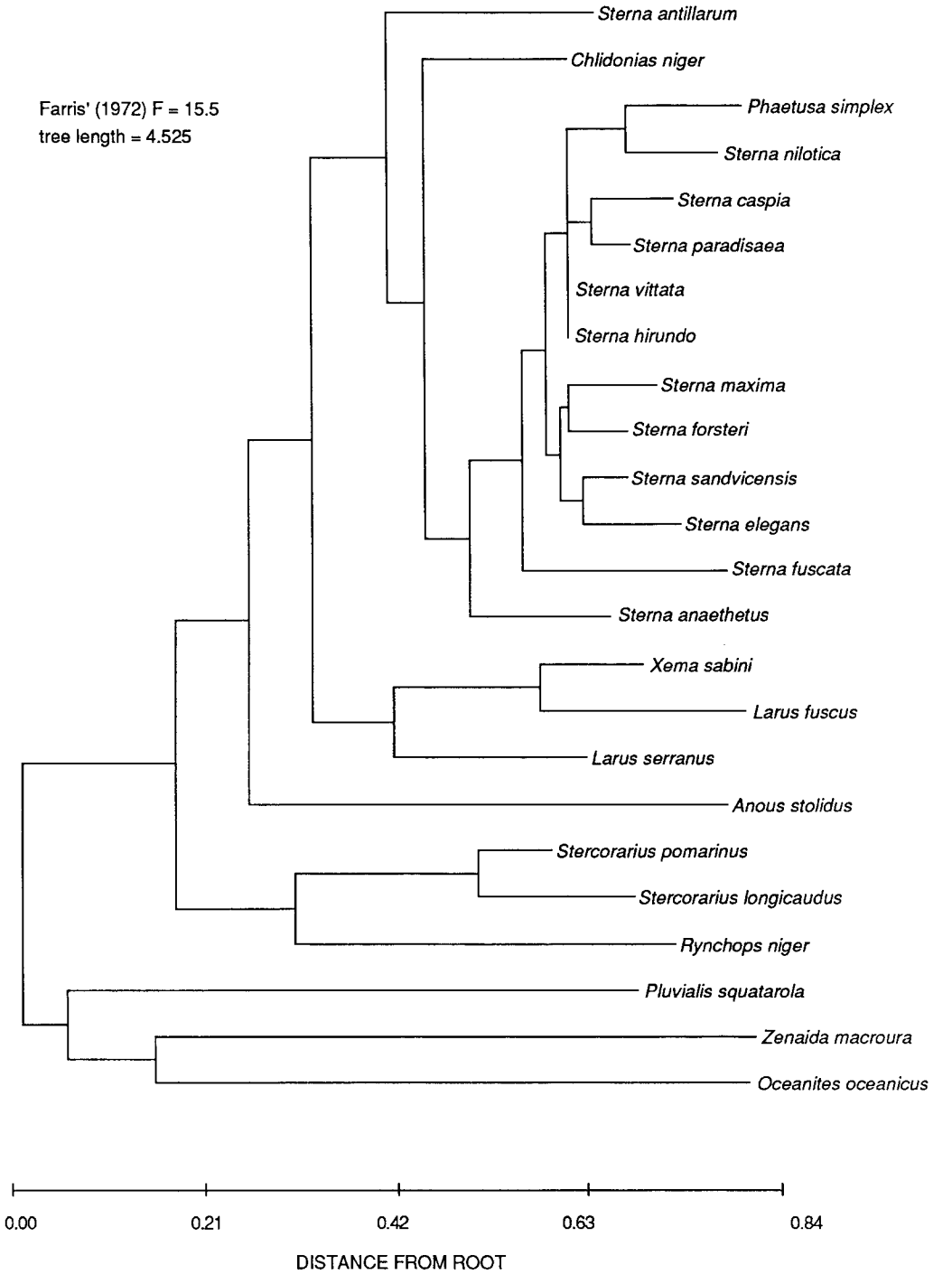

FIGURE 2. Distance-Wagner tree based on Rogers' (1972) genetic distances representing the most variation uncovered in this study (Table 6).

stolidus in the same monophyletic clade as the rest of the Sterninae on the basis of the three synapomorphies it shares with various members of the Sterninae (Table 8). *Anous stolidus* has autapomorphies at 10 loci (ADA, EST, GOT1, GOT2, ICD1, LDH, ME1, GPI, PGM1, PP), but this taxon shared three derived alleles with other members of the Sterninae, including the synapomorphy at ME2 that unites the Sterninae as a monophyletic lineage. It also shared a synapomorphy with *S. antillarum* at LGG, and with *P. simplex* and *S. anaethetus* at NP.

EFFECTS OF HIDDEN VARIATION IN PHYLOGENY RECONSTRUCTION

As mentioned above, the only example of hidden variation at commonly used buffer types occurs at the GPI locus. To test the effects of this hidden variation, I performed UPGMA and Distance-Wagner analyses on the matrix of the maximum amount of genetic variation, replacing the scoring at the GPI locus with that of the buffer type showing the hidden variation (i.e., condition 2). These branching diagrams (not shown) are identical to those derived from the matrix of the maximum amount of genetic variation (Fig. 1, 2). Thus, hidden variation (hidden under commonly used buffer types) had no effect on detection of evolutionary patterns. I conclude, therefore, that most protein electrophoretic studies that examine a reasonably large number of loci and employ a few commonly used buffer types are not going to be compromised by the presence of hidden variation.

DISCUSSION

GENETIC VARIATION AND DIFFERENTIATION

Electrophoretic studies of avian proteins have shown that birds have average individual heterozygosities comparable to other vertebrates, but between pairs of avian taxa measures of genetic differentiation average an order of magnitude less than comparable values for other vertebrates (Avise and Aquadro 1982). Results of my sequential electrophoretic analysis of the Laridae were consistent with these two generalizations.

With the exception of the genus *Thalasseus* (AOU 1957), the genetic distance values (Nei 1978) between larid congeners average higher than those reported between some oscine congeners (genetic distances averaging 0.04; Barrowclough 1980), and closer to those reported by Johnson et al. (1988) for the genera *Vireo* and *Hylophilus*, averaging 0.29 and 0.30 respectively. My results suggest a greater age for nonpasserine genera than some oscine genera, if one assumes that genetic distance measures are roughly proportional to time since taxa last shared a common ancestor. At the familial level, interspecific genetic distances between passerines average 0.21 (Barrowclough and Corbin 1978), whereas genetic distances for nonpasserine taxa range from 0.50 (Laridae) to 0.32 (a conservative estimate of Ramphastidae; Lanyon and Zink 1987); genetic distances between non-nine-primaried oscines also average higher than 0.21 (e.g., 0.35 in Vireonidae; Johnson et al. 1988). These estimates of genetic distances, however, are still an order of magnitude smaller than genetic distances reported between other vertebrates.

Several factors could account for reduced avian intertaxon genetic differentiation. These include: (1) significant levels of hidden variation; (2) over-splitting of avian taxa; (3) recent origin of avian taxa; (4) selective constraints on avian proteins; and (5) differences between birds and other vertebrates in the patterns of genetic variation at enzyme loci within demes. I discuss the first three factors; the other factors are beyond the scope of this paper.

Significant levels of hidden variation could obscure potential synapomorphies that would unite taxa and allow reconstruction of phylogenies. Also, in instances with few observed derived states, detection of additional variation increases confidence in the patterns found. In my sequential electrophoretic analysis, variation was hidden by buffer types that are *rarely* used in avian electrophoretic studies. This variation, although comprising 15% of the total, does not have notable significance with respect to interpretation of avian studies because only those electrophoretic surveys employing rarely used buffer types would be biased by hidden variation. The only exception to this generalization may involve the GPI locus, but, as discussed above, hidden variation at this locus did not alter the suggested pattern of relationships among taxa. I conclude, then, that there is no taxonomically significant hidden variation in the Laridae. Also, the generalization of conservative levels of avian genetic differentiation seems not to be biased by biochemical methodology. My results corroborate those of Aquadro and Avise (1982), but differ substantially from the findings concerning effects of hidden variation on the resolution of phylo-

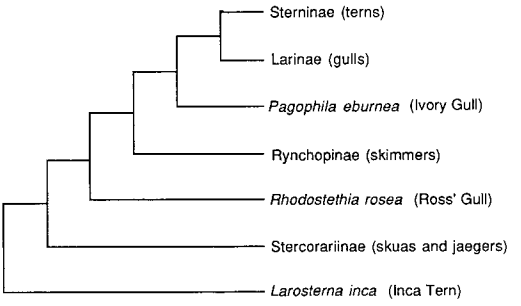

FIGURE 3. Major groups in Laridae as determined by Schnell (1970b, fig. 29).

genetic relationships among species of *Drosophila* (Coyne et al. 1979). My analyses documenting the extent of hidden variation in the Laridae, however, should be extended to other groups of birds.

If hidden variation is not responsible for low levels of genetic divergence between avian taxa, what of the other factors mentioned above? If extant taxa are simply of recent origin, information from the fossil record of birds should be relevant. Unfortunately, the fossil record for the Laridae is spotty and marred by erroneous dating and identification (Olson 1985). Over-splitting of avian taxa has been proposed by Sibley and Ahlquist (1982) to explain the reduced intertaxon genetic divergence. Avise and Aquadro (1982) suggested that birds are not over-split at the species level, and demonstrated that, if a family of birds were reduced to a genus, it would contain three times as many species as a typical amphibian or reptile genus, and the genetic distances in that avian "genus" would still be much lower than those found in the amphibian genus. Likewise, to obtain avian intraspecific genetic distances comparable to other vertebrates, many valid congeneric species would have to be made conspecific (e.g., all species within *Melospiza, Dendroica,* or *Sterna*).

RELATIONSHIPS AMONG LARID TAXA: ALLOZYMES VS. MORPHOMETRICS

The results of Schnell's (1970a, 1970b) morphometric study of the Laridae are summarized in Figures 3 and 4; for comparison, only those taxa that I examined electrophoretically are included in Figure 4. In his analysis, Larinae, Sterninae, Rynchopinae, and Stercorariinae are each distinct groups (Fig. 3). Larinae and Sterninae are most similar phenetically; Rynchopinae is closest to the Larinae/Sterninae group; the most distinct group is the Stercorariinae. These morphometric results differ from the electrophoretic study in which both Stercorariinae and Rynchopinae are equally distant from the Larinae/Sterninae group. Within the terns (see Fig. 4), *A. stolidus, C. niger, S. antillarum,* and *S. anaethetus* are morphologically the most distinct; genetically this is true as well. The morphological and genetic trees differ most in their placement of *S. maxima,* which genetically is most closely related to *S. forsteri,* whereas morphologically is most closely related to *S. caspia.* Unfortunately, no tissue samples were available for *Larosterna inca,* the species that Schnell found to be most distinctive among the Laridae.

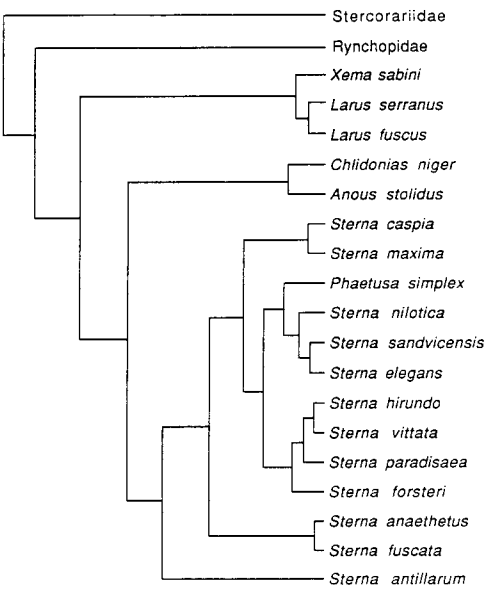

FIGURE 4. Relationships within Laridae determined by clustering morphological distances. This figure was taken from Schnell (1970b, fig. 29) and was redrawn using only the taxa analyzed electrophoretically in the present study.

The phylogenetic placement of *A. stolidus* is dependent on whether a phenetic or cladistic analysis of alleles is used. In analyses using both genetic and morphological distances, this taxon is placed outside the Larinae and Sterninae, such that one might question its taxonomic placement in the Sterninae. The cladistic assessment of alleles groups *A. stolidus* with the rest of the terns. In a distance analysis, however, autapomorphies in *A. stolidus* inflate genetic distances and cause

the taxon to fall outside the subfamily in which it is currently placed. The cladistic assessment of alleles and the genetic distance analysis both provide information about evolution in that taxon. Although *A. stolidus* is probably a tern (based on the cladistic assessment of allelic states), it may be evolving at a much faster rate than other members of the Sterninae that I examined (based on the high number of autapomorphies). A relative rate test also shows *A. stolidus* to be evolving faster than other members of the Sterninae. It would be interesting to sample its congeners—*A. minutus, A. tenuirostris,* as well as the putative close relative *Procelsterna cerulea*—to determine if this species is anomalous, or if the group as a whole is evolving at a much faster rate than other tern species. Because the Distance-Wagner procedure allows for unequal rates of evolution, it may be the most appropriate estimate of evolutionary history in the Laridae.

Both genetically and morphologically, *S. vittata* closely resembles *S. hirundo* (it is identical to *S. hirundo* at the 23 protein loci examined in this study). Suspension of migration in some larid species has been proposed to result in speciation, with *paradisaea/vittata* as an example. For example, Murphy (1936) proposed that *S. vittata* evolved from a population of wintering *S. paradisaea* that failed to migrate back to the breeding grounds. This hypothesis is not supported by my genetic analysis. *Sterna vittata* could have arisen from a *S. hirundo* population that was isolated in the Antarctic, but direct evidence for this is lacking. *Sterna hirundo* is a common, widespread tern that winters as far south as Tierra del Fuego. If *S. vittata* did evolve from *S. hirundo,* the genetic identity between these species implies a recent split.

TAXONOMIC RECOMMENDATIONS

The merger of the genera *Thalasseus, Hydroprogne,* and *Gelochelidon* into *Sterna* by the AOU (1983) is justified, to the limited extent that genetic analyses affect decisions of this kind (see Johnson and Zink 1983; also Johnson et al. 1988). Members of the genus *Thalasseus* (*maxima, elegans,* and *sandvicensis*) do not form a distinct clade apart from other species in *Sterna.*

The genus *Sterna* (sensu AOU 1983) is not monophyletic. More species in the Sterninae need to be examined before taxonomic decisions about this genus can be proposed. I would recommend, however, that classificatory schemes place *S. vittata* next to *S. hirundo* and not *S. paradisaea.* The consistent close association of *S. nilotica* with *P. simplex* implies that they should be placed next to each other in classificatory schemes. *Anous stolidus* should remain in the Sterninae and its putative close relatives examined to clarify their systematic position.

I do not find genetic support for the AOU's (1983) decision to give subfamilial status to the Stercorariinae, Larinae, Sterninae, and Rynchopinae. The Sterninae and Larinae are sister taxa (see Fig. 2, also supported by the cladistic analysis). Thus, I recommend a return to the classifications of Wetmore (1960) and Peters (1934), who gave familial status to the Stercorariidae, Laridae (subfamilies Larinae and Sterninae), and Rynchopidae.

ACKNOWLEDGMENTS

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APPENDIX 1. Species studied, English names, sample sizes, and collecting locality. Sample size and previous genus name (AOU 1957) are in parentheses after scientific name.

Species	English name	Collecting locality
Stercorariinae		
Stercorarius pomarinus (1)	Pomarine Jaeger	North Carolina, Dare County
S. longicaudus (1)	Long-tailed Jaeger	North Carolina, Dare County
Larinae		
Larus fuscus (1)	Lesser Black-backed Gull	Louisiana, Cameron Parish
L. serranus (1)	Andean Gull	Peru, Dpto. Huánuco
Xema sabini (1)	Sabine's Gull	California, Riverside County
Sterninae		
Chidonias niger (2)	Black Tern	Louisiana, Cameron Parish
Phaetusa simplex (1)	Large-billed Tern	Peru, Dpto. Loreto
Sterna (Gelochelidon) nilotica (2)	Gull-billed Tern	Louisiana, Cameron Parish
S. (Hydroprogne) caspia (2)	Caspian Tern	Louisiana, Cameron Parish
S. paradisaea (1)	Arctic Tern	Washington, Grays Harbor County
S. hirundo (2)	Common Tern	Louisiana, Cameron Parish
S. vittata (1)	Antarctic Tern	Antarctica, Flanders Bay
S. forsteri (25)	Forster's Tern	Louisiana, Cameron Parish
S. anaethetus (1)	Bridled Tern	North Carolina, Dare County
S. fuscus (1)	Sooty Tern	Louisiana, Cameron Parish
S. antillarum (2)	Least Tern	Louisiana, Cameron Parish
S. (Thalasseus) maximus (2)	Royal Tern	Louisiana, Cameron Parish
S. (Thalasseus) elegans (1)	Elegant Tern	California
S. (Thalasseus) sandvicensis (2)	Sandwich Tern	Louisiana, Cameron Parish
Anous stolidus (1)	Brown Noddy	Guam
Rynchopinae		
Rynchops niger (1)	Black Skimmer	Louisiana, Cameron Parish
Outgroups		
Oceanites oceanicus (1)	Wilson's Storm-Petrel	North Carolina, Dare County
Pluvialis squatarola (1)	Black-bellied Plover	Louisiana, Cameron Parish
Zenaida macroura (1)	Mourning Dove	Louisiana, Cameron Parish

APPENDIX 2. Allelic frequencies incorporating all genetic variation for 23 presumptive genetic loci examined in this study. Numbers in parentheses are frequencies of alleles at a locus. A single letter denotes a sample fixed for that allele. Abbreviations for loci can be found in Table 4 and for taxa in Table 6.

Locus	SPOM	SLON	LFUS	LSER	XSAB	SNIL	SCAS	SMAX	SELE
ACON	a	a	d	a	a (0.50) c (0.50)	a	a	a	a
ACP	d	d	b	b	b	b	b	b	b
ADA	d	d	a	a	a	b	a	a	a
EST	f	g	f	f	f	a	a	a (0.50) c (0.50)	e
GOT1	d	d	d	d	d	a	a	a	a
GOT2	a	a	a	a	a	a	a	a	a
aGPD	a	a	c	d	c	a	a (0.75) b (0.25)	a	a
ICD1	a	a	a	a	a (0.50) c (0.50)	a	a	a	a
ICD2	a	a	a	a	a	a	a	a	a
LA	b	b	a	a	a	a	a	a	a
LDH	f	e	a	a	a	a	a	a	a
LGG	e	e	c	c	c	c	b	b	b
MDH1	a	a	a	a	a	a	a	a	a
MDH2	a	a	a	a	a	a	a	a	a
ME1	g	g	c	f	c	c	c	c	c
ME2	d	d	b	d	b (0.50) c (0.50)	a	a	a	a
MPI	a	b	a	a	a	a (0.50) c (0.50)	a	a	a (0.50) g (0.50)
NP	k	k (0.50) l (0.50)	i	j	i	a	a (0.50) e (0.50)	f	a (0.50) g (0.50)
6PGD	a	a (0.50) d (0.50)	b	a	a	a	a	a	a
PGI	i	i	b	b	d (0.50) e (0.50)	b	d	b	b
PGM1	c	c	c	c	a (0.50) b (0.50)	c	c	e	f (0.50) g (0.50)
PP	g	g	g	a	f	a	a	a	a
SDH	b	b	a	a	a	a	a	a	a

Locus	SSAN	SHIR	SPAR	SVIT	SFOR	SANT	SANA	SFUS	PSIM
ACON	a	a	a	a	a	a	a	a	a
ACP	b	b	b	b	b	a	c	b	b
ADA	a	a	a	a	a	a	a	b	a
EST	d	a	a	a	a (0.50) c (0.50)	a	a	a	a
GOT1	a	a	a	a	a	a	a	a	a
GOT2	a	a	a	a	a	a	a	a	a
aGPD	a	a	a	a	a	a	a	b	a
ICD1	a	a	a	a	a	a	a	a	a
ICD2	a	a	a	a	a	a	a	a	a
LA	a	a	a	a	a	a	a	a	a
LDH	a	a	a	a	a	a	a	c	a
LGG	b	b	b	b	b (0.50) d (0.50)	a	b	b	c
MDH1	a	a	a	a	a	a	a	a	a
MDH2	a	a	a	a	a	a	a	a	a
ME1	c	c	c	c	c	a	c	c	e
ME2	a	a	a	a	a	a	a	a	a
MPI	a	a	a	a	a	a	a	a	a
NP	a	a	h	a	a	a (0.50) b (0.50)	b	a	b

APPENDIX 2. Continued.

Locus	SPOM	SLON	LFUS	LSER	XSAB	SNIL	SCAS	SMAX	SELE
6PGD	a	a	a	a	a	a	a	a	a
PGI	b	b	b	b	b	a	b	b	b
PGM1	f	c	c	c	a	a (0.75) b (0.25)	a (0.50) b (0.50)	e	c
PP	a	a	a	a	a	a	e	d	a
SDH	a	a	a	a	a	a	a	a	a

Locus	CNIG	ASTO	RNIG	OOCE	PSQU	ZMAC
ACON	a	a	e	h	f	g
ACP	b	b	e	h	f	g
ADA	a	c	e	h	f	g
EST	a	b	h	k	i	j
GOT1	a	b	c	g	d	e
GOT2	a	b	a	a	a	a
aGPD	a	a	a	g	e	f
ICD1	a	b	a	g	e	f
ICD2	a	a	a	d	b	c
LA	a	a	c	e	c	d
LDH	a	b	e	h	f	g
LGG	b (0.50) c (0.50)	a	f	h	f	g
MDH1	a	a	a	b	a	b
MDH2	a	a	a	e	b (0.50) c (0.50)	d
ME1	b (0.50) c (0.50)	d	h	k	i	j
ME2	a	a	d	f	e	e
MPI	a	a	a	f	d	e
NP	c (0.75) d (0.25)	b	m	p	n	o
6PGD	a	a	a	b	a	c
PGI	b	c	j	g (0.50) h (0.50)	j	f
PGM1	a (0.50) c (0.50)	d	k	k	i	j
PP	a (0.50) b (0.50)	c	g	j	h	i
SDH	a	a	b	d	c	d

APPENDIX 3. Allelic frequencies incorporating the least amount of genetic variation for 23 presumptive genetic loci examined in this study. Numbers in parentheses are frequencies of alleles at a locus. A single letter denotes a sample fixed for that allele. Abbreviations for loci can be found in Table 4 and for taxa in Table 6.

Locus	SPOM	SLON	LFUS	LSER	XSAB	SNIL	SCAS	SMAX	SELE
ACON	a	a	d	a	a	a	a	a	a
ACP	a	b	a	a	a	a	a	a	a
ADA	d	d	a	a	a	b	a	a	a
EST	c	a	c	c	c	a	a	a	a
GOT1	c	c	c	c	c	a	a	a	a
GOT2	a	a	a	a	a	a	a	a	a
aGPD	a	a	c	d	c	a	a (0.75) b (0.25)	a	a
ICD1	a	a	a	a	a (0.50) c (0.50)	a	a	a	a
ICD2	a	a	a	a	a	a	a	a	a
LA	b	b	a	a	a	a	a	a	a
LDH	a	a	a	a	a	a	a	a	a
LGG	e	e	c	c	c	c	b	b	b
MDH1	a	a	a	a	a	a	a	a	a
MDH2	a	a	a	a	a	a	a	a	a
ME1	g	g	c	f	c	c	c	c	c
ME2	d	d	b	d	b (0.50) c (0.50)	a	a	a	a
MPI	b	b	a	a	a	a (0.50) c (0.50)	a	a	a (0.50) g (0.50)
NP	a	a	a	a	a	d	d (0.50) f (0.50)	e	e
6PGD	a	a	a	a	a (0.50) b (0.50)	a	a	a	a
PGI	e	e	a	a	c (0.50) d (0.50)	a	a	a	a
PGM1	c	c	c	c	a (0.50) b (0.50)	c	c	e	f (0.50) g (0.50)
PP	a	a	a	a	c	a	a	a	a
SDH	b	b	a	a	a	a	a	a	a

Locus	SSAN	SHIR	SPAR	SVIT	SFOR	SANT	SANA	SFUS	PSIM
ACON	a	a	a	a	a	a	a	a	a
ACP	a	a	a	a	a	a	a	a	a
ADA	a	a	a	a	a	a	a	b	a
EST	b	a	a	a	a	a	a	a	a
GOT1	a	a	a	a	a	a	a	a	a
GOT2	a	a	a	a	a	a	a	a	a
aGPD	a	a	a	a	a	a	a	b	a
ICD1	a	a	a	a	a	a	a	a	a
ICD2	a	a	a	a	a	a	a	a	a
LA	a	a	a	a	a	a	a	a	a
LDH	a	a	a	a	a	a	a	c	a
LGG	b	b	b	b	b (0.50) d (0.50)	a	b	b	c
MDH1	a	a	a	a	a	a	a	a	a
MDH2	a	a	a	a	a	a	a	a	a
ME1	c	c	c	c	c	a	c	c	e
ME2	a	a	a	a	a	a	a	a	a
MPI	a	a	a	a	a	a	a	a	a
NP	d	d	e	d	d	b	b	d	d
6PGD	a	a	a	a	a	a	a	a	a
PGI	a	a	a	a	a	a	a	a	a
PGM1	f	c	c	c	a	a (0.75) b (0.25)	a (0.50) b (0.50)	e	c

APPENDIX 3. Continued.

Locus	SPOM	SLON	LFUS	LSER	XSAB	SNIL	SCAS	SMAX	SELE
PP	a	a	a	a	a	a	a	a	a
SDH	a	a	a	a	a	a	a	a	a

Locus	CNIG	ASTO	RNIG	OOCE	PSQU	ZMAC
ACON	a	a	e	h	f	g
ACP	a	a	c	f	d	e
ADA	a	c	e	h	f	g
EST	a	a	c	f	d	e
GOT1	a	b	c	f	d	e
GOT2	a	b	a	a	a	a
aGPD	a	a	a	g	e	f
ICD1	a	b	a	a	e	f
ICD2	a	a	a	d	b	c
LA	a	a	c	e	c	d
LDH	a	b	a	f	d	e
LGG	b (0.50) c (0.50)	a	a	i	g	h
MDH1	a	a	a	b	a	c
MDH2	a	a	a	e	b (0.50) c (0.50)	d
ME1	b (0.50) c (0.50)	d	h	k	i	j
ME2	a	a	d	f	e	e
MPI	a	a	a	f	d	e
NP	b	b	f	h	b	g
6PGD	a	a	a	d (0.50) e (0.50)	a	c
PGI	a	b	f	i (0.50) j (0.50)	g	h
PGM1	a (0.50) c (0.50)	d	h	k	i	j
PP	a	b	a	f	d	e
SDH	a	a	b	d	c	d