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PHYLOGENETIC PATTERNS IN THE GENUS *HELIODOXA* (AVES: TROCHILIDAE): AN ALLOZYMIC PERSPECTIVE

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ABSTRACT.—Patterns of genetic variation at 42 presumptive genetic loci were analyzed in *Heliodoxa* (seven species), *Polyplancta aurescens, Urosticte benjamini,* and *Schistes geoffroyi.* Thirty-three loci were variable, either within or among taxa. Heterozygosity values for two representative taxa, *H. leadbeateri* ($H = 0.017$) and *H. xanthogonys* ($H = 0.015$) were low compared to other birds. Conversely, genetic distance values were high when compared to other birds. D(avg) for species in the genus *Heliodoxa* was 0.240.

Phenetic and phylogenetic analyses of the genetic data resolve several clusters within *Heliodoxa*: (1) the phenotypically similar *H. jacula* and *H. leadbeateri* are genetically similar ($D = 0.025$) and form a sister-group to *H. rubinoides,* (2) the phenotypically similar *H. branickii* and *H. gularis* were sister taxa but were genetically distinct ($D = 0.090$), (3) *H. xanthogonys* was the most genetically distinct member of *Heliodoxa,* and (4) *H. schreibersii* was most closely related to *P. aurescens.* Our data suggest that *Heliodoxa* is paraphyletic, and we recommend that *P. aurescens* be moved to *Heliodoxa. Urosticte benjamini* and *S. geoffroyi* were genetically distinct from all other taxa. If our phylogeny is correct, throat color (pink, blue or both) evolved twice (in parallel) within *Heliodoxa.* *Received 28 Feb. 1988, accepted 15 Feb. 1989.*

The Trochilidae (hummingbirds), with approximately 325 species, is one of the most diverse bird families. Although much research has focused on ecological and behavioral aspects of hummingbird biology, there have been few modern attempts to infer phylogenetic relationships at any taxonomic level (Zusi and Bentz 1982; Zusi 1985; Schuchmann 1987; C. G. Sibley, unpubl. data). The current taxonomic arrangement of trochilid genera (Morony et al. 1975) differs little from that used by Peters (1945).

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Biochemical groupings of hummingbirds of the genus *Heliodoxa* and relatives, all adult males; four upper left birds, left–right, top–bottom: *H. jacula, H. rubinoides, H. leadbeateri, H. imperatrix*; two upper right birds, top–bottom: *H. gularis, H. branickii*; lower left two birds, left–right: *Polyplancta aurescens, H. schreibersii*; lower right bird: *H. xanthogonys.* From a mixed media painting by John P. O'Neill.

Reasons for this arrangement of taxa are unclear because few explicit character analyses have been performed; presumably, taxa have been grouped by overall morphological resemblance. In addition, 60 of 110 (55%) trochilid genera (ca 110) are monotypic, which likely indicates confusion concerning their systematic relationships (Platnick 1976, 1977). These monotypic genera often exhibit distinctive but unique plumage and morphological features, and thus few synapomorphies exist that reveal phylogenetic affinities of these genera. Understanding the phylogenetic relationships within the family must precede evolutionary interpretations of behavioral and ecological attributes (Felsenstein 1985a), and construction of informative classifications (Wiley 1981).

Biochemical systematic methods offer powerful ways to infer phylogenetic relationships, and especially are useful in groups in which morphological analysis is compromised by either plesiomorphy, extreme divergence, or convergence. We constructed a phylogeny using starch-gel electrophoresis of proteins for one group of hummingbirds, namely, the genus *Heliodoxa* (brilliants). *Polyplancta aurescens* (Gould's Jewelfront), a species in a monotypic genus, was included to test its purported close relationship to *Heliodoxa* (Zimmer 1951). Males of these taxa are illustrated in the frontispiece. No modern systematic studies of *Heliodoxa* exist and the intrageneric classification of *Heliodoxa* is likely based on overall (phenetic) morphological resemblance, which may not reflect phylogenetic relationships (Wiley 1981). Although molecular systematic studies are appearing with increasing frequency in ornithology (e.g., Lanyon and Zink 1987), no biochemical systematic studies of trochilids have as yet been published. We compare our estimate of phylogenetic relationships with traditional classifications, which in effect represents a comparison of genic and morphological evolution. Genetic distance values and heterozygosity estimates (for two taxa) are reported, and compared to temperate passerines. A classification following phylogenetic principles is presented.

Distribution and previous taxonomy.—Members of the genus *Heliodoxa* are found primarily in South America, although *H. jacula* occurs mostly in Central America (Meyer de Schauensee 1966). The genus *Heliodoxa* (Meyer de Schauensee 1966, Morony et al. 1975) includes eight species: *leadbeateri* (Violet-fronted Brilliant), *jacula* (Green-crowned Brilliant), *xanthogonys* (Velvet-browed Brilliant), *rubinoides* (Fawn-breasted Brilliant), *schreibersii* (Black-throated Brilliant), *branickii* (Rufous-webbed Brilliant), *gularis* (Pink-throated Brilliant), and *imperatrix* (Empress Brilliant). Previous workers (in Peters [1945] and Zimmer [1951]) have treated these taxa as members of six different genera ("*Heliodoxa*" *leadbeateri, jacula,* and *xanthogonys*; "*Phaiolaima*" *rubinoides*; "*Ionolaima*" *schrei-*

bersii; "*Agapeta*" *gularis*; "*Lampraster*" *branickii*; "*Eugenia*" *imperatrix*). As an example of taxonomic uncertainty, note that Zimmer (1951) suggested that *H. branickii* and *H. gularis* were conspecific, rather than members of separate genera (Peters 1945). Zimmer (1951) merged the eight species listed above into *Heliodoxa* because he believed that morphological characters previously used to delimit genera were only sufficient to delimit species, and he proposed the linear sequence (classification) given above. The brilliants possess a forward extension of feathering covering the nasal operculum, a potential synapomorphy for *Heliodoxa*. However, Zimmer (1951) concluded that "*Polyplancta* and *Clytolaema* possibly belong in the same assemblage." *Polyplancta* and *Clytolaema* have traditionally been placed adjacent to *Heliodoxa*.

METHODS

Starch-gel electrophoresis was used to analyze proteins occurring in extracts of liver, muscle, and heart tissue from 30 specimens representing 10 taxa within the Trochilidae and one from the Apodidae (Appendix I). We lacked tissue of one member of *Heliodoxa* (*imperatrix*). In addition to *Polyplancta aurescens, Urosticte benjamini* was included because it is a putative near-relative of *Heliodoxa* (Peters 1945, Zimmer 1951, Meyer de Schauensee 1966). *Schistes geoffroyi* was included because it is considered a distant relative of *Heliodoxa* (Zusi 1985) and served as an additional outgroup. We lacked samples of *Clytolaema*. Nomenclature follows Meyer de Schauensee (1966). Specimens were collected during several expeditions to various regions of the New World tropics (Appendix I). Samples of tissue were preserved in liquid nitrogen in the field and held at −70°C at the Louisiana State Univ. Museum of Natural Science (LSUMNS), where tissue vouchers remain (see Johnson et al. 1984 for details on collection and preservation methods).

Electrophoretic procedures basically followed Selander et al. (1971), Harris and Hopkinson (1976), and Johnson et al. (1984). Forty-two presumptive genetic loci were scored. For multiple isozymes at a locus, the most anodal one on a gel was scored as a "1" (i.e., *sMDH-1*). Alleles at each locus were coded by reference to their mobility from the origin. Acronyms for loci follow the International Union of Biochemistry Nomenclature Committee (IUBNC 1984). We entered individual genotypes into the computer program BIOSYS-1 (Swofford and Selander 1981), which generated a table of allelic frequencies, Nei's (1978) and Rogers' (1972) genetic distances, a UPGMA phenogram (Sneath and Sokal 1973), and several distance-Wagner trees (Farris 1972, 1981; Swofford 1981). Three monomorphic loci (two general proteins ["AB"] and *mACOH* [Enzyme Commission 4.2.1.3] were removed from the analysis to accommodate current program dimensions (this has very minor effects on estimates of genic variation). Distance-Wagner trees were generated by specifying (in BIOSYS-1) the Multiple Addition Criterion and allowing for 30 partial networks to be used during each successive step. Prager and Wilson's (1976) "F" value was used to determine which partial networks would be saved. Distance-Wagner trees were rooted using the Fork-tailed Palm-Swift (*Reinarda squamata*) (Apodidae) as the outgroup. To evaluate the robustness of the distance-Wagner trees, we used the bootstrap procedure (Felsenstein 1985b) to resample with replacement phylogenetically informative loci 100 times. From each of the 100 bootstrapped replicates of loci, we produced a distance-Wagner tree. A majority-rule consensus tree was then produced from the 100 trees.

Much controversy surrounds the cladistic analysis of alleles (Patton and Avise 1983, Buth

1984, Swofford and Berlocher 1987). One can consider the locus as the character and alleles as (unordered) character states, or, consider each allele as a character and the states as present or absent. Buth (1984) strongly recommends the former approach, because coding alleles as present or absent can lead to ancestral nodes having "no" alleles. The coding of polymorphisms is also unresolved, and there are several alternatives. We coded each locus as a character and alleles at each locus as unordered states. In the case of polymorphism, the most frequent allele was considered the state; this approach, as most, ignores frequency information, which is a definite drawback. These data were analyzed using the computer program HENNIG86 (written by James S. Farris). HENNIG86 was used to find all most parsimonious trees. We present this analysis as a compromise of coding and analysis (see Dittmann et al. 1989 for a similar approach).

The use of genetic distance data to infer phylogenies is a much debated issue (see Farris 1985, 1986; Felsenstein 1986), as is the use of a phenetic vs a cladistic algorithm (Nei 1987). Thus, we present results of both of these methodologies.

RESULTS

Genetic variation.—Of 42 loci scored, 33 (79%) showed at least two allelic variants across all taxa (Table 1). Attempts at scoring and analyzing five other loci were unsuccessful (*AK-1* [E.C. 2.7.4.3], *ALDO* [4.1.2.13] *GLUDH* [1.4.1.–], *mGOT* [2.6.1.1], *mSOD-1* [1.15.1.1]). Nine loci were monomorphic and fixed for the same allele in all taxa: *ACP* (3.1.32), *EST-2* (3.1.1.–), *HK* (2.7.1.1), *LAP* (3.4.–.–), *LDH-1* (1.1.1.27), *mSOD-2* (1.15.1.1), and the three loci listed above. One locus, *ADH* (1.1.1.1), was nearly fixed, except for a single variant allele. At 13 loci, the Trochilidae shared a single allele, but one different from that in the swift.

Because some measures of within-sample genetic variation are especially dependent on sample size, we considered only our largest samples (*H. leadbeateri* [N = 9] and *H. xanthogonys* [N = 5]). For these taxa, observed mean direct-count heterozygosity H(obs) is 0.017 ± 0.013 [SD] and 0.015 ± 0.007; percentage of polymorphic loci (95% criterion) is 10.26 and 7.69; and the average number of alleles per locus is 1.15 and 1.08, respectively.

Genetic distances.—The average Nei's genetic distance among the 10 Trochilidae examined is 0.331 ± 0.138 [SD] (N = 45) (Table 2). Within *Heliodoxa* interspecific genetic distances range from 0.025 (*H. leadbeateri* vs *H. jacula*) to 0.367 (*H. rubinoides* vs *H. branickii*); the average is 0.240 ± 0.088 (N = 21). Within *Heliodoxa* including *Polyplancta* the average genetic distance is 0.241 ± 0.080 (N = 28). Genetic distance values for *Polyplancta* vs *Heliodoxa* range from 0.128 (vs *H. schreibersii*) to 0.283 (vs *H. xanthogonys*).

Branching diagrams.—Dendrograms depicting hypothesized relationships (Figs. 1 and 2) reveal several common features. Three genetically defined subgroups exist within the brilliants, one consisting of *H. leadbeateri, H. jacula,* and *H. rubinoides,* one of *H. schreibersii* and *P. au-*

rescens, and the third of *H. gularis, H. branickii,* and possibly *H. xanthogonys. Urosticte benjamini* is placed adjacent to the *Heliodoxa* group; however, this warrants comment. The placement of *Urosticte* varies when two alternative, nearly equal-length distance-Wagner trees are compared. We portray the distance-Wagner tree that is consistent with the majority of our branching diagrams. The three subgroups of taxa discussed above were present in the consensus distance-Wagner tree (not shown) based on 100 bootstrapped replicates of loci, which corroborates the trees depicted in Figs. 1 and 2. *Schistes geoffroyi* is consistently placed as a sister taxon to all other hummingbirds.

Cladistic analysis.—Patterns of allelic distribution among taxa reveal the basic phylogenetic framework implied by our data. We found no shared alleles that unite the genus *Heliodoxa* as currently recognized into a monophyletic group. There are, however, alleles at two loci (*DIA, PGM-1*) shared by all *Heliodoxa* (including *Polyplancta*) except *H. xanthogonys.* Within the *Heliodoxa,* several sister groups were identified. One group has *H. leadbeateri* and *H. jacula* as sister taxa linked with *H. rubinoides. Heliodoxa leadbeateri* and *H. jacula* share apparently derived alleles at four loci (*sMDHP, MPI, NP, PEPD*) and there are only frequency differences between these taxa at other loci. *Heliodoxa rubinoides* shares alleles at two loci (*AK-2* and *G6PDH*) with these taxa. The phenotypically similar *H. xanthogonys* shares one allele at *GPT* with the *leadbeateri-jacula-rubinoides* group. An allele at *GPT* supports *H. schreibersii* and *P. aurescens* as sister taxa; these taxa share an allele at *sMDHP* with *U. benjamini* (a possible example of parallelism).

Zimmer (1951) suggested that the allopatric taxa *H. gularis* and *H. branickii* were conspecific. Two alleles (at *ADA, mMDH2*) support the monophyly of this species pair. Six differences, however, were found between these taxa, four of which were apparently fixed (*GPT, sIDH, NP, PEPD*); these taxa are likely not conspecific. Two loci (*sMDHP, SORD*) support the grouping of *H. branickii, H. gularis,* and *H. xanthogonys*; the latter species has five autapomorphies. At *G6PDH* these three taxa share a derived allele with *H. schreibersii.*

In comparing *Urosticte* with *Heliodoxa,* we found 12 fixed differences and seven alleles that *Urosticte* shares with at least one other member of the *Heliodoxa* (relative to *Schistes*). *Schistes* exhibited 14 unique alleles and few shared with *Heliodoxa,* consistent with its designation as a sister taxon to the other hummingbirds.

Coding loci cladistically (Appendix II) resulted in 22 informative characters. HENNIG86 found 27 equally parsimonious trees (not shown); we do not know how many trees are one or a few steps longer. Of interest are groups that occur in high frequency: *H. leadbeateri, H. jacula,* and *H.*

TABLE 1

ALLELIC FREQUENCIES FOR VARIABLE LOCI. NUMBERS IN PARENTHESES ARE FREQUENCIES OF ALLELES NOT FIXED FOR THAT LOCUS. ALLELIC DESIGNATIONS BY LETTER INDICATE FIXATION AT THAT LOCUS

Locus (E.C. No.)	Taxon[a]										
	1	2	3	4	5	6	7	8	9	10	11
sACOH (4.2.1.3)	A	A	A	A	B	A	A	A	D	A	C
ADA (3.5.4.4)	A	A	B	B	A	A	A	A	D	C	E
ADH (1.1.1.1)	A	A	A	A (0.75) B (0.25)	A	A	A	A	A	A	A
ADK (2.7.4.3)	A	A	C	C	C	C	A	C	C	B	C
CK1 (2.7.3.2)	A	A	A	A	A	A	A	A	A	A	B
EAP (2.7.3.2)	A	A	A	A	A	A	A	A	A	A	B
ESTD (3.1.1.–)	A	A	A	A	B	C	G	E	H	D	F
FUMH (4.2.1.2)	A	A	A	A	A	A	A	A	A	A	B
G6PDH (1.1.1.49)	A	A	C	C	C	C	A	F (0.67) G (0.33)	H	E	D
GDA (3.5.4.3)	A	A	A	A	A	A	A	A	A	A	B
sGOT (2.6.1.1)	A	A	A	A (0.75) B (0.25)	A	A	A	A	A	A	C
GPT (2.6.1.2)	A	A	B	C	A	F	A	F	D	G	E (0.50) H (0.50)
GR (1.6.4.2)	A	A	A	A	A (0.90) B (0.10)	A	A	A	A	C	D
sIDH (1.1.1.42)	A	A	A	B	A	A	A	A	C	A	D
mIDH (1.1.1.42)	A	A	A	A	A	A	A	A	B	A	C
LA (3.4.–.–)	A	A (0.83) B (0.17)	A	A	A (0.50) C (0.50)	A	A	A	D	A	E
LDH2 (1.1.1.27)	A	A	A	A	A	A	A	A	A	A	B
LGG (3.4.–.–)	A (0.89) B (0.06) C (0.06)	A	A	A	A	A	A	A	A	D	E (0.50) F (0.50)

TABLE 1
CONTINUED

αGPD (1.1.1.8)	A	A	A	A	A	A	A	A	A	A	B
sMDH1 (1.1.1.40)	A	A	A	A	A	A	A	A	A	A	B
mMDH2 (1.1.1.40)	A	A	B	B	A	A	A	D	E	A	C
sMDHP (1.1.1.40)	A (0.89) H (0.11)	A	B	B	B	D	C	D	F	D	E (0.50) G (0.50)
MP1 (5.3.1.8)	A	A	C	C	C	C	B	C	C	D	C
NP (2.4.2.1)	A (0.61) C (0.28) D (0.11)	C	E (0.50) I (0.50)	B	D (0.90) E (0.10)	H	J	J (0.83) K (0.17)	L	F	G
6PGD (1.1.1.44)	A	A	A	A	A	A	A	A	A	A	B
PGI (5.3.1.9)	A	A (0.83) C (0.17)	A	A	A	A	A	A	A	A	B
PGM1 (5.4.2.2)	A	A (0.83) C (0.17)	A	A	C	A	A	A (0.75) C (0.13) E (0.13)	B	D	C
PGM2 (5.4.2.2)	A	A	A	A	A	A	A	A	A	A	B
PPRO (3.4.–.–)	A (0.89) B (0.11)	B (0.33) D (0.50) E (0.17)	E	D (0.75) F (0.25)	D	D (0.83) E (0.17)	G	D	D	E	C
PK (2.7.1.40)	A	A	A	A	A	A	A	A	A	A	B
SDH (1.1.1.14)	A	A	C	C	C	A	A	B	E	D	A
XDH	A	A	A	A	A	A	A	A	A	A	B
DIA (1.6.–.–)	A	A	A	A	B	A	A	A	C	E	D

[a] Taxon designations: 1. *H. leadbeateri*; 2. *H. jacula*; 3. *H. gularis*; 4. *H. branickii*; 5. *H. xanthogonys*; 6. *H. schreibersii*; 7. *H. rubinoides*; 8. *P. aurescens*; 9. *S. geoffroyi*; 10. *U. benjamini*; 11. *R. squamata.*

TABLE 2

MATRIX OF NEI'S (1978; BELOW DIAGONAL) AND ROGERS' (1972; ABOVE DIAGONAL) GENETIC DISTANCES

	Species										
	1	2	3	4	5	6	7	8	9	10	11
1 *H. leadbeateri*	—	0.055	0.248	0.288	0.291	0.199	0.124	0.253	0.432	0.351	0.776
2 *H. jacula*	0.025	—	0.259	0.291	0.280	0.201	0.137	0.249	0.422	0.359	0.772
3 *H. gularis*	0.271	0.272	—	0.109	0.241	0.197	0.279	0.228	0.381	0.356	0.755
4 *H. branickii*	0.318	0.312	0.090	—	0.264	0.224	0.318	0.251	0.378	0.421	0.760
5 *H. xanthogonys*	0.325	0.303	0.255	0.276	—	0.224	0.296	0.261	0.357	0.395	0.727
6 *H. schreibersii*	0.213	0.204	0.207	0.234	0.238	—	0.203	0.133	0.363	0.329	0.731
7 *H. rubinoides*	0.125	0.129	0.318	0.367	0.341	0.225	—	0.238	0.436	0.359	0.785
8 *P. aurescens*	0.276	0.263	0.240	0.259	0.283	0.128	0.259	—	0.351	0.351	0.749
9 *S. geoffroyi*	0.562	0.535	0.473	0.456	0.436	0.447	0.573	0.427	—	0.487	0.759
10 *U. benjamini*	0.429	0.429	0.432	0.530	0.494	0.395	0.445	0.427	0.668	—	0.810
11 *R. squamata*	1.528	1.501	1.413	1.435	1.309	1.317	1.544	1.395	1.426	1.678	—

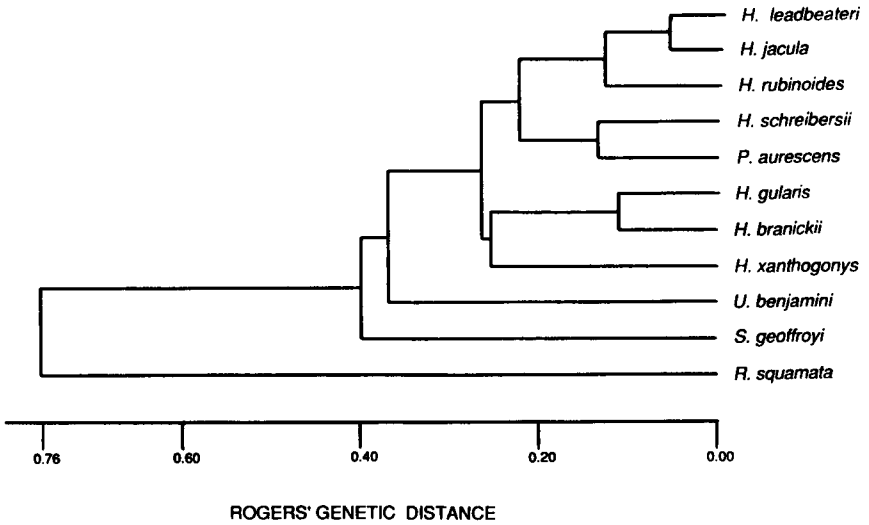

FIG. 1. UPGMA phenogram based on Rogers' D values (Table 2). Cophenetic correlation coefficient equals 0.989, indicating that the phenogram faithfully represents the original distance matrix (Sneath and Sokal 1973).

rubinoides (27 of 27 trees, 100%), *H. schreibersii* and *P. aurescens* (23 of 27 trees, 85%), and *H. gularis* and *H. branickii* (18 of 27 trees, 67%). There is little consensus among the 27 trees concerning other relationships. However, in 15 of 27 trees (56%), the *P. aurescens-H. schreibersii* clade and *H. leadbeateri-rubinoides-jacula* clade were sister taxa. The affinities of *H. xanthogonys* are uncertain, and different placements of this taxon contributed to the lack of strict consensus among the 27 trees; the relationships of this taxon require further research.

DISCUSSION

Genetic variation.—Two taxa (*H. leadbeateri* and *H. xanthogonys*) for which a sufficient number (see Nei 1978) of individuals was available for analysis exhibited low values of *H*(avg) (0.017, 0.015) relative to other birds (*H*[avg] = 0.05; Barrowclough 1980, Corbin 1983). Low heterozygosity estimates have been reported for some insular species (Selander 1976, Yang and Patton 1981). *Heliodoxa xanthogonys* is, in fact, an "insular" species in that populations inhabit islands of submontane vegetation ("tepuis") in southeast Venezuela and therefore are almost certainly isolated from other populations. Insularity might explain the low *H*(avg) value of *xanthogonys*; however, these isolated populations often exist at high density (J. P. O'Neill pers. comm.). *Heliodoxa leadbeateri,* on the

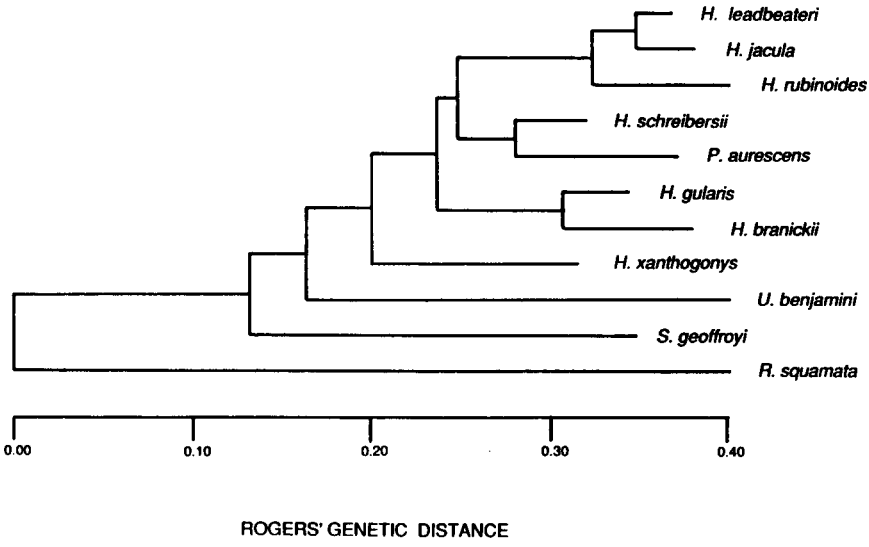

FIG. 2. Distance-Wagner tree (optimized) rooted by the outgroup method (Farris 1972). Units are in Rogers' D. Cophenetic correlation coefficient equals 0.987 and the %SD equals 4.41.

other hand, is distributed from Colombia and Venezuela south to Bolivia (Meyer de Schauensee 1966). Nei et al. (1975) have shown that low values of *H* are expected if the total population of a species has passed through a lengthy bottleneck or has existed at very low density for many generations. Although little is known of the natural history of many hummingbird taxa, *H. leadbeateri* is not currently a low-density taxon (Davis 1986, J. V. Remsen pers. comm.). It is, therefore, unclear why *H* is low in this taxon. It is unknown if low *H* values are a general phenomenon in hummingbirds. In any event, heterozygosity levels at allozyme loci are dubious predictors of adaptive potential or "genetic health" (Lande and Barrowclough 1987). Past demographic events may leave a signature in patterns of heterozygosity, but discovering these events and their biological significance is difficult. Therefore, the significance, if any, of low levels of heterozygosity is unclear.

Genetic differentiation.—Genetic differentiation among avian taxa, particularly passerines, is low relative to other vertebrates (Barrowclough and Corbin 1978; Avise et al. 1980a, b; Barrowclough et al. 1981; Avise and Aquadro 1982; Zink 1982). Relatively few workers, however, have investigated genetic differentiation in nonpasserines (Guttman et al. 1980, Barrowclough et al. 1981, Gutiérrez et al. 1983, Johnson and Zink 1983,

Lanyon and Zink 1987, Zink et al. 1987, Hackett 1989). Although values of H may be low, genetic distance values at all taxonomic levels in the hummingbirds exceed those previously reported for other birds (see Barrowclough 1980). Our average value for congeners, 0.241, is four times higher than that observed between most congeneric species of oscines (Avise and Aquadro 1982). Also, Johnson et al. (1988) report an average D among species within *Vireo* and *Hylophilus* of 0.293 (see also Marten and Johnson 1986, Christidis 1987); they also argue that there are too few vireo genera, which inflates genetic distance values among congeners. Hackett (1989) estimated an average interspecific genetic distance of 0.103 ± 0.061 for the nonpasserine genus *Sterna*. Our within-family value of 0.404 is similar to values obtained in studies of other nonpasserines (Gutiérrez et al. 1983, Lanyon and Zink 1987). Our between-family value of 1.45 (swift vs hummingbirds) is also high, but the number of independent comparisons at this level is small; this value is reported for future comparison.

Factors that could increase genetic differentiation in hummingbirds relative to temperate birds include (1) increased age of lineages; (2) aspects of social systems (e.g., polygyny; see Wilson et al. 1975); and (3) aspects of demography (fluctuating effective population sizes) thought to accelerate divergence for selectively neutral characters (Nei 1987). It is unknown which of these factors contributes most, if at all, to the increased levels of genetic differentiation observed in hummingbirds surveyed herein. If allelic substitutions in birds are selectively neutral (Barrowclough et al. 1985) and accrue at a constant rate (molecular clock hypothesis), then species of *Heliodoxa* are on average older than species of temperate birds. Regarding (2), some species of hummingbirds are lekking, a mating structure that reduces the variance effective population size and might increase rate of genetic drift. The magnitude of this effect is unknown in *Heliodoxa*; most species exhibit loosely organized leks (T. A. Parker pers. comm.). In other hummingbirds (*Phaethornis*) a high level of genetic differentiation has been found between some lekking species (Gill and Gerwin unpubl. data). Concerning (3), some species of hummingbirds are known to be isolated and/or restricted in distribution, which might cause Ne to fluctuate, and at least one of these (*H. xanthogonys*) shows an increased level of genetic divergence relative to most of its congeners. Other evidence (Braun and Parker 1985, Capparella 1987) reveals that Neotropical birds show greater genetic differentiation than temperate birds, which argues for the "greater age" hypothesis because these taxa do not all share aspects (2) and (3). Disentangling alternative causal factors is difficult and all might contribute to increased genetic differentiation among hummingbirds.

Protein evolution and body temperature.—Avise and Aquadro (1982) suggested that high avian body temperature limits the number of tolerable alleles at enzyme loci. As a consequence, reduced genic diversity would lead to a smaller substrate for genetic divergence, accounting for the conservative nature of avian intertaxon genetic differentation. Hummingbirds possess the highest body temperature among birds (Welty 1982), and hummingbirds surveyed genetically to date consistently exhibit low heterozygosity relative to other birds. However, genetic differentiation among hummingbird taxa exceeds that observed for most other birds. These observations illustrate the potential interaction of factors influencing protein evolution, such as high body temperature (reduced genetic variation) and greater antiquity of hummingbird clades (increased genetic differentiation). Avise and Aquadro's (1982) interesting hypothesis deserves continued attention because our results seem partly consistent with their predictions. Experimental tests of enzyme kinetics also might reveal constraints on amino-acid substitutions in avian enzymes.

Relationships among taxa.—Relationships within the Trochilidae in general are uncertain, with only a few studies addressing phylogenetic or taxonomic relationships (see Zimmer 1951; Graves 1980, 1986; Stiles 1983; Schuchmann 1987, unpubl. data; Zusi and Bentz 1982; Gerwin unpubl. data). This problem is not unique to hummingbirds; that is, there are few studies investigating phylogenetic relationships for most Neotropical avian taxa. For *Heliodoxa* and its relatives, we referred to published checklists as a starting point for hypothesized relationships and our results are compared to Zimmer's (1951) linear sequence.

Thirteen alleles are shared by the 10 trochilid members, uniting them into a monophyletic group when compared to *R. squamata.* Doubts about the hummingbirds' nearest relative have existed for some time (Sibley and Ahlquist 1972, Zusi pers. comm.). DNA-DNA hybridization data support the placement of the Apodidae as the sister group, albeit a rather distant one (Sibley et al. 1988). Our electrophoretic data confirm only that the swift is a distant outgroup from the trochilid taxa surveyed (we would need to survey other taxa to confirm a swift-hummingbird sister-group relationship). We found, however, 10 alleles shared by both the hummingbirds and the swift.

In our analyses, several phylogenetic patterns emerged consistently. The well differentiated (from each other and other taxa) *S. geoffroyi* and *U. benjamini* branch off first and second, respectively. Compared with members in the genus *Heliodoxa,* their average genetic distances are high (0.489 $\pm$ 0.059 and 0.448 $\pm$ 0.043, respectively), suggesting a relatively ancient connection with *Heliodoxa.* However, the placement of *U. benjamini* varied when several alternative distance-Wagner trees were generated.

This ambiguity is the result of conflicting allelic distributions. *Urosticte benjamini* shares common alleles with some member of *Heliodoxa* at five loci and an allele (at *sMDHP*) with *H. schreibersii* and *P. aurescens.* These results represent convergence of alleles or retentions of ancestral states. *Urosticte* has always been placed near *Heliodoxa* in taxonomic treatments and has been tentatively placed in a higher-level clade defined as "Andean" hummingbirds (R. Zusi, pers. comm.). Recently, Schuchmann (1987) proposed that *Urosticte* and *Ocreatus underwoodii* (Booted Racket-tail) are sister taxa, and that these plus *Eriocnemis* and *Haplophaedia* form a monophyletic group. The sharing of six common alleles by *Urosticte* with various *Heliodoxa* may reflect its inclusion in a group whose only members studied at this time were *Heliodoxa.* The high number (12) of genetic differences lead us to advocate its continued exclusion from *Heliodoxa-Polyplancta.* Protein comparisons with proposed relatives should clarify the relationships of *Urosticte* to other taxa. In addition the monophyly of *Heliodoxa* would be tested.

The genus *Heliodoxa,* as currently recognized, is paraphyletic because our results indicate that *Polyplancta* is a sister taxon to *H. schreibersii.* However, no synapomorphies unite the genus *Heliodoxa* as a monophyletic group, even when *Polyplancta* is included. Recent data on mating behavior and vocalizations also support the conclusion that *Polyplancta* is a member of the *Heliodoxa* assemblage (Schuchmann pers. comm.). We suggest that the monotypic genus *Polyplancta* be moved to *Heliodoxa,* and the monophyly of the resultant group studied further.

In sum, our phenetic and cladistic analyses of the protein data support the following groupings: (1) *H. jacula-leadbeateri-rubinoides,* (2) *H. branickii-gularis,* and (3) *H. schreibersii-P. aurescens.* We hypothesize that groups 1 and 3 are sister groups, and we are uncertain as to the placement of *H. xanthogonys* and group 2. Comparing the genetic groupings with the patterns of resemblance in external morphology (frontispiece) reveals the difficulty in inferring phylogeny from the latter. Apart from the obvious synopomorphy (white crissum) linking *branickii-gularis,* systematic affinities are obscured, potentially by sexual selection for male plumage traits.

Phenotypic evolution: a genetic perspective.—In theory, sexual selection can yield rapid phenotypic differentiation and speciation (West-Eberhard 1983). The phenotypic diversity observed in hummingbirds, especially in male plumages, might be a result of sexual selection (Futuyma 1987). If so, then these speciation events were not recent, owing to the relatively high genetic differentiation observed among *Heliodoxa* sister-taxa, excluding *leadbeateri-jacula* ($D = 0.025$). In some north temperate species, such as *Dendroica* warblers, plumage differentiation, perhaps via sexual

selection, and speciation have occurred with little or no allozymic differentiation (Barrowclough and Corbin 1978). Additional comparisons of levels of allozymic divergence in sexually dimorphic species might clarify the role of sexual selection in avian speciation.

Classifications, by virtue of their linear sequences of taxa, have long reflected that throat color is evolutionarily plastic, or subject to parallel evolution, in hummingbirds. Classifications have not grouped all taxa with similar throat colors. Nonetheless, throat color, when used with other characters, could indicate systematic relationships. Within the *Heliodoxa* assemblage exist pink (*rubinoides, branickii, gularis*), blue (*jacula, xanthogonys*), green (*leadbeateri*), black (*schreibersii*) and green and black (*P. aurescens*) throat colors (see frontispiece). We hypothesize that these "states" are homologous, both in terms of "throat color" as a character, and in instances (pink, blue) when more than one species share the same character states. For instance, although we recognize that the pink throats of *branickii, gularis,* and *rubinoides* exhibit slight differences, we assume for argument that the pink throats are homologous.

The most closely related taxa in *Heliodoxa* are *leadbeateri* and *jacula* (D = 0.025), and they have distinct throat colors (green and blue, respectively). This contrasts with the situation in the sister taxa *branickii* and *gularis,* which have pink throats. Males of these taxa are overall very similar phenotypically (in addition to throat color they also share white undertail coverts, and their overall body plumage color is similar), and yet their genetic distance value (D = 0.090) is 3.5 times higher than *leadbeateri* vs *jacula.* The pink-throated *rubinoides* is placed adjacent to the *leadbeateri-jacula* cluster, and is genetically quite distinct from the other pink-throated forms. Although the body plumage colors of *H. schreibersii* and *P. aurescens* are strikingly different, the genetic data unite them as sister taxa. Perhaps most surprising is the placement of *xanthogonys.* Genetically, it is the most distinct member of the group (D[avg] 0.289). Phenotypically, however, its throat pattern closely resembles *jacula.* In our analysis *xanthogonys* is genetically similar to *branickii-gularis,* although we are not confident of a sister-group relationship. The high level of genetic differentiation, coupled with an apparent lack of phenotypic differentiation between *xanthogonys* and the *leadbeateri-jacula* cluster, highlights one aspect of this analysis—the complexity and plasticity of phenotypic change in hummingbirds relative to patterns of genetic affinities. If our phylogenetic hypothesis is correct, then the pink and blue throat colors appear to have arisen in parallel. One could not use throat color, per se, to unite taxa into a classification reflecting phylogeny, because parallel evolution prevents this (Wiley 1981, Christidis 1987). Although plasticity in throat color has been suspected in hummingbirds,

based on arrangements of past classifications, our data represent the first direct evidence.

Classification of brilliants and Gould's Jewelfront.—We advocate a phylogenetic classification (Wiley 1981), one which preserves the branching order (genealogy) of our phylogeny (Fig. 2). Within any level of the hierarchy we follow the "sequencing convention" of Wiley (1981) to reflect phylogenetic positions of taxa. Thus, phylogenetic patterns can be recovered fully from our classification.

Genus *Heliodoxa*
- *H. imperatrix incertae sedis*
- Division 1
  - *H. xanthogonys*
- Division 2
  - Subdivision 1
    - *H. gularis*
    - *H. branickii*
  - Subdivision 2
    - Section 1
      - *H. schreibersii*
      - *H. (Polyplancta) aurescens*
    - Section 2
      - *H. rubinoides*
      - *H. jacula*
      - *H. leadbeateri*

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APPENDIX I

SPECIES STUDIED, SAMPLE SIZES, AND REGIONS FOR SPECIMENS (PRECISE LOCALITIES AVAILABLE FROM THE AUTHORS)

Species	N	Region
Violet-fronted Brilliant *Heliodoxa leadbeateri*	9	Ecuador (8), Peru (1)
Green-crowned Brilliant *H. jacula*	3	Peru (2), Ecuador (1)
Fawn-breasted Brilliant *H. rubinoides*	1	Peru
Black-throated Brilliant *H. schreibersii*	3	Peru
Rufous-webbed Brilliant *H. branickii*	2	Peru
Pink-throated Brilliant *H. gularis*	1	Peru
Velvet-browed Brilliant *H. xanthogonys*	5	Venezuela
Gould's Jewelfront *Polyplancta aurescens*	4	Peru (2), Bolivia (1), Venezuela (1)
White-tip *Urosticte benjamini*	1	Peru
Wedge-billed Hummingbird *Schistes geoffroyi*	1	Ecuador
Fork-tailed Palm-Swift *Reinarda* (*Tachornis*) *squamata*	1	Peru

APPENDIX II

STATES OF LOCI USED AS INPUT INTO THE COMPUTER PROGRAM HENNIG86. STATES FOR POLYMORPHIC LOCI WERE THOSE REPRESENTING THE MOST COMMON ALLELE AT A LOCUS. CHARACTERS WERE CODED AS NONADDITIVE. ONLY PHYLOGENETICALLY INFORMATIVE LOCI WERE USED, AND THE TWO MORE DISTANT OUTGROUPS (*U. BENJAMINI* AND *R. SQUAMATA*) WERE EXCLUDED

	Locus[a,b]																					
Taxon	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
H. leadbeateri	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
H. jacula	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	C	A	A	C	A	A
H. gularis	A	B	A	B	A	B	A	B	A	A	A	A	A	B	B	C	D	A	A	B	B	A
H. branickii	A	B	A	B	A	B	A	C	A	B	A	A	A	B	B	C	B	A	A	C	B	A
H. xanthogonys	B	A	A	B	B	B	A	A	A	A	A	B	A	A	B	C	E	A	C	D	B	B
H. schreibersii	A	A	A	B	C	B	A	E	A	A	A	A	A	A	D	C	F	A	A	C	A	A
H. rubinoides	A	A	A	A	D	A	A	A	A	A	A	A	A	A	C	B	G	A	A	D	A	A
P. aurescens	A	A	A	B	E	C	A	E	A	A	A	A	A	C	D	C	G	A	A	C	C	A
S. geoffroyi	C	C	A	B	F	D	A	D	A	C	B	C	A	D	E	C	H	A	C	C	D	C

[a] 1 = *sACOH*, 2 = *ADA*, 3 = *ADH*, 4 = *ADK*, 5 = *ESTD*, 6 = *G6PDH*, 7 = *sGOT*, 8 = *GPT*, 9 = *GR*, 10 = *sIDH*, 11 = *mIDH*, 12 = *LA*, 13 = *LGG*, 14 = *mMDH2*, 15 = *sMDHP*, 16 = *MPI*, 17 = *NP*, 18 = *PGI*, 19 = *PGM1*, 20 = *PPRO*, 21 = *SDH*, 22 = *DIA*. EC numbers in Table 1.

[b] Characters 3, 7, 9, 13, and 18 had polymorphisms (Table 1) which could be considered informative; however, these characters we coded as invariant here.