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LITERATURE CITED

DAAN, S., C. DIJKSTRA, R. H. DRENT, AND T. MEIJER. 1988. Food supply and the annual timing of avian reproduction. Pages 392-407 *in* Acta XIX Congressus Internationalis Ornithologici (H. Ouellet, Ed.). Ottawa, Ontario, 1986. National Museum of Natural Science, Ottawa.

EDEN, S. F., A. G. HORN, AND M. L. LEONARD. 1989. Food provisioning lowers inter-clutch interval in Moorhens *Gallinula chloropus*. Ibis 131:429-432.

ENS, B. J., M. KERSTEN, A. BRENNINKMEIJER, AND J. B. HULSCHER. 1992. Territory quality, parental effort and reproductive success of Oystercatchers (*Haemapotus ostralegus*). J. Anim. Ecol. 61:703-716.

KIRKWOOD, J. K. 1983. A limit to metabolizable energy intake in mammals and birds. Comp. Biochem. Physiol. A. 75:1-3.

LUNDBERG, A., AND R. V. ALATALO. 1991. The Pied Flycatcher. T. & A. D. Poyser, London.

MARTIN, T. E. 1987. Food as a limit on breeding birds: A life history perspective. Annu. Rev. Ecol. Syst. 18:453-487.

MCCULLAGH, P., AND J. A. NELDER. 1983. Generalized linear models. Chapman and Hall, London, New York.

SIMONS, L. S., AND T. E. MARTIN. 1990. Food limitation of avian reproduction: An experiment with the Cactus Wren. Ecology 71:869-876.

VAN BALEN, J. H. 1973. A comparative study of the breeding ecology of the Great Tit *Parus major* in different habitats. Ardea 61:1-93.

WILLIAMS, G. C. 1966. Natural selection, the costs of reproduction, and a refinement of Lack's principle. Am. Nat. 100:687-690.

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Allozyme Phylogeny of *Spheniscus* Penguins

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There are four species of *Spheniscus* penguins, all with distributions limited to the Southern Hemisphere. The Humboldt (Peruvian) Penguin (*S. humboldti*) is found on the west coast of South America in the cold, upwelled waters of the Humboldt Current (Stonehouse 1975). The Magellanic Penguin (*S. magellanicus*) occurs to the south of the Humboldt Penguin on the west coast and on the south-eastern coast of South America. The Jackass Penguin (*S. demersus*) occurs on the west and south coasts of southern Africa in the cold Benguela Current, while the Galapagos Penguin (*S. mendiculus*) is restricted to the tropical Galapagos Archipelago.

The phylogenetic relations among these birds are uncertain. Murphy (1936) suggested that the Humboldt and Galapagos penguins were sister taxa and that the Magellanic and Jackass penguins were sister taxa, basing this conclusion on several morphological features and geographic distributions. However, a recent analysis of measurements of external and skeletal structures showed that the Humboldt and the Magellanic penguins were phenetically more similar to each other and to the Jackass Penguin than to the Galapagos Penguin (Livezey 1989). The larger skeletal analysis in the latter study revealed that of the four congeners, *S. magellanicus* and *S. humboldti* were most similar (Livezey 1989). These similarities, however, may not reflect phylogenetic relations because of convergence.

In this study, we estimated a phylogenetic tree from allozyme frequencies for three of these taxa (the Humboldt, Magellanic, and Jackass penguins) and used the closely related Rockhopper (*Eudyptes chrysocome*) and Macaroni (*E. chrysolophus*) penguins from the Southern Ocean as outgroups. We also examined tissue expressions of loci to search for changes in expression during speciation (Mindell and Sites 1987) and compared the average heterozygosities among taxa to search for historical population bottlenecks.

Methods.—We collected tissues from 45 Jackass Penguins on 19 December 1986 at Stony Point (34°20'S, 18°53'E), Cape Province, South Africa about six hours after a leopard (*Pantherus pardus*) killed the birds. Samples of cardiac and breast muscle, liver, and vitreous fluid were removed for electrophoretic analysis. Four

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birds were in juvenile plumage. We also included three downy Jackass Penguins recently dead at the nest and three juveniles killed by oil pollution near Cape Town. Tissues from frozen carcasses of captively grown Magellanic and Humboldt penguins were collected at the Sea World Research Institute, San Diego, California. Macaroni and Rockhopper penguins were collected on Marion Island (46°52'S, 37°51'E). Tissue samples were held at −70°C until electrophoretic analysis. Aqueous proteins were extracted with a 0.1 M phosphate (pH = 7.4) buffer and, after centrifugation at 5,000 × *g* for 10 min, the clear supernatant was used for electrophoresis. Stain protocols followed Harris and Hopkinson (1976), and loci were designated numerically by the mobilities of their products beginning at the anodal end of a gel.

We detected the products of 30 loci with 22 active-enzyme stains. We used a Tris-citric acid-lithium hydroxide discontinuous buffer system (pH = 8.7; Ridgway et al. 1970) for aspartate aminotransferase (*Aat*), alcohol dehydrogenase (*Adh-1, Adh-2*), esterase (*Est-1, Est-2, Est-3, Est-4*), creatine kinase (*Ck-A, Ck-B*), glucosephosphate isomerase (*Gpi*), lactate dehydrogenase (*Ldh-A, Ldh-B*), phosphoglucomutase (*Pgm-1, Pgm-2*), superoxide dismutase (Sod), a Tris-citrate buffer (pH = 6.9; Whitt 1970) for acid phospatase (*Acp*), adenylate kinase (*Ak*), glyceraldehydephosphate dehydrogenase (*Gap-1, Gap-2, Gap-3*), glycerol-3-phosphate dehydrogenase (*Gpd-1, Gpd-2*), isocitrate dehydrogenase (*Icd-A, Icd-B*), malate dehydrogenase (*mMdh, sMdh*), phosphogluconate dehydrogenase (*Pgd*), and a Tris-borate-NaEDTA buffer (pH = 8.7; Markert and Faulhaber 1965) for aldolase (*Ald*), fumerase (*Fum-1, Fum-2*), glutamate pyruvate transaminase (*Gpt*), mannose-phosphate isomerase (*Mpi*), nucleoside phosphorylase (*Np*), and peptidases with the substrates glycyl-leucine (*Pep-A, Pep-C*), leucyl-glycyl-glycine (*Pep-B1, Pep-B2*), and phenylalanyl-proline (*Pep-D1, Pep-D2*).

Results and Discussion.—Several enzyme systems showed developmental differences in gene expression among downy chicks, juveniles, and adults of the Jackass Penguin (Table 1). *Adh-2* and *Gap-3* were expressed only in chicks. *Adh-1, Icd-B,* and *Ldh-A* were not expressed in chicks. Both *Ldh-A* and *Ldh-B* were expressed in juveniles and adults, and interlocus heterotetrameric bands appeared between the homotetrameric products of each locus. All other loci were expressed in each age group. There were no differences in the tissue distributions of gene expression between the adults of *Spheniscus* and those of *Eudyptes.* No significant deviations from Hardy-Weinberg expectations were detected with the *G*-test and Levene's (1949) correction for small samples for any of the polymorphic loci in any of the taxa. *Ck-A, Est-3, Fum-1, Fum-2, Gpi, Gpd-1, Icd-B, Ldh-B, sMdh, Mpi, Pep-A, Pep-C, Pep-B1, Pgm-2,* and *Sod* were polymorphic within one or more species (Table 2). Three loci *Gap-1, Ldh-A,* and *Pgm-1* were fixed for different alleles between genera, and 13 loci, *Ald, Ak, Ck-B, Est-2, Est-4, Gap-2, Gda, Icd-A, mMdh, Me-2, Pep-B2, Pep-D2,* and *Pgd* were invariant among taxa.

TABLE 1. Locus expression in Jackass Penguins.[a]

Locus	Muscle	Heart	Liver	Eye
Acp	+	++	+++	−
Adh-1[b]	−	−	+++	−
Adh-2[c]	−	−	+++	−
Ak[b]	+++	−	−	−
Est-1	−	++	+++	+
Est-2	−	−	++	−
Est-3	+	++	+++	+
Est-4	−	+	++	−
Ck-A[b]	+++	−	−	−
Ck-B	−	+++	−	−
Fum-1	++	+++	+	−
Fum-2	+++	++	+	−
Gpi	+++	+++	+++	+++
Gpt	+++	++	+	−
Gap-1	++	+++	+++	+
Gap-2[b]	+	−	++	−
Gap-3[b]	−	+	+++	−
Gpd-1	++	+	+++	−
Gpd-2	+	+	+++	−
Gda	++	+	+++	−
Icd-A[b]	+++	+++	++	−
Icd-B	+	++	+++	−
Ldh-A[b]	+++	−	++	+
Ldh-B[b]	−	+++	+++	−
mMdh	+	+++	+++	+
sMdh	++	+++	+	+++
Mpi	+++	+++	+++	++
Np	−	−	+	+++
Pep-A	++	+++	+++	+
Pep-C	+	+	+++	−
Pep-B1	+++	+++	+++	+
Pep-B2	+	++	+++	−
Php-D1	+	++	++	−
Php-D2	+	++	+++	−
Pgd	+	++	+++	+
Pgm-1	−	−	+++	−
Pgm-2	+++	+	+	−
Sod	++	++	+++	−

[a] (−) banding absent; (+) banding just perceptible; (++) banding moderately dense; (+++) banding very dense.

[b] Banding absent or much reduced in chicks.

[c] Banding absent in adults.

We estimated unbiased average heterozygosity and its variance (Nei and Roychoudhury 1974, Nei 1978) for each taxon. For the Jackass Penguin, only the birds from Stony Point were used to estimate heterozygosity, because they presumably represented a single population. Expected heterozygosities averaged over 30 loci were 0.031 and 0.046 for Rockhopper and Macaroni penguins, respectively, and 0.025, 0.054, and 0.081 for Humboldt, Magellanic and Jackass penguins, respectively. Jackknifed estimates of heterozygosities (Weir 1990) were similar to the simple averages over loci. The distributions of single-locus

TABLE 2. Allelic frequencies for penguins in the genera *Spheniscus* and *Eudyptes*.[a]

Locus	Allele	Species				
		Jackass	Magellanic	Humboldt	Rockhopper	Macaroni
Est-3[b]	90	—	—	—	1.000	—
	95	—	0.083	—	—	0.944
Fum-2	50	—	—	—	—	0.050
Gpi	600	—	—	—	0.100	—
	150	—	—	1.000	0.900	1.000
	−300	0.189	—	—	—	—
Gap-1	−100	1.000	1.000	1.000	—	—
	−200	—	—	—	1.000	1.000
Gpd-1	−5	—	—	0.333	—	—
	−20	0.411	0.500	0.667	—	—
	−100	0.589	0.500	—	1.000	1.000
Icd-B	150	0.378	0.083	—	—	—
Ldh-A	−500	—	—	—	1.000	1.000
Ldh-B	210	—	0.083	—	—	—
	180	—	—	—	1.000	1.000
	130	—	0.167	—	—	—
mMdh	−100	1.000	1.000	1.000	1.000	0.950
	−200	—	—	—	—	0.050
Mpi	90	—	—	—	0.150	—
	105	0.533	0.167	—	—	—
Pep-A	85	0.422	—	0.143	—	—
	95	—	—	—	0.222	0.350
	110	—	—	—	0.778	0.650
Pep-C	95	0.033	—	—	—	—
	110	—	—	—	1.000	1.000
Pep-B1	40	0.011	—	—	—	—
	180	—	—	—	—	0.050
Pgm-1	300	—	—	—	—	1.000
Pgm-2	150	—	—	—	0.950	1.000
	200	—	—	—	0.050	—
Sod	40	—	—	1.000	—	—
	105	0.044	—	—	—	—
	110	—	—	—	—	0.350
	190	—	—	—	1.000	0.650

[a] *Adh-2, Ald, Ak, Ck-A, Ck-B, Est-2, Est-4, Gap-2, Gda, ldh-A, sMdh, Me-2, Pep-D2,* and *Pgd* fixed for same allele in all taxa. Sample sizes (*n*): Jackass, 45; Magellanic, 6; Humboldt, 9; Rockhopper, 10; Macaroni, 10.

[b] Frequencies for anodally migrating common (100) alleles obtained by subtraction.

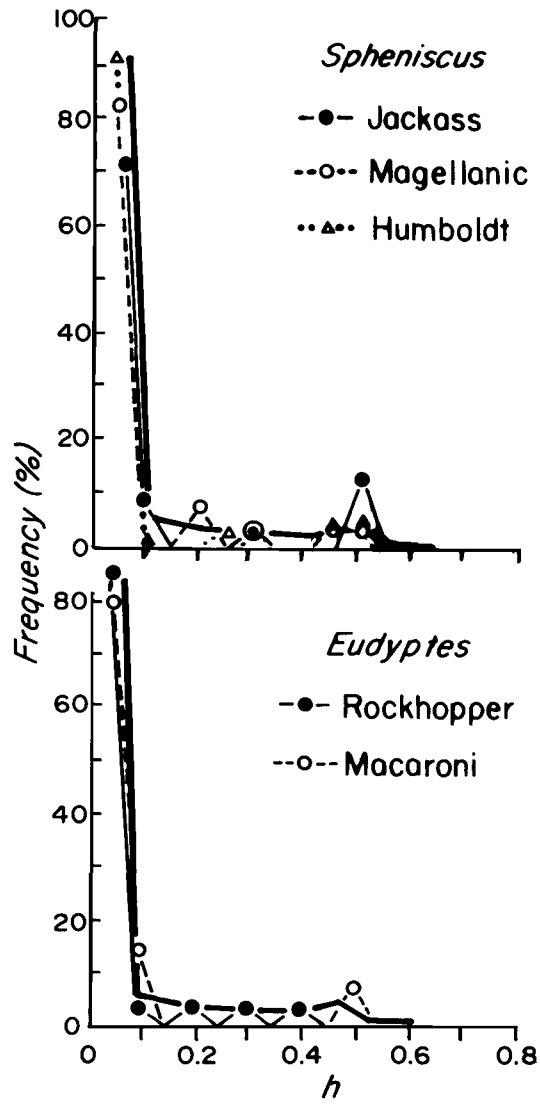

Fig. 1. Distributions of single-locus heterozygosities in *Spheniscus* and *Eudyptes* penguins.

heterozygosities for each of the penguin taxa (Fig. 1) showed a strong L-shaped distribution and appeared to fit the predictions of the infinite-alleles model for populations in equilibrium with mutation and drift (Fuerst et al. 1977).

To test for differences in heterozygosity (H) between species, we first used a t-test on arcsine-transformed single-locus heterozygosities, and this suggested that none of the differences in H was significant. However, since the power of these tests (probability of detecting a true difference) was about 30% or less (Archie 1985:fig. 6), we then used the jackknifed procedure (Weir 1990) to produce an estimate of H^* and its variance. These comparisons showed that H^* for the Jackass Penguin was significantly greater ($P <$ 0.05) than the values for each of the other taxa. The H^* for the Magellanic Penguin was significantly greater ($P < 0.05$) than those for the Humboldt and Rockhopper penguins, and the H^* for the Macaroni Penguin was significantly greater ($P < 0.05$) than that of the Humboldt Penguin.

The smaller heterozygosities for the Humboldt and Magellanic penguins may be because we took these samples from a captive colony, and inbreeding may have led to the loss of heterozygosity. The value of H of 0.081 for Jackass Penguin is larger than the average for other birds (H = 0.044; Evans 1987). The natural colonies of Jackass Penguins have historically been very large. For instance, a single colony on Das-

TABLE 3. Nei's modified genetic distance (Nei 1978, Hillis 1984) below diagonal and standard error above diagonal penguin-species pairs.

	Jack-ass	Magel-lanic	Hum-boldt	Rock-hopper	Maca-roni
Jackass		0.008	0.050	0.120	0.128
Magellanic	0.017		0.052	0.119	0.126
Humboldt	0.107	0.080		0.117	0.123
Rockhopper	0.389	0.370	0.357		0.050
Macaroni	0.436	0.411	0.403	0.073	

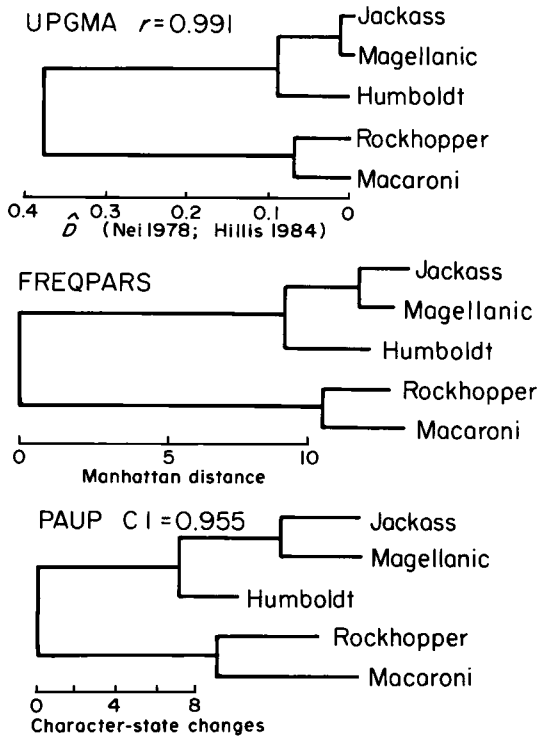

Fig. 2. Phenetic and cladistic trees for *Spheniscus* and *Eudyptes* penguins (*r*, cophenetic correlation; *CI*, consistency index.

sen Island, South Africa once numbered over 1.5 million birds (Shelton et al. 1984). Both the Humboldt and Magellanic penguins also occur in large colonies (Stonehouse 1975). On the other hand, the smaller average heterozygosities for the *Eudyptes* penguins may indicate smaller populations or historical bottlenecks (Nei et al. 1975).

We inferred phylogenies from allozyme frequencies in three ways. We calculated Nei's (1978) unbiased genetic distance (Table 3) with Hillis' (1984) modified $\hat{D}$ between pairs of taxa and used the UPGMA cluster analysis to produce a phenetic tree. Since a phenetic tree may not be the best estimate of the true phylogeny, we used two parsimony methods to produce cladistic trees: FREQPARS (Swofford and Berlocher 1987) to infer a parsimony tree directly from allelic frequencies; and PAUP (Swofford 1985) with Wagnerian parsimony and an exhaustive search to infer a tree from unordered allelic states (independent allele model). Since only 4 of the 30 loci could be ordered by allelic gains and losses with two *Eudyptes* species as outgroups, we could not produce a tree from ordered states. All of the trees had the same topology (Fig. 2). Jackass and Magellanic penguins were most closely related with a genetic distance $\hat{D}$ of 0.017 ± SE of 0.008 (Table 2). The average distance between these taxa and the Humboldt Penguin was 0.094. The average $\hat{D}$ between species of *Spheniscus* and *Eudyptes* was 0.394, and between the Rockhopper and Macaroni penguins was 0.073 ± 0.050.

Our trees agree with the phylogeny suggested by Murphy (1936) to the extent that the Magellanic and Jackass penguins are closely related. Inferences about the Galapagos Penguin are not possible with these data; there are several positions the Galapogos Penguin may fit into the tree. The small amount of genetic divergence between the Jackass and Magellanic penguins was unexpected because these species are located on different continents. A distance of 0.017 between these taxa is more typical of that found between conspecific populations than of a distance between congeneric species (Thorpe 1982). One explanation is that there is long-distance migration and gene flow between the two species. Magellanic Penguins have traveled as far east as South Georgia in the mid-Atlantic Ocean (Tickell 1965), and penguins with two neck bands (Magellanic Penguins?) have been reported in southern Africa (e.g., Donnelly 1965, Rowlands 1965). The second band in these latter penguins, however, is always much narrower than that of the Magellanic Penguin, and many Jackass Penguins show a partial second band that may be an atavism rather than evidence of hybridization. An alternative explanation is that these two species have only recently diverged from each other.

The relatively small genetic distances between pairs of the three *Spheniscus* penguins indicate that speciation events may have been associated with dispersal and founder events in the Pliocene or Pleistocene rather than with continental rifting between South America and Africa in the Cenozoic. Although several fossils have been described from southern South America and Africa, none is clearly ancestral to modern species (Fordyce and Jones 1990). If we assume a molecular-clock calibration of a distance (*D*) of 1.0 equaling five million years (Nei 1975), the two major *Spheniscus* clades diverged about 500,000 mya in the mid-Pleistocene, and the Jackass and Magellanic penguins appeared in the late Pleistocene.

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Literature Cited

Archie, J. W. 1985. Statistical analysis of heterozygosity data: Independent sample comparisons. Evolution 39:623–637.

Donnelly, B. G. 1965. Megellanic [sic] penguin *Spheniscus magellanicus* (Forster) on Lambert Island, Lambert's Bay. Ostrich 36:44–45.

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

Fordyce, R. E., and C. M. Jones. 1990. Penguin history and new fossil material from New Zealand. Pages 419–446 *in* Penguin biology (L. S. Davis and J. T. Darby, Eds.). Academic Press, San Diego.

Fuerst, P. A., R. Chakraborty, and M. Nei. 1977. Statistical studies on protein polymorphism in natural populations. I. Distributions of single locus heterozygosity. Genetics 86:455–483.

Harris, H., and D. A. Hopkinson. 1976. Handbook of enzyme electrophoresis in human genetics. American Elsevier, New York.

Hillis, D. M. 1984. Misuse and modification of Nei's genetic distance. Syst. Zool. 33:138–240.

Levene, H. 1949. On a matching problem arising in genetics. Ann. Math. Stat. 20:91–94.

Livezey, B. C. 1989. Morphometric patterns of Recent and fossil penguins (Aves, Sphenisciformes). J. Zool. (Lond.) 219:269–307.

Markert, C. L., and I. Faulhaber. 1965. Lactate dehydrogenase isozymes patterns of fish. J. Exp. Zool. 159:319–332.

Mindell, D. P., and J. W. Sites, Jr. 1987. Tissue expression patterns of avian isozymes: A preliminary study of phylogenetic applications. Syst. Zool. 36:137–152.

Murphy, R. C. 1936. Oceanic birds of South America, vol. 1. Macmillan, London.

Nei, M. 1975. Molecular population genetics and evolution. American Elsevier, New York.

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

Nei, M., and A. K. Roychoudhury. 1974. Sampling variances of heterozygosity and genetic distance. Genetics 76:379–390.

Nei, M., T. Maruyama, and R. Chakraborty. 1975. The bottleneck effect and genetic variability in populations. Evolution 29:1–10.

Ridgway, G. J., S. W. Sherburne, and R. D. Lewis. 1970. Polymorphism in the esterase of Atlantic herring. Trans. Am. Fish. Soc. 99:147–151.

Rowlands, B. 1965. Abberations in the neck-banding of the Jackass Penguin, *Spheniscus demersus*. Ostrich 56:225–226.

Shelton, P. A., R. J. M. Crawford, J. Cooper, and R. M. Brooke. 1984. Distribution, population size and conservation of the Jackass Penguin, *Spheniscus demersus*. S. Afr. J. Mar. Sci. 2:217–257.

Stonehouse, B. 1975. Introduction: The Spheniscidae. Pages 1–15 *in* The biology of penguins (B. Stonehouse, Ed.). MacMillan, London.

Swofford, D. L. 1985. PAUP: Phylogenetic analysis using parsimony. Illinois Natural History Survey, Champaign.

Swofford, D. L., and S. H. Berlocher. 1987. Inferring evolutionary trees from gene frequency data under the principle of maximum parsimony. Syst. Zool. 36:293–325.

Thorpe, J. 1982. The molecular clock hypothesis: Biochemical evolution, genetic differentiation and systematics. Annu. Rev. Ecol. Syst. 13:139–168.

Tickell, W. L. N. 1965. New records for South Georgia. Ibis 107:389–399.

Weir, B. S. 1990. Genetic data analysis. Sinauer Associates, Sunderland, Massachusetts.

Whitt, G. S. 1970. Developmental genetics of the lactate dehydrogenase isozymes of fish. J. Exp. Zool. 175:1–35.

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