SPECIATION, HETEROCHRONY, AND GENETIC VARIATION IN HISPANIOLAN PALM-TANAGERS

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ABSTRACT.—We documented levels of genetic variability for two species of Hispaniolan palm-tanagers. Significant differences between age classes in levels of genetic heterozygosity were concordant with an age dimorphism in foraging behavior and morphology in *Phaenicophilus palmarum*; juveniles ($H = 0.121$) were almost twice as heterozygous as adults ($H = 0.074$). *Phaenicophilus poliocephalus* ($H = 0.104$) was not characterized by a distinct age dimorphism in any character examined. Although *P. poliocephalus* resembled juvenile *P. palmarum* in morphology and behavior, it was not significantly different from either adult or juvenile *P. palmarum* in levels of genetic variability. Both species of *Phaenicophilus* possess levels of genetic variability (9–10%) that are high for birds, and they differ in allele frequencies and presence of private alleles, although they are not characterized by fixed allelic differences. *Phaenicophilus poliocephalus* was probably derived from small founding populations (ca. 50,000–260,000 yr BP), composed mostly of juvenile *P. palmarum* that colonized the south island of Hispaniola formed during the Pleistocene. Rapid divergence between species is consistent with predictions from models of heterochrony by paedomorphosis and speciation by a founder event. *Received 25 August 1989, accepted 28 April 1990.*

HETEROCHRONY (i.e. shifts in developmental patterns) provides a framework for understanding how population processes lead to evolutionary change (Gould 1977). Alterations in body size and shape, fecundity, age-structure, and significant changes in social structure within populations can result by changing individual growth rates or age of sexual maturity (Geist 1971, Gould 1977, Lawton and Lawton 1986). Life history characteristics, such as fecundity and growth rates, are correlated to levels of genetic variability (Cothran et al. 1983, Mitton and Grant 1984), although there is no unifying theory to predict the genetic consequences of heterochrony. Differential selection on various life history stages due to heterochrony can lead to genetic changes within and between species, and it can provide a basis from which to develop a general model predicting the genetic consequences of heterochrony. Furthermore, avian groups are known to be morphologically diverse (Wyles et al. 1983) but genetically conservative (Avise and Aquadro 1982) when compared with other vertebrates. A model of heterochrony may explain this paradox if speciation occurs primarily by regulatory gene changes, as proposed by Gould (1977), although he did not believe heterochrony to be significant in avian evolution.

Heterochrony can be achieved in two ways: *peramorphosis* (i.e. terminal deletion) and *paedomorphosis* (i.e. terminal addition) (Kluge and Strauss 1985). Whereas peramorphosis results in the acquisition of novel characters, paedomorphosis results in the retention of juvenile characters in reproductively capable individuals (Gould 1977, Lawton and Lawton 1986) and it may be achieved by small changes in regulatory genes early in development (Larson 1980). Paedomorphosis may occur by delaying somatic maturation (i.e. *neoteny*) or accelerating sexual maturation (i.e. *progenesis*). Gould (1977) characterized neoteny as a response to limiting resources (i.e. *K*-selected strategy) and progenesis as a response to plentiful resources (i.e. *r*-selected). Only recently has neoteny been equated with delayed maturation in birds (Lawton and Lawton 1986, Foster 1987), although delayed maturation has already been documented for numerous bird species (Selander 1965, Rohwer et al. 1980, Flood 1984, Hamerstrom 1986). Avian groups that are paedomorphic may rep-

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resent ideal systems from which to develop a coordinated theory relating ecological constraints, life history traits, and genetic variability to rapid speciation.

The Neotropical tanagers are good candidates for the study of heterochrony because of their adaptive radiation into a number of ecological niches in the tropics. Moreover, in at least 39 species, first-year birds can be distinguished from adults by plumage. Because molt often does not occur until after the first potential breeding season, many tanagers can breed in juvenile (or subadult) plumage (Isler and Isler 1987). We chose to examine the evolution and speciation of Hispaniolan palm-tanagers using the predictions from the model of heterochrony, because the similarities between the two species suggest one is paedomorphic to the other and may have undergone rapid speciation on a small island (McDonald 1988).

Gray-crowned Palm-Tanagers (*Phaenicophilus poliocephalus*) resemble juvenile Black-crowned Palm-Tanagers (*P. palmarum*) in foraging behavior and morphology. They are also smaller in body size, are more social relative to Black-crowned Palm-Tanagers, and are most likely derived from them (McDonald and Smith in press). We propose that age-related genetic differences observed for *P. palmarum* may be related to the other observed phenotypic differences in this species, and may have relevance to the evolution of *P. poliocephalus*.

Our purpose is to describe the patterns of genetic variation within and between two palm-tanager species and to relate these patterns to their possible mode of speciation. Our specific objectives are to describe age-specific genetic variation within these species and relate it to differences in behavior and morphology, and to quantify the degree of genetic differentiation between these species and estimate divergence time. We discuss speciation of these species as it relates to isolation, founder effect, and genetic variability, and test whether the observed genetic variation is consistent with a paedomorphic derivation of one species from the other.

METHODS

Collections were made of two species of *Phaenicophilus* and their hybrids in Haiti from May through September, 1985. Hybrid specimens and representatives of both parental species are deposited in the American Museum of Natural History. The remaining specimens are deposited in the Florida Museum of Natural History. Tissue samples are deposited in the Louisiana State University Museum of Zoology. Field identifications were based on crown and chin characters (McDonald and Smith in press). Juveniles of both species could be identified in hand by the presence of a yellow wash in the plumage. This plumage character was correlated with a gray—instead of red-brown—iris observed for adults, gray crown, small gonads, and rictal flanges in six of eight specimens.

Because dry ice or liquid nitrogen was not available in Haiti, tissues were stored in 1.5% buffered 2-phenoxyethanol solution 0.5–4.0 h after collection, and vials were stored in a conventional freezer at −20°C within a week after collection until September 1985. Tissues were stored at −60°C thereafter. Little or no degradation, as indicated by mobility changes and subbanding, occurred in most enzymes after 2 weeks storage of tissues in the preservative at room temperature and after 4 months storage in a conventional freezer (McDonald MS).

Liver and muscle extracts were ground in the phenoxyethanol solution. Samples were centrifuged for 5 s to clear the supernatant of particulate matter. Tissue extracts were analyzed using horizontal starch gel electrophoresis, according to the modified methods of Selander et al. (1971) and Harris and Hopkinson (1976).

We assayed for 39 presumptive loci. Locus designations, abbreviations, and buffer conditions are given in Table 1, except where listed below. Loci were numbered according to the mobility of the products (with the most anodal as 1) when two or more isozymes appeared on the same gel. Monomorphic loci included AAT-2 (analyzed using Amine Citrate 6.2 and Tris Maleate 7.4 buffers), CK-1, FH-2, αGPD-1, lactate dehydrogenase (LDH)-1 and -2, malate dehydrogenase (MDH)-1 and -2, malic enzyme (ME), and SOD-2 (all on Amine Citrate 6.2 buffer), and general proteins (GP)-1 and -2 (Lithium Hydroxide 8.2 buffer). Alleles were designated alphabetically, with *A* corresponding to the allele with the fastest migrating product. No locus had more than three alleles. All loci were scored independently from the fresh gels by one or both authors, and then rescored from the pictures by both authors. Photographs are archived with the data at the Savannah River Ecology Laboratory.

General statistical tests were conducted with the Statistical Analysis System (SAS 1985) or Statistical Package for the Social Sciences (SPSS, Norusis 1985). Significance was set at $P \leq 0.05$; highly significant rejection of null hypotheses occurred when $P \leq 0.01$. Acceptance levels for multiple comparisons involving the same data set were adjusted to an experimentwide error of $P_1 \leq 0.05$ (Harris 1975). Tests are reported as two-tailed except where noted.

Allele frequencies and genetic variability were

measured by the proportion of heterozygous loci determined by direct count per individual averaged across 39 loci (H), the average number of alleles per locus, and the percent loci polymorphic with the criterion of a secondary allele frequency of no more than 0.01 (Table 1). The average number of loci observed per individual varied because some of the samples were taken in the absence of ice or cool temperatures. Genetic data were analyzed with BIOSYS-1 (Swofford and Selander 1981). Using a t-test, we performed statistical analyses of individual heterozygosities that were transformed by taking the square-root of the arcsine of the value (Archie 1985). Slatkin's (1985) rare allele model was used to estimate gene flow across palm-tanagers. Hybrids were excluded from this latter analysis. Standard error for the gene flow estimate was evaluated by jackknifing (Lanyon 1987) each of the 31 unique alleles found in one or the other species.

To estimate the relative size of the founding populations required for colonization and maintenance of current heterozygosity levels in *P. poliocephalus*, we calculated expected heterozygosity (H_e) for the colonists as follows (Baker and Moeed 1987):

$$H_e = (1 - 1/2N_o)H_o,$$

where N_o is the size of the founding population and H_o is heterozygosity of the founders. Founding populations derived from ancestral *P. palmarum* were assumed for the calculation to consist of one of three groups: all adults, all juveniles, or a mixture of adults and juveniles collected at random.

Results

Genetic distance (Nei 1978) between *P. palmarum* and *P. poliocephalus* was $D = 0.01$. No significant differences in genetic heterozygosity existed between the two parental species ($t = -0.81$, df = 40), between *P. palmarum* and hybrids ($t = -0.17$, df = 34), or between *P. poliocephalus* and hybrids ($t = 0.54$, df = 32) (Table 1). No fixed allelic differences were found between the species although there were highly significant shifts in five loci between the two species (i.e. AAT-1, DIA-2, FH-1, IDH, and PGD-1).

We found *"private" alleles*, defined as those observed in only one group (Slatkin 1985), at 21 of the 27 polymorphic loci. *Phaenicophilus poliocephalus* had 15 unique alleles in 63 alleles, and *P. palmarum* had 16 in 64. Sample size for each species was sufficient to detect 56.2% and 74.1%, respectively, of the private alleles in at least one individual at the observed frequencies in the other species. The estimated number of migrants per generation was 0.963 (SE 0.011) based on the rare allele method. There were 9 private alleles in juvenile ($n = 8$) and 6 private alleles in adult ($n = 14$) *P. palmarum* at 11 of the 39 loci. Private alleles may be expected to occur in the larger adult sample, but not in the smaller juvenile sample.

Differences in heterozygosity were significant between juvenile ($H = 0.121$) and adult *P. palmarum* ($H = 0.074$) ($t = -3.04$, df = 19, $P_1 \leq 0.007$). We found no significant differences in heterozygosity between age classes within *P. poliocephalus* ($H = 0.104$) ($t = -1.73$, df = 18, $P_1 = 0.10$) or within the hybrids ($t = -1.40$, df = 12, $P_1 = 0.18$), although adult *P. poliocephalus* ($H = 0.115$) were more heterozygous than juveniles ($H = 0.069$). Only five juvenile *P. poliocephalus* and three juvenile hybrids were available for this test. Juvenile or adult *P. palmarum* did not differ significantly from either *P. poliocephalus* or the hybrids in genetic heterozygosity.

Sampling biases due to taking juveniles from the same nest are not likely to account for the observed heterozygosity difference between age classes because 5 of the 8 juvenile *P. palmarum* were collected with another adult in the same habitat, and these habitats were distributed across Haiti. Habitats included cloud forest, disturbed pines, woodland, farms, rural areas, and deserts. One juvenile was collected alone. Two juveniles were collected in the same habitat and could be potential nest mates. Of the 5 juvenile-adult pairs, 4 juveniles had higher levels of heterozygosity than the adults. Of the 2 juveniles collected in the same habitat, 1 ranked highest for individual heterozygosity ($H = 0.143$); the other ranked second to the lowest for juveniles ($H = 0.094$).

Of the 17 variable loci in *P. palmarum* (αGPD-2 was omitted because of low sample sizes), only 4 did not have higher levels of heterozygosity in juveniles than in adults. Data from the 4 loci (GDH, PGM-1, PepA1, and XDH) that did not follow this trend were excluded from the jackknife procedure because removal of these data would result in higher juvenile heterozygosities and bias the following tests. We jackknifed (Lanyon 1987) the remaining data to evaluate single-locus effects on heterozygosity differences between age classes. The data for one locus at a time were removed for the 13 variable loci, and heterozygosities were computed for each age class with the data for the remaining 38 loci. Average heterozygosity for each age

TABLE 1. Allele frequencies, direct count heterozygosity (H), percent polymorphic loci ($P_{0.01}$) (common allele ≥0.99), and mean number of alleles ($\bar{A}$) across 27 variables of 39 enzyme loci. BPA = Adult *Phaenicophilus palmarum*; BPI = juvenile *P. palmarum*; GPT = *P. poliocephalus*; HYB = hybrids; sample sizes are in parentheses in Sample columns.

Name (EC No.)[a]	Buffer pH[b]	Locus/ Alleles	Sample[c] BPA	BPI	GPT	HYB
Aspartate aminotransferase (2.6.1.1)	AC 6.2	AAT-1	(14)	(8)	(20)	(14)
	TM 7.4	A	0.143	0.188	0.000	0.036
		B	0.857	0.813	0.850	0.929
		C	0.000	0.000	0.150	0.036
Aconitate hydratase (4.2.1.3)	AC 6.2	ACON-1	(13)	(8)	(17)	(13)
		A	0.000	0.000	0.029	0.000
		B	1.000	1.000	0.971	1.000
		ACON-2	(12)	(6)	(16)	(12)
		A	0.000	0.083	0.063	0.042
		B	1.000	0.917	0.938	0.958
Catalase (1.11.1.6)	TC 8.0	CAT	(14)	(8)	(20)	(14)
		A	0.000	0.000	0.100	0.214
		B	0.857	0.875	0.750	0.750
		C	0.143	0.125	0.150	0.036
Creatine kinase (2.7.3.2)	AC 6.2	CK-2	(14)	(8)	(16)	(13)
		A	0.000	0.000	0.063	0.038
		B	1.000	1.000	0.938	0.962
Diaphorase (1.6.2.2)	TC 8.0	DIA-2	(12)	(7)	(12)	(12)
		A	0.167	0.143	0.000	0.000
		B	0.792	0.643	1.000	1.000
		C	0.042	0.214	0.000	0.000
(1.6.4.3)		DIA-3	(14)	(8)	(19)	(13)
		A	0.036	0.000	0.000	0.000
		B	0.929	0.875	0.921	0.923
		C	0.036	0.125	0.079	0.077
Fructose diphosphate aldolase (4.1.2.13)	AC 6.2	FDA-1	(8)	(6)	(15)	(8)
		A	0.000	0.000	0.000	0.063
		B	1.000	1.000	1.000	0.938
Fumarate hydratase (4.2.1.2)	AC 6.2	FH-1	(13)	(8)	(16)	(13)
		A	0.885	0.750	1.000	0.962
		B	0.115	0.250	0.000	0.038
Glucose dehydrogenase (1.1.1.47)	TM 7.4	GDH	(12)	(6)	(17)	(12)
		A	0.042	0.000	0.000	0.042
		B	0.958	1.000	0.912	0.917
		C	0.000	0.000	0.088	0.042
Alpha glycerophosphate dehydrogenase (1.1.1.8)	AC 6.2	αGPD2	(1)	(3)	(20)	(9)
		A	0.000	0.167	0.000	0.000
		B	1.000	0.333	1.000	0.833
		C	0.000	0.500	0.000	0.167
Glucose phosphate isomerase (5.3.1.9)	AC 6.2	GPI	(14)	(8)	(20)	(14)
		A	0.214	0.188	0.200	0.107
		B	0.000	0.000	0.000	0.036
		C	0.786	0.813	0.800	0.857
Beta-glucuronidase (3.2.1.31)	TC 8.0	βGUS	(14)	(8)	(20)	(13)
		A	0.000	0.188	0.050	0.192
		B	1.000	0.750	0.925	0.808
		C	0.000	0.063	0.025	0.000
Hexokinase (2.7.1.1)	AC 6.2	HK	(9)	(6)	(17)	(12)
		A	0.000	0.000	0.000	0.125
		B	1.000	1.000	1.000	0.833
		C	0.000	0.000	0.000	0.042
Isocitrate dehydrogenase (1.1.1.42)	JRP 7.1	ICD-1	(14)	(8)	(17)	(14)
	TC 8.0	A	0.000	0.000	0.118	0.000
		B	1.000	1.000	0.853	1.000
		C	0.000	0.000	0.029	0.000

TABLE 1. Continued.

Name (EC No.)[a]	Buffer pH[b]	Locus/ Alleles	Sample[c] BPA	BPI	GPT	HYB
Mannose phosphate isomerase (5.3.1.8)	TC 8.0	MPI	(9)	(5)	(14)	(10)
	EDTA 8.6	A	0.000	0.100	0.000	0.000
		B	1.000	0.800	1.000	1.000
		C	0.000	0.100	0.000	0.000
Purine nucleoside phosphorylase (2.4.2.1)	EDTA 8.6	NP	(14)	(7)	(19)	(12)
		A	0.143	0.214	0.316	0.083
		B	0.107	0.214	0.158	0.833
		C	0.750	0.571	0.526	0.083
Phosphoglucomutase (2.7.5.1)	AC 6.2	PGM-1	(12)	(7)	(17)	(14)
		A	0.042	0.000	0.000	0.036
		B	0.917	1.000	1.000	0.821
		C	0.042	0.000	0.000	0.143
		PGM-2	(5)	(6)	(14)	(9)
		A	1.000	1.000	0.929	1.000
		B	0.000	0.000	0.071	0.000
		PGM-3	(4)	(3)	(13)	(13)
		A	0.000	0.000	0.038	0.000
		B	1.000	1.000	0.962	0.962
		C	0.000	0.000	0.000	0.038
Peptidase[d] (3.4.11)	TM 7.4	PEP-A1	(9)	(6)	(15)	(14)
		A	0.000	0.000	0.000	0.036
		B	1.000	1.000	0.967	0.893
		C	0.000	0.000	0.033	0.071
		PEP-G	(13)	(8)	(16)	(14)
		A	0.038	0.063	0.000	0.143
		B	0.962	0.938	1.000	0.857
		PEP-A2	(14)	(7)	(18)	(14)
		A	0.000	0.071	0.000	0.036
		B	1.000	0.929	0.889	0.964
		C	0.000	0.000	0.111	0.000
		PEP-L	(14)	(7)	(16)	(14)
		A	0.071	0.000	0.000	0.000
		B	0.929	1.000	0.906	1.000
		C	0.000	0.000	0.094	0.000
Phosphogluconate dehydrogenase (1.1.1.44)	TM 7.4	6PGD-1	(14)	(8)	(16)	(12)
		A	0.000	0.000	0.219	0.000
		B	1.000	1.000	0.781	1.000
Superoxide dismutase (1.15.1.1)	AC 6.2	SOD-1	(7)	(6)	(18)	(7)
		A	0.000	0.000	0.167	0.429
		B	1.000	0.917	0.833	0.571
		C	0.000	0.083	0.000	0.000
Xanthine oxidase (1.2.3.2)	TC 8.0	XDH	(10)	(7)	(19)	(14)
		A	0.100	0.000	0.079	0.000
		B	0.900	1.000	0.895	0.964
		C	0.000	0.000	0.026	0.036
Direct count heterozygosity[e]			0.074	0.121	0.104	0.095
SE			0.011	0.009	0.012	0.011
Mean number of alleles			1.4	1.5	1.6	1.7
% polymorphic loci			30.8	35.9	48.7	51.3

[a] Enzyme names and numbers recommended by the Commission on Biological Nomenclature (1973).

[b] Abbreviations for buffers are: AC = Amine Citrate (Clayton and Tretiak 1972); JRP = Tris Citrate 7.0 (Ayala et al. 1972); TM = Tris Maleate; TC = Tris Citrate 8.0; EDTA = Ethylenediamine Tetraacetic Acid (Selander et al. 1971, Harris and Hopkinson 1976). When more than one buffer condition is specified, both were routinely used to assay for multiple loci.

[c] Sample sizes of adults and juveniles for *P. poliocephalus* were 15 and 5, respectively, and for hybrids were 9 and 3.

[d] Peptidase substrates used were PEP-A1 and PEP-A2 = L-leucyl-L-alanine; PEP-G = DL-leucylglycylglycine; PEP-L = L-leucyl-L-leucine.

[e] Genetic variability pooled for adult and juvenile Black-crowned Palm-Tanagers: $H = 0.091$; SE = 0.009; $\bar{A} = 1.6$; $P_{0.01} = 46.2$.

TABLE 2. Mean % heterozygosity for adult (AD) and juvenile (JUV) *Phaenicophilus palmarum* after jackknife simulation (Lanyon 1987) for 13 variable loci. A *t*-test was performed to test the null hypothesis that the average difference across all 13 loci between age classes is not significant. Differences were highly significant ($t = 24.3$, df = 24, $P < 0.001$).

Locus[c] removed	% heterozygosity[a,b] after removal		Locus % heterozygosity before removal		Sample size	
	AD	JUV	AD	JUV	AD	JUV
ACON-2	7.6	12.0	0.0	16.7	12	6
CAT	7.3	11.6	14.3	25.0	14	8
DIA-2	7.0	11.4	25.0	42.9	12	7
DIA-3	7.2	11.7	14.3	25.0	14	8
FH-1	7.0	10.9	23.1	50.0	13	8
AAT-2	6.6	11.3	28.6	37.5	14	8
GPI	6.9	11.3	28.6	37.5	14	8
βGUS	7.7	10.9	0.0	50.0	14	8
MPI	7.6	11.9	0.0	20.0	9	5
NP	6.4	11.0	35.7	57.1	14	7
PEP-C	7.7	12.0	0.0	14.3	14	7
PEP-B	7.4	12.1	7.7	12.5	13	8
SOD-1	7.7	12.1	0.0	16.7	7	6

[a] Standard errors ranged from 0.010 to 0.013 for adults and 0.007 to 0.013 for juveniles.

[b] Probabilities, after removal, ranged from 0.004 to 0.037 for single locus *t*-tests.

[c] Abbreviations for loci in Table 1.

class was then computed across 13 combinations of 38 loci. Heterozygosity differences between the averages for each age class were tested under the hypothesis that the differences were not due to significant single-locus effects. Levels of heterozygosity were still highly significantly different between age classes ($t = -24.33$, df = 24). Therefore, differences in heterozygosity between age classes could not be attributed to the effects of any single variable locus in the study. Moreover, the direction of the difference between age classes remained unchanged. Juvenile *P. palmarum* were always more heterozygous then adults were, regardless of which data were removed (Table 2).

The manner in which the paedomorphic form (i.e. *P. poliocephalus*) was derived could be important in determining its level of genetic variability. Equal or reduced heterozygosity in the derived form relative to that in the antecedent would be consistent with a vicariance model, but equal levels in both forms would not normally be expected given limited dispersal to a relatively isolated area. The required number

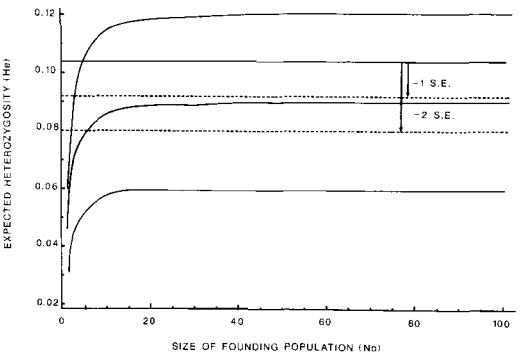

Fig. 1. Expected heterozygosity (H_e) of colonists based on founding population size (N_o) and observed heterozygosity (H_o) in the founders. The lower curve was generated based on the assumption that H_o was contributed by colonists consisting only of adult *Phaenicophilus palmarum*; the middle curve assumed a mixture of adults and juveniles; the upper curve assumed colonists were all juveniles. The solid horizontal straight line is the current heterozygosity observed in Gray-crowned Palm-Tanagers, *P. poliocephalus*. The dotted straight lines are one and two standard errors below this, respectively.

of dispersers to maintain certain levels of heterozygosity may be so high as to make the dispersal model unreasonable. Therefore, we used a modification of Crow and Kimura's (1970) equation to estimate the size of founding populations for given levels of heterozygosity (Baker and Moeed 1987). Three curves were generated and compared with the current levels of heterozygosity in *P. poliocephalus* ($H = 0.104$) (Fig. 1). The curve based on a founding population of all juvenile *P. palmarum* had an expected heterozygosity (H_e) = 0.106 at a founding population size (N_o) = 4, where the curve based on a mixed group of adult and juvenile founders had $H_e = 0.090$ at $N_o = 40$. This latter value is more than one standard error below the current level of heterozygosity of *P. poliocephalus*. The curve based on a founding population of all adults reached an asymptote at $H_e = 0.061$ and $N_o = 10$, more than two standard errors below the current heterozygosity level of *P. poliocephalus*.

DISCUSSION

Paedomorphosis in palm-tanagers.—Before we can discuss the evolutionary patterns observed in *Phaenicophilus* under a model of hetero-

chrony, the polarity of evolutionary relationships (i.e. which species is derived from which) must be established. The problem is to detect whether a terminal addition (peramorphosis) or terminal deletion (paedomorphosis) has been made. This may be resolved by the use of outgroup analysis (Kluge and Strauss 1985). Outgroup analysis involves the choice of a taxon (i.e. sister group) that is closely related to the taxa being compared to determine whether a character is common or unique to one or more of the taxa examined. If a character is shared with the sister group by one but not the other taxa, the first taxon is most likely antecedent to the second.

Three criteria were significant to demonstrate the derivation of *P. poliocephalus* from *P. palmarum*. First, neoteny occurs in *P. palmarum* but not in *P. poliocephalus*. Neoteny is observed in *Piranga*, the most likely sister group to *Phaenicophilus*, based on DNA-DNA hybridization data (Sibley in litt.) and on biochemical evidence (McDonald 1988). Second, *Phaenicophilus poliocephalus* has five alleles not shared with *Piranga*, whereas *P. palmarum* has only three. Third, the presence of a distinct white chin against a gray throat is unique to *P. poliocephalus*. In addition, *P. poliocephalus* is smaller, resembles juvenile *P. palmarum* in foraging behavior, and is relatively more social than is *P. palmarum* (McDonald and Smith in press), a tendency predicted by the retention of juvenile morphology (Lawton and Lawton 1986). The absence of neoteny, the presence of a unique plumage character, the number of unshared alleles with the sister group, and the similarity in behavior and morphology of *P. poliocephalus* to juvenile *P. palmarum* argue strongly in favor of the derivation of the former from the latter species. Once this polarity is accepted, the absence of a black crown in adult *P. poliocephalus* (i.e. a terminal deletion) supports the hypothesis that it is paedomorphic to *P. palmarum*.

Understanding the importance of ecological constraints to the model of paedomorphosis for palm-tanagers is critical to evaluating their probable mode of speciation. There are several assumptions relative to the model. Early sexual maturation is favored when all other factors are equal. However, limiting resources may impose strong intraspecific competition on individuals that attempt to breed. Under these conditions, the relative increase in fitness accrued by inexperienced individuals that breed at an earlier age would be offset by fitness decreased because of intense intraspecific competition for scarce resources. As a result, inexperienced individuals in a resource-limited environment may delay somatic maturation and benefit from cryptic or deceptive morphology by a decrease in intraspecific competition (Rohwer 1978, Lawton and Lawton 1986, Foster 1987). When resources are not severely limiting, these individuals may breed. For example, subadult Northern Harriers (*Circus cyaneus*) breed only when *Microtus* densities are high, but refrain from breeding at other times (Hamerstrom 1986). Paedomorphosis can be achieved by two different reproductive strategies in response to differential resource availability: neoteny and progenesis (Gould 1977). Neotenic species can be characterized by an age dimorphism in morphology and possibly in behavior.

In contrast, progenesis occurs when resources may be relatively more abundant. Species colonizing new habitat represent potential for progenesis to occur. Under these circumstances, early sexual maturation is no longer constrained, nor is there strong competition between age classes for resources. With attainment of sexual maturity, somatic development slows down or stops (Gould 1977), which results in individuals with smaller body size, juvenile morphology, juvenile behaviors associated with juvenile morphology, and a concomitant increase in group behaviors (Geist 1971, Gould 1977, Lawton and Lawton 1986). If the colonizing population becomes isolated from the main stock, divergence can occur, with genetic changes primarily expected at regulatory loci.

Age-related differences in heterozygosity.—Age-related genetic differences may be an expected consequence of neoteny, as are changes in morphology and behavior, particularly because age-related differences are most likely selected in environments with limited resources. We found age-related differences in *P. palmarum* but not in the derived paedomorphic form, *P. poliocephalus* (Table 1). Age-class differences in single- and multi-locus heterozygosity have also been observed for grouse, lizards, deer, toads, and mosquitofish (Redfield 1973, Tinkle and Selander 1973, Cothran et al. 1983, Samollow and Soule 1983, Smith et al. 1989), although higher genetic variability is not always found for juveniles as in *P. palmarum*. Increased heterozy-

gosity seems to be favored in age classes under intense differential mortality or selection (Samollow and Soule 1983). Juvenile *P. palmarum* ($H = 0.121$) are significantly more heterozygous than adults ($H = 0.074$) of their own species. Selection could account for this difference, but other explanations must be considered also.

Age-related differences in heterozygosity in *P. palmarum* might be spurious because of small sample sizes. Because of the small sample size in this study, the magnitude of the differences in heterozygosity between age classes had to be large to yield a probability of <0.007 for rejection of the null hypothesis. It seems unlikely that stochastic processes that were due to small sample size produced such large differences. It is also possible that one or a few loci are responsible for the age-related differences, but higher levels of heterozygosity were observed for juvenile *P. palmarum* for 13 out of 17 variable loci (Table 2). In addition, removal of the data for any single locus did not alter the overall difference (Table 2). Because juveniles were collected across sites, a sampling bias due to their coming from a limited number of nests is not likely. Age-related difference in heterozygosity seems to be a multilocus phenomenon and not due to small-sample biases. However, this result does not provide a basis for understanding the mechanism(s) that generates the differences.

The differences in genetic variability across life history stages may be due to developmental changes in isozyme patterns, negative assortative mating, or nonrandom dispersal of individuals with different genotypes. These processes are not likely to produce the consistent decrease in heterozygosity for adults observed for 13 loci. The decrease in the number of rare alleles in juveniles compared with adults indicates selection is probably operating (Samollow and Soule 1983). Private alleles observed only in juveniles are probably not completely lost in adults, but may occur at much lower frequencies than in juveniles. Our sample size was not sufficient to detect these alleles. Selection may act on both life history stages—first increasing, then decreasing genetic variability—but this hypothesis remains to be tested.

The importance of age-related differences in genetic variability extends beyond its concordance with behavior and morphology in *P. palmarum* and may be related to the process of rapid divergence in *P. poliocephalus*. Lower genetic variability is not characteristic of adults in all species (Cothran et al. 1983, Smith et al. 1989), even for *Phaenicophilus*. Although significant differences in heterozygosity did not exist between age classes within *P. poliocephalus* ($P = 0.10$), the trends are reversed from *P. palmarum*, with adult *P. poliocephalus* ($H = 0.115$) relatively more heterozygous than juveniles ($H = 0.069$). Furthermore, levels of heterozygosity are similar in adult *P. poliocephalus* ($H = 0.115$) and juvenile *P. palmarum* ($H = 0.121$). The similarity in terms of genetic variability between adult *P. poliocephalus* and juvenile *P. palmarum* may be serendipitous, but the pattern across several character sets (i.e. behavior and morphology; McDonald and Smith in press) suggests there may be a single underlying cause related to how and when *Phaenicophilus* diverged.

The genetic divergence between the two *Phaenicophilus* taxa was low ($D = 0.01$), even for avian species where the average $D = 0.044$ (Barrowclough 1980). However, Johnson and Zink (1983) reported $D = 0.004$ for two closely related sympatric species of sapsuckers (*Sphyrapicus*) that were undergoing character displacement and assortative mating. Genetic distance should not be used a priori as the only criterion for species recognition in birds (Hepp et al. 1988). The low D for *Phaenicophilus* is likely an indication of two recently diverged but distinct species. There were no fixed allelic differences between the two taxa (Table 1), but there were a significant number of private alleles (31) not shared by them and significant frequency shifts in alleles for 5 loci. It takes at least one migrant per generation to maintain allelic equivalence between taxa (Crow and Kimura 1970). The significant difference in frequencies for 5 of 17 variable loci and the large number of private alleles supports our contention that gene flow is severely restricted between the two taxa. These characteristics are consistent with the specific designation of the two recently diverged *Phaenicophilus* taxa.

The estimated time of divergence (Nei 1975, Gutiérrez et al. 1983, Marten and Johnson 1986) is from 5.0×10^4 to 2.6×10^5 yr before present (BP). This range in the estimate corresponds remarkably well to the time of the most recent interglacial period, when sea levels rose 8–10 m some 6.5×10^4 yr BP, and multiple times throughout the Pliocene and Pleistocene (Pregill and Olson 1981). The rise in sea level would have inundated the Cul-de-Sac Plain, which runs from west to east across Hispaniola, and

cleaved it into north and south islands. This plain is presently below sea level; during interglacial periods when glaciers melted and sea levels rose it would have formed an open water barrier to gene flow (Pregill and Olson 1981).

Model of speciation.—We propose that *Phaenicophilus* speciated in allopatry on the two islands. Current distributions of the two species suggest that *P. poliocephalus* arose on or dispersed to the south island. If the divergence were a result of a vicariance event, then *P. poliocephalus* would be expected to be similar to *P. palmarum* in having a distinct age dimorphism in behavior, morphology, and genetics, unless neoteny was derived in *P. palmarum* after divergence occurred. A vicariance model might explain similarities in overall genetic variability between the two species, but is inadequate to explain the pattern of similarity of *P. poliocephalus* to juvenile *P. palmarum* in behavior and morphology. In contrast, a model of heterochrony can explain the lack of an age dimorphism in the derived species, the retention of juvenile behavior and morphology in that species, its smaller body size and increased sociality (McDonald and Smith in press), and the retention of high levels of genetic variability (Fig. 1). The absence of a striking age dimorphism in *P. poliocephalus* could have resulted if it were derived from neotenic *P. palmarum* after colonization to the south island. Low competition and relatively abundant resources on the south island would favor the evolution of progenesis, which is characterized by earlier sexual maturation often at smaller body size than normally expected (Gould 1977). *Phaenicophilus poliocephalus* is smaller than *P. palmarum*, as expected for progenetic species. If the colonizers were few in number, then genetic variability might be expected to have declined because of founder effect (Crow and Kimura 1970). Both species have surprisingly high levels of heterozygosity ($H = 9$–10%) for island species (Nevo et al. 1984, but see Yang and Patton 1981). A decline in heterozygosity could be avoided by rapid population growth after colonization (likely for progenetic species), a large number of founders, and multiple invasions.

The simplest explanation for the current situation is that heterozygosity was not reduced on the south island in ancestral *P. poliocephalus*. Multiple invasions or large populations of colonizers could explain the retention of high levels of heterozygosity, but are inconsistent with subsequent divergence and with Templeton's (1980) model of speciation by founder effect. The size of the founder population should be just small enough to cause a rapid accumulation of inbreeding without a severe reduction in genetic variability (Templeton 1980). These conditions enhance the probability of the reorganization of the genome, primarily for regulatory genes (Templeton 1980). Therefore, fixed allelic differences in palm-tanagers may not be an expected consequence of speciation. Changes in regulatory genes are consistent with a model of heterochrony (Gould 1977). Even small changes in regulatory genes can effect significant phenotypic changes (Larson 1980) and provide for the establishment of isolating mechanisms. Few differences in structural loci and radical shifts in ecological niches are expected in either Templeton's (1980) or Gould's (1977) models.

Large or multiple founding populations that consisted mostly of adult *P. palmarum* would not have achieved the high levels of heterozygosity, regardless of the numbers of colonizers (Fig. 1). A small mixed group (<40) of ancestral *P. palmarum* adult and juvenile colonizers would have retained sufficient levels of heterozygosity currently observed in *P. poliocephalus* (Fig. 1). Juvenile dispersal is common in birds (Greenwood and Harvey 1982). Flocks of neotenic Brown Jays (*Cyanocorax morio*) that colonize recently cleared habitats have lower mean ages than flocks in the main populations (Lawton and Lawton 1985). Geographic isolation, small numbers of founders, and few founding events set the stage for rapid speciation (Templeton 1980), and paedomorphosis provides the basis for explaining the resemblance of *P. poliocephalus* to juvenile *P. palmarum* across several character sets and a mechanism (via progenesis) for rapid population growth after founding. An alternative explanation based on a vicariance model does not explain adequately similar levels of genetic variability in both species, the absence of an age dimorphism in *P. poliocephalus*, few structural gene differences, and the resemblance of adult *P. poliocephalus* to juvenile *P. palmarum*.

We conclude that neoteny in *P. palmarum* is expressed both behaviorally and morphologically, and the age dimorphism in these characters is congruent with observed heterozygosity differences between age classes. Hispaniolan palm-tanagers have recently diverged, most likely during the Pleistocene, when higher sea

levels separated Hispaniola into north and south islands. *Phaenicophilus poliocephalus* is distinct from *P. palmarum* based on the distribution of private alleles. Gene flow between species is low. *Phaenicophilus poliocephalus* resembles juvenile *P. palmarum* in behavior and morphology. Higher juvenile genetic variability in *P. palmarum*, combined with a greater amount of juvenile dispersal and the high genetic variability observed in *P. poliocephalus*, is consistent with the derivation of *P. poliocephalus* from small founding populations that consisted mostly of juvenile *P. palmarum* on the south island of Hispaniola during the Pleistocene. The generality of this model of speciation should be investigated for other groups, particularly the tanagers.

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