iour of Goldeneye, with notes on population composition. Wildfowl 20:75–77.

MADSEN, J., T. BREGNBALLE, AND F. MEHLUM. 1989. Study of the breeding ecology and behaviour of the Svalbard population of Light-bellied Brent Goose *Branta bernicla hrota*. Polar Res. 7:1–21.

MAJ, M. B. 1983. Analysis of Trumpeter Swan habitat on the Targhee National Forest of Idaho and Wyoming. M.S. thesis, Montana State Univ., Bozeman.

MCKELVEY, R. W., AND N. A. VERBEEK. 1988. Habitat use, behaviour, and management of Trumpeter Swans, *Cygnus buccinator*, wintering at Comox, British Columbia. Can. Field-Nat. 102:434–441.

MOULTON, D. W., AND M. W. WELLER. 1984. Biology and conservation of the Laysan Duck (*Anas laysanensis*). Condor 86:105–117.

NILSSON, L. 1970. Food-seeking activity of south Swedish diving ducks in the non-breeding season. Oikos 21:145–154.

OWEN, M., AND C. J. CADBURY. 1975. The ecology and mortality of swans at Ouse Washes, England. Wildfowl 26:31–42.

OWEN, M., AND J. KEAR. 1972. Food and feeding habits. Pages 58–77 *in* The swans (P. Scott, Ed.). Houghton Mifflin Co., Boston.

PAULUS, S. L. 1984. Activity budgets of nonbreeding Gadwalls in Louisiana. J. Wildl. Manage. 48:371–380.

PAULUS, S. L. 1988. Time-activity budgets of nonbreeding Anatidae: A review. Pages 135–152 *in* Waterfowl in winter (M. W. Weller, Ed.). Univ. Minnesota Press, Minneapolis.

PEDROLI, J. 1982. Activity and time-budget of Tufted Ducks on Swiss lakes during winter. Wildfowl 33:105–112.

RAVELING, D., W. E. CREWS, AND W. D. KLIMSTRA. 1972. Activity patterns of Canada Geese during winter. Wilson Bull. 84:278–295.

RYDER, J. P. 1970. A possible factor in the evolution of clutch size in Ross' Goose. Wilson Bull. 82:5–13.

SHEA, R. E. 1979. The ecology of the Trumpeter Swan in Yellowstone National Park and vicinity. M.S. thesis, Univ. Montana, Missoula.

SKUTCH, A. F. 1976. Parent birds and their young. Univ. Texas Press, Austin.

SWANSON, G. A., AND A. B. SARGEANT. 1972. Observation of nighttime feeding behavior of ducks. J. Wildl. Manage. 36:959–961.

TAMISIER, A. 1974. Etho-ecological studies of teal wintering in the Camargue (Rhone Delta, France). Wildfowl 25:123–133.

TAMISIER, A. 1976. Diurnal activities of Green-winged Teal and Pintail wintering in Louisiana. Wildfowl 27:19–32.

THOMPSON, S. C., AND D. G. RAVELING. 1987. Incubation behavior of Emperor Geese compared with other geese: Interactions of predation, body size, and energetics. Auk 104:707–716.

YDENBERG, R. C., H. H. TH. PRINS, AND J. VAN DIJK. 1984. A lunar rhythm in the nocturnal foraging activities of wintering Barnacle Geese. Wildfowl 35:93–96.

ZAR, J. H. 1984. Biostatistical analysis, 2nd ed. Prentice-Hall, Englewood Cliffs, New Jersey.

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Patterns of Genetic Polymorphism in Five Species of Penguins

NINA N. THUMSER AND JEFFREY D. KARRON
Department of Biological Sciences, University of Wisconsin–Milwaukee, Milwaukee, Wisconsin 53201, USA

Conservation programs benefit from increased knowledge of the basic biology and systematics of endangered species (Haig et al. 1990). This study focuses on relationships in the genus *Spheniscus*, which includes: Jackass Penguin (*S. demersus*), Galapagos Penguin (*S. mendiculus*), Humboldt Penguin (*S. humboldti*), and Magellanic Penguin (*S. magellanicus*). The first three taxa are considered threatened or endangered (U.S. Fish and Wildlife Service 1990, 1993). However, Jackass, Humboldt, and Magellanic penguins are quite abundant in captivity, making this group well-suited for genetic and behavioral studies.

In addition to facilitating penguin research, captivity has led to mixed-species exhibits and interbreeding between *Spheniscus* species. Fertile hybrids between Jackass and Humboldt penguins and between Humboldt and Magellanic penguins have been reported in captivity (Conway 1965, Araya 1983). This raises questions concerning the species status of members of this group. The Galapagos and Jackass pen-

guins are geographically isolated from other members of the genus. However, the Humboldt and Magellanic penguins' ranges overlap by about 10° of latitude. For these latter taxa it is generally believed that different breeding periods and breeding grounds prevent hybridization in the wild. In fact, there are no confirmed cases of natural hybrids, although suspected instances have been reported in the literature (Murphy 1936, Araya 1983).

The ability of *Spheniscus* penguins to interbreed in captivity and occasional reports of birds with Jackass Penguin markings in Magellanic Penguin colonies in South America led Clancey (1966) to suggest that Jackass Penguins be considered a subspecies of the Magellanic Penguin. Sibley and Monroe (1990) recommended that *S. demersus* be viewed as a superspecies containing *demersus, humboldti,* and *magellanicus.* O'Hare (1989) used 22 morphological characters to differentiate *Spheniscus* from other penguin genera. However, he was unable to determine the phylogenetic relationships among species of the genus. Also, the fossil record for *Spheniscus* is not sufficiently detailed to distinguish species-level differences (Simpson 1976).

Molecular and biochemical approaches may provide valuable information concerning phylogenetic relationships among closely related species (Avise 1974, Buth 1984). These techniques are advantageous because they are less likely than many morphological characters to be influenced by natural selection (Hillis 1987). Even though allozymes are not necessarily neutral, they may be subject to different selective forces than morphological markers and provide additional information concerning phylogenies. In our study, protein polymorphism was determined for three *Spheniscus* species (Jackass, Humboldt, and Magellanic penguins) and two outgroups (Rockhopper Penguin, *Eudyptes chrysocome;* King Penguin, *Aptenodytes patagonicus*). Galapagos Penguins were not included because no animals occur in captivity and it was not possible to sample blood from naturally occurring populations of this endangered species. The outgroups provide a means of estimating ancestral character states of the *Spheniscus* species (Matson 1984). Rockhopper Penguins were chosen as an outgroup because they occur in the same geographic area as the *Spheniscus* penguins and belong to one of two genera thought to be the most closely related to *Spheniscus* (Zusi 1975, Jouventin 1982). The King Penguin serves as a more distantly related outgroup.

Methods.—Blood samples were obtained from 57 captive and 50 wild Humboldt Penguins, 33 captive Jackass Penguins, 16 wild Magellanic Penguins, 8 captive Rockhopper Penguins, and 1 captive King Penguin. The Humboldt Penguin blood samples were obtained from the Brookfield Zoo (5), the Milwaukee County Zoo (21), Hubbs Sea World (7), the St. Louis Zoo (10), the Washington Park Zoo (10), the Woodland Park Zoo (4) and two populations in Chile (Al-

TABLE 1. Allele frequencies at nine polymorphic loci for (1) Humboldt, (2) Washington Park Humboldt, (3) Jackass, (4) Magellanic, (5) Rockhopper, and (6) King penguins. Sample size (*n*) indicated in first row for each locus.

	Species					
Allele	1	2	3	4	5	6
			Est-1			
	97	10	33	16	8	1
a	0.000	0.000	0.000	0.000	0.000	1.000
b	1.000	1.000	1.000	1.000	0.000	0.000
c	0.000	0.000	0.000	0.000	1.000	0.000
			Got-1			
	97	10	33	16	8	1
a	0.000	0.000	0.076	0.063	0.000	0.000
b	1.000	1.000	0.924	0.906	1.000	1.000
c	0.000	0.000	0.000	0.031	0.000	0.000
			Ldh-1			
	97	10	33	16	8	1
a	0.000	0.000	0.000	0.000	1.000	0.000
b	0.000	0.000	0.000	0.000	0.000	1.000
c	1.000	1.000	1.000	1.000	0.000	0.000
			Ldh-2			
	97	10	33	16	8	1
a	0.000	0.000	0.000	0.000	1.000	0.000
b	0.000	0.000	0.000	0.000	0.000	1.000
c	1.000	1.000	1.000	1.000	0.000	0.000
			Mdh-1			
	97	10	33	16	8	1
a	0.005	0.000	0.030	0.000	0.000	1.000
b	0.995	1.000	0.970	1.000	1.000	0.000
			Mdh-2			
	97	10	33	16	8	1
a	0.000	0.000	0.000	0.000	0.000	1.000
b	1.000	1.000	1.000	1.000	1.000	0.000
			Pab-1			
	97	10	33	16	8	1
a	0.000	0.000	0.000	0.000	0.000	1.000
b	1.000	0.000	0.000	0.000	0.000	0.000
c	0.000	0.000	1.000	1.000	0.000	0.000
d	0.000	1.000	0.000	0.000	1.000	0.000
			Pgi-1			
	97	10	33	16	8	1
a	1.000	1.000	0.742	1.000	1.000	1.000
b	0.000	0.000	0.258	0.000	0.000	0.000
			Sod-1			
	97	10	33	16	8	1
a	0.000	0.000	0.000	0.000	1.000	0.000
b	0.000	0.000	0.000	0.000	0.000	1.000
c	0.000	0.000	1.000	1.000	0.000	0.000
d	1.000	1.000	0.000	0.000	0.000	0.000

TABLE 2. Nei (1978) unbiased genetic distance for five penguin species, with Washington Park (WP) Humboldt Penguin specimens treated separately.

Penguin species	1	2	3	4	5
1 Humboldt					
2 WP Humboldt	0.057				
3 Jackass	0.124	0.124			
4 Magellanic	0.119	0.119	0.003		
5 Rockhopper	0.326	0.251	0.337	0.328	
6 King	0.492	0.492	0.504	0.496	0.492

garrobo [30] and Cachagua [20]). The Jackass Penguin blood samples were obtained from the Baltimore Zoo (14), the Denver Zoo (12), and the New York Aquarium (7). The Magellanic Penguin blood samples were obtained from wild birds being rehabilitated at the Sao Paulo Zoo, Brazil (16). The Rockhopper Penguin blood samples were obtained from the Milwaukee County Zoo (3) and the St. Louis Zoo (5). The King Penguin blood sample was obtained from the Milwaukee County Zoo (1). The samples were analyzed for unique population and species variants using starch-gel electrophoresis (methods described in Lacy 1982). The following enzyme systems were surveyed: Ldh, Mdh, and Got resolved on potassium phosphate buffer (pH 6.7) of Selander et al. (1971); Pgi, 6-Pgd, and Adk resolved on tris citrate buffer (pH 6.3) of Selander et al. (1971); Pgm and Sod resolved on lithium borate tris buffer (pH 8.1) of Ridgeway et al. (1970); Acp resolved on morpholine citrate buffer (pH 6.1) of Clayton and Tretiak (1972); and Est and three non-enzyme protein loci (Pab, Hbo, and Alb) resolved on sodium borate buffer (pH 8.2) of Poulik (1957). To minimize error in scoring of variants, each gel contained representatives from several populations of each species.

Phenetic analyses were conducted using the BIOSYS-1 computer package (Swofford and Selander 1989). Nei (1972, 1978), Rogers (1972), modified Rogers (Wright 1978), and Cavalli-Sforza and Edwards (1967) genetic distances were calculated from the genotype frequencies. These were used to construct UPGMA and distance-Wagner trees. Parsimony analyses were carried out using the PAUP 3.0s computer package (Swofford 1991). Data were analyzed in two ways. First, each locus was considered to be a character and the alleles were character states. Second, each allele was treated as a character and the states were presence or absence.

Results.—Blood proteins coded by 18 loci were consistently scorable in all five species. Nine loci (Ldh-3, Pgm-1, Pgm-2, Acp, Hbo, Alb, Pgd, Adk-1, Adk-2) were monomorphic across all taxa examined. The other nine loci were polymorphic, with most differences being fixed between species (Table 1). Intraspecific polymorphism was low in all *Spheniscus* species. Humboldt and Magellanic penguins had 5.6% polymorphic loci, and Jackass Penguins had 16.7% polymorphic loci. The only population-level variation found was in one captive group of Humboldt Penguins. The Washington Park birds had a fixed difference, which distinguished them from the other Humboldt Penguins; we treated the Washington Park Humboldt Penguins separately. Table 2 shows Nei's (1978) unbiased genetic distances between pairs of taxa.

In the phenetic analysis, all UPGMA trees had the same topology. The *Spheniscus* formed a single group with the Magellanic and Jackass penguins being most closely associated. Figure 1A shows the tree obtained using the modified Rogers distance (Wright 1978) which had the best fit (cophenetic correlation = 0.997). The distance-Wagner trees had topologies similar to each other and similar to the UPGMA trees. The rooted distance-Wagner tree using the Cavalli-Sforza and Edwards (1967) chord distance shown in Figure 1B was one of the best-fitting trees (cophenetic correlation = 0.998).

Cladistic analysis using loci as characters and allele variants as character states yielded 21 most-parsimonious trees. After 100 bootstrap replications with a heuristic search, the 50% majority-rule consensus tree resolved only the Jackass and Magellanic penguins as a separate group. Examination of each of the most-parsimonious trees indicated that King Penguins never clustered with another penguin species, while Jackass and Magellanic penguins clustered together in over 80% of the trees. The positions of Rockhopper and Humboldt penguins varied considerably among the trees, with no consistent pattern.

The analysis using alleles as characters and their presence or absence as character states produced a single most-parsimonious tree with a length of 27. The heuristic search with 100 bootstrap replications produced the 50% majority-rule consensus tree shown in Figure 1C. This tree matches those generated using the phenetic methods and strongly suggests that *Spheniscus* penguins are a monophyletic group, and that Jackass and Magellanic penguins are sister taxa.

Discussion.—The electrophoretic data generally agree with other findings concerning the systematics of *Spheniscus* penguins. For example, these results support earlier morphological studies which indicate that the *Spheniscus* penguins form a monophyletic group (O'Hare 1989). Data on protein polymorphism also support several other studies suggesting that *Eudyptes*

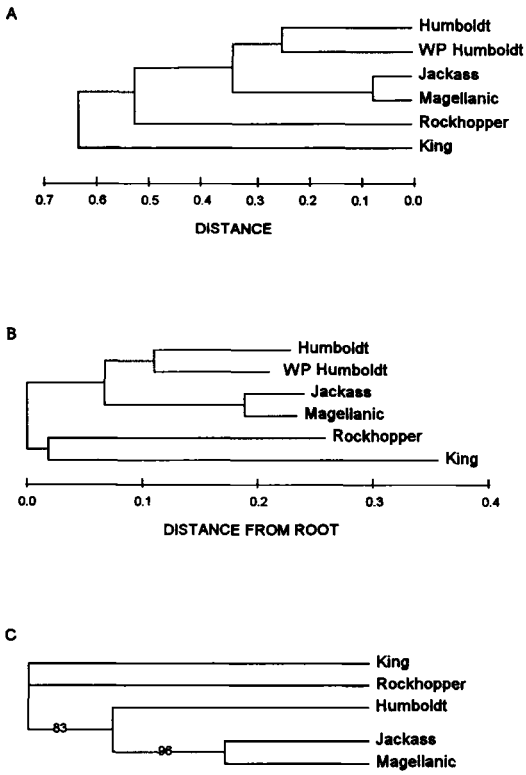

Fig. 1. (A) UPGMA tree derived from modified Rogers distance (Wright 1978); (B) Wagner tree based on Cavalli-Sforza & Edwards (1967) chord distance; and (C) majority-rule consensus tree based on binary coding of presence or absence of 18 alleles for five species (with Humboldt Penguins divided into two groups). Wagner tree optimized and rooted using outgroup method. Majority-rule consensus tree numbers along branches indicate percentage of times clade was distinguished in 100 bootstrapped trees.

is more closely related than *Aptenodytes* to the *Spheniscus* penguins (Jouventin 1982, Schreiweis 1982, Sibley and Ahlquist 1972, Taylor 1965, Zusi 1975).

The occurrence of a fixed difference within the Humboldt Penguins was surprising, especially since such low levels of polymorphism were found. Birds often have reduced polymorphism (Barrowclough and Corbin 1978), but the level (5.6%) for Humboldt and Magellanic penguins is only one-third the level normally found. A possible explanation for the fixed difference in the Washington Park Zoo population is that they are derived from wild-caught founders from Peru, whereas most of the other captive and wild population samples are thought to come from Chile. It would be useful to sample extensively over the Humboldt Penguin's entire natural range to determine what patterns exist in allele frequencies.

The most unexpected result was the small genetic distance (Nei $D = 0.002$) between the Magellanic and Jackass penguins, reflecting the absence of fixed differences between these two taxa. In fact, the distance is less than that between the two groups of Humboldt Penguins. It also is lower than the normal range of within-species variation in birds (Barrowclough 1980). Genetic distances between Humboldt Penguins and the other two *Spheniscus* penguins (0.119 and 0.124) are within the range of congeneric species. Genetic distances between outgroup taxa and the *Spheniscus* penguins fall in the confamilial range (Barrowclough 1980) as expected.

Because the sample size of 18 loci is relatively small, no conclusive statement can be made concerning the relationship between Magellanic and Jackass penguins. However, the possibility of gene flow between these taxa merits further investigation. Occurrences of birds with a Magellanic Penguin morphology in African Jackass Penguin colonies have been well documented from the 1960s to the present (Boswell and MacIver 1975, R. Wilson pers. comm.). In addition to the presence of morphological variation, the patterns of ocean currents and the life history of Magellanic Penguins may allow for the exchange of genetic material between Jackass Penguins in Africa and Magellanic Penguins in South America. The currents between Africa and South America are circular (Stommel 1957), allowing potential movement in both directions. Magellanic Penguins differ from all the other *Spheniscus* in having a single breeding season and spending over six months of the year at sea (Boersma et al. 1990). More research on the genetic relationship between the Jackass and Magellanic penguins and the behavior of the latter when at sea is warranted.

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Literature Cited

Araya, M. B. 1983. A preliminary report on the status and distribution of the Humboldt Penguin in Chile. Proceedings of the International Foundation for the Conservation of Birds, Los Angeles, California.

Avise, J. C. 1974. Systematic value of electrophoretic data. Syst. Zool. 23:465-481.

Barrowclough, G. F. 1980. Genetic and phenotypic

differentiation in a wood warbler (genus *Dendroica*) hybrid zone. Auk 97:655–668.

BARROWCLOUGH, G. F., AND K. W. CORBIN. 1978. Genetic variation and differentiation in the Parulidae. Auk 95:691–702.

BOERSMA, P. D., D. L. STOKES, AND P. M. YORIO. 1990. Reproductive variability and historical change of Magellanic Penguins (*Spheniscus magellanicus*) at Punta Tombo, Argentina. Pages 15–43 *in* Penguin biology (L. S. Davis and J. T. Darby, Eds.). Academic Press, San Diego.

BOSWELL, J., AND D. MACIVER. 1975. The Magellanic Penguin *Spheniscus magellanicus*. Pages 271–305 *in* The biology of penguins (B. Stonehouse, Ed.). Macmillan, New York.

BUTH, D. G. 1984. The application of electrophoretic data in systematic studies. Annu. Rev. Ecol. Syst. 15:501–522.

CAVALLI-SFORZA, L. L., AND A. W. F. EDWARDS. 1967. Phylogenetic analysis: Models and estimation procedures. Evolution 21:550–570.

CLANCEY, P. A. 1966. The penguins *Spheniscus demersus* Linnaeus and *Spheniscus magellanicus* Forster. Ostrich 37:237.

CLAYTON, J. W., AND D. N. TRETIAK. 1972. Amine-citrate buffers of pH control in starch gel electrophoresis. J. Fish. Res. Board Can. 29:1169–1172.

CONWAY, G. 1965. The penguin metropolis of Punta Tombo. Anim. Kingdom 68:115–123.

HAIG, S. M., J. D. BALLOU, AND S. R. DERRICKSON. 1990. Management options for preserving genetic diversity: Reintroduction of Guam Rails to the wild. Conserv. Biol. 4:290–300.

HILLIS, D. 1987. Molecular versus morphological approaches to systematics. Annu. Rev. Ecol. Syst. 18:23–42.

JOUVENTIN, P. 1982. Visual and vocal signals in penguins, their evolution and adaptive characters. Adv. Ethol. 24.

LACY, R. C. 1982. Population biology of mycophagous Drosophilidae in the eastern United States. Ph.D. dissertation, Cornell Univ., Ithaca, New York.

MATSON, R. H. 1984. Application of electrophoretic data in avian systematics. Auk 101:717–729.

MURPHY, R. C. 1936. Oceanic birds of South America. Macmillan, New York.

NEI, M. 1972. Genetic distance between populations. Am. Nat. 106:283–292.

NEI, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89:583–590.

O'HARE, R. J. 1989. Systematics and the study of natural history, with an estimate of the phylogeny of living penguins (Aves: Spheniscidae). Ph.D. dissertation, Harvard Univ., Boston, Massachusetts.

POULIK, M. D. 1957. Starch gel electrophoresis in a discontinuous system of buffers. Nature 180:1477–1479.

RIDGEWAY, G. J., S. W. SHERBURNE, AND R. D. LEWIS. 1970. Polymorphism in the esterases of Atlantic herring. Trans. Am. Fish. Soc. 99:147–151.

ROGERS, J. S. 1972. Measures of genetic similarity and genetic distances. Studies in Genetics VII. Univ. Texas Publ. 7213:145–153.

SCHREIWEIS, D. O. 1982. A comparative study of appendicular musculature of penguins (Aves: Sphenisciformes). Smithson. Contrib. Zool. 341.

SELANDER, R. K., M. H. SMITH, S. Y. YANG, W. E. JOHNSON, AND J. B. GENTRY. 1971. Biochemical polymorphism and systematics in the genus *Peromyscus*. I. Variation in the old-field mouse (*Peromyscus polionotus*). Studies in Genetics VI. Univ. Texas Publ. 7103:49–90.

SIBLEY, C. G., AND J. E. AHLQUIST. 1972. A comparative study of egg-white proteins of non-passerine birds. Peabody Mus. Nat. Hist. Bull. 39.

SIBLEY, C. G., AND B. L. MONROE, JR. 1990. Distribution and taxonomy of birds of the world. Yale Univ. Press, New Haven.

SIMPSON, G. G. 1976. Penguins past and present, here and there. Yale Univ. Press, New Haven.

STOMMEL, H. 1957. A survey of ocean current theory. Deep Sea Res. 4:149–184.

SWOFFORD, D. 1991. PAUP: Phylogenetic analysis using parsimony, version 3.0s. Illinois Natural History Survey, Champaign.

SWOFFORD, D., AND R. B. SELANDER. 1989. BIOSYS-1, release 1.7. Illinois Natural History Survey, Champaign.

TAYLOR, C. 1965. A study of the egg shells of the Spheniciformes. J. Zool. 147:1–19.

U.S. FISH AND WILDLIFE SERVICE. 1990. Appendices I, II, III to the Convention on International Trade in Endangered Species of Wild Fauna and Flora. 50 CFR 23.23.

U.S. FISH AND WILDLIFE SERVICE. 1993. Endangered and threatened wildlife and plants. Fed. Register 50 CFR 17.11 and 17.12.

WRIGHT, S. 1978. Evolution and the genetics of populations. Vol. 4, Variability within and among natural populations. Univ. Chicago Press, Chicago.

ZUSI, R. L. 1975. An interpretation of skull structure in penguins. Pages 59–84 *in* The biology of penguins (B. Stonehouse, Ed.). Macmillan, New York.

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