GENIC VARIATION, SYSTEMATIC, AND BIOGEOGRAPHIC RELATIONSHIPS OF SOME GALLIFORM BIRDS

R. J. GUTIÉRREZ,[1] ROBERT M. ZINK, AND SUH Y. YANG[2]
Museum of Vertebrate Zoology, University of California, Berkeley, California 94720 USA

ABSTRACT.—Starch gel electrophoresis was used to evaluate levels and patterns of genic differentiation among 10 species of galliform birds in the Phasianidae (9) and Tetraonidae (1). The phasianids included an Old World quail, a partridge, a pheasant, and six species of New World quail. Measures of within-species genetic variation included heterozygosity, percentage polymorphic loci, and number of alleles per polymorphic locus. These values were similar to but lower than those reported for other birds. Genetic distances among conspecific populations and among congeneric species were low compared to other avian results. Genetic distances among noncongeners both within and between families were considerably higher, however, than those reported for passerine birds. Thus, more studies of levels of genic differentiation among nonpasserines are required to complement the literature on genic divergence among passerines and to enable us to make general statements about genic evolution in birds.

Phenograms and phylogenetic trees suggested that *Phasianus colchicus, Tympanuchus pallidicinctus, Coturnix coturnix, Alectoris chukar,* and the New World quail (Odontophorinae) are genically distinct taxa. The branching sequence among the non-Odontophorine taxa is unresolved by our data. The branching order among taxa in the Odontophorinae from a common ancestor is: *Cyrtonyx montezumae, Oreortyx pictus, Colinus virginianus, Callipepla squamata, Lophortyx gambelii,* and *L. californicus.* The genera *Cyrtonyx, Oreortyx,* and *Colinus* are clearly distinct from *Callipepla* and *Lophortyx,* which are quite similar to each other genically.

We use a fossil species from the mid-Miocene of Nebraska to calibrate our genetic distances. We estimate dates of divergence of taxa in the Odontophorinae and offer a hypothesis on their historical biogeography. Our analysis suggests that three east–west range disjunctions could account for the origin of *Oreortyx* (12.6 MYBP), *Colinus* (7.0 MYBP), and *Callipepla-Lophortyx* (2.8 MYBP). We suggest that *L. californicus* and *L. gambelii* should be considered distinct species because of an apparent lack of panmixia in zones of sympatry, even though the *D* between them is typical of that found between subspecies of other birds. *Oreortyx* and *Colinus* should remain as distinct genera, while our data are equivocal on the status of *Callipepla* and *Lophortyx.*[3] *Received 9 March 1982, accepted 5 July 1982.*

ALLOZYME electrophoresis has been used less frequently to examine genetic variation within and among groups of birds than in other vertebrates (see review in Nevo 1978). Some workers have examined patterns of intraspecific genic variation in passerines (e.g. Barrowclough 1980, Johnson and Brown 1980, Corbin 1981, and references therein), and a few have examined avian intrafamilial relationships (e.g. Barrowclough and Corbin 1978, Avise et al. 1980a–c). They have found that passerine birds possess considerably lower levels of genic (= allozymic) differentiation than other vertebrate taxa, at comparable taxonomic levels.

Several workers have compared the level of genic divergence and taxonomic rank for various vertebrate and invertebrate taxa (e.g. Ayala 1975, Avise et al. 1980b). Barrowclough et al. (1981) present similar data for birds but discuss reasons why comparisons across different groups of organisms may be inappropriate; these include taxonomic artifacts [e.g. avian, mammalian, and reptilian genera may not be comparable because of the way in which taxonomists partition variation (Sibley and Ahl-

[1] Present address: Department of Wildlife Management, Humboldt State University, Arcata, California 95521 USA.

[2] Present address: Department of Biology, Inha University, Inchon, Korea.

[3] *Lophortyx* has been merged with *Callipepla* in the Thirty-fourth Supplement to the American Ornithologists' Union check-list of North American Birds [Auk 99 (3, Suppl.): 5CC].

quist 1982)] and differences in rates of evolution, mating systems, effective population sizes, recency of common ancestry, and dispersal parameters. Whether or not low levels of genic divergence typify birds as a group is unclear, because only Barrowclough et al. (1981) studied a nonpasserine taxon. They found higher levels of differentiation among some procellariiform taxa than those usually found among passerines. Because the levels of genetic differentiation are sometimes used to make inferences about evolutionary processes (Avise et al. 1980c, Templeton 1980), we clearly need additional studies of nonpasserines before we can make general statements about genic evolution in birds.

The patterns of genic differences can also be used to infer phylogenetic relationships (e.g. Barrowclough and Corbin 1978, Zink 1982). In this paper we examine levels of genic variation and phylogenetic relationships among 10 species from 5 of 10 genera of New World quail, an Old World quail, a partridge, a pheasant, and a grouse. We use our phylogenetic hypothesis to construct an estimate of the evolutionary history of some New World quail. We also evaluate previous statements about the taxonomic relationships of these galliform birds.

MATERIALS AND METHODS

We examined 217 specimens of galliform birds representing 10 species. Species, localities, sample sizes, and the taxonomic framework used in this paper are given in Appendix 1. Nomenclature follows the A.O.U. check-list (1957, 1973).

Liver, heart, and kidney tissue were excised in the field within 4 h of death and frozen in liquid nitrogen. Tissues were homogenized using the methods of Selander et al. (1971), and extracts were stored at −76°C until used for electrophoresis. Combined tissue extracts were subjected to horizontal starch gel electrophoresis as described by Selander et al. (1971). Gel and buffer systems for the loci listed in Appendix 2 were essentially the same as those described by Yang and Patton (1981). More detailed information regarding electrophoretic conditions is available from the authors.

We assume that our electrophoretically detectable variants (= electromorphs) at a locus differ genetically; hence, we refer to them as alleles. Alleles at a locus were coded by their mobility from the origin. The most anodal allele was designated as a, with slower alleles denoted as b, c, d, etc. Isozyme nomenclature follows Yang and Patton (1981). Heterozygosity ($\bar{H}$) was defined as the number of heterozygous genotypes recorded in a sample divided by the product of the number of loci and the number of individuals assayed (see Corbin 1981 for a discussion of calculating $\bar{H}$). Estimates of percentage polymorphism were based on the number of loci having more than one allele divided by the total number of loci (27) examined.

The measures of Nei (1978) and Rogers (1972) were used to estimate genetic distances between taxa. Cluster analyses, summarizing the matrix of Rogers' D-values, were performed with both the unweighted and weighted pair-group methods, using arithmetic means (UPGMA and WPGMA, respectively). The cophenetic correlation coefficient, r_{cc}, was used to evaluate how well the resultant phenograms represent the original distance matrix. Sneath and Sokal (1973) provide details on these phenetic methods. Phylogenetic trees, also based on Rogers' D-values, were constructed according to the methods of Farris (1972; Wagner trees) and Fitch and Margoliash (1967; F-M trees). The Wagner tree is an approximation of the most parsimonious tree. The F-M procedure constructs a number of trees by altering the branching structure and branch lengths. Alternative trees were evaluated by the percentage standard deviation (%SD) and by the number of negative branches (the fewer the better). A lower %SD means a better fit of distances implied by the tree to the original distance matrix (Fitch and Margoliash 1967). A cladistic analysis, *sensu* Hennig (1966), using alleles as character states (see Wake 1981), basically corroborated the above methods. The allele in *T. pallidicinctus* (Tetraonidae) was considered "primitive" when comparing the pattern of allele distribution in the remaining taxa (Phasianidae), i.e. *T. pallidicinctus* was used as an "outgroup" to the phasianids.

RESULTS

Protein variation.—Twenty structural proteins and enzymes encoded by 27 presumptive genetic loci were examined in all individuals. Allelic frequencies for the 23 variable loci, percentage polymorphism and heterozygosity, and number of alleles per polymorphic locus are given in Appendix 2. Four loci (Mdh-1, Mdh-2, Lap, Pt-1) were monomorphic and fixed for the same electromorph across species. Seven loci (αGpd-1, Got-2, Udh, Gdh, Ldh-1, Ldh-2, Pept-2) were monomorphic within species but exhibited interspecific differences. The remaining loci were polymorphic in some species and also showed interspecific fixed differences.

We exclude the laboratory strains of *Coturnix* and *Alectoris* from discussions of within-species variation, because these levels of variation may

TABLE 1. Matrix of genetic distances between 17 taxa of galliform birds. Distances computed by methods of Nei (1978) above diagonal and Rogers (1972) below diagonal.

Species	Species						
	1	2	3	4	5	6	7
1. *Tympanuchus pallidicinctus*	—	1.041	1.308	1.291	1.326	1.201	1.483
2. *Phasianus colchicus*	0.649	—	1.446	1.282	1.310	1.172	1.654
3. *Coturnix coturnix* A	0.721	0.749	—	0.064	0.059	1.185	1.340
4. *C. coturnix* B	0.713	0.714	0.109	—	0.056	1.136	1.209
5. *C. coturnix* C	0.729	0.721	0.086	0.101	—	1.190	1.346
6. *Alectoris chukar*	0.695	0.682	0.685	0.665	0.689	—	0.962
7. *Lophortyx gambelii*	0.769	0.799	0.725	0.692	0.730	0.616	—
8. *L. californicus* A	0.773	0.797	0.728	0.690	0.730	0.617	0.028
9. *L. californicus* B	0.774	0.801	0.732	0.694	0.734	0.617	0.032
10. *L. californicus* C	0.768	0.795	0.727	0.688	0.728	0.610	0.037
11. *Callipepla squamata* A	0.773	0.758	0.752	0.727	0.761	0.652	0.106
12. *C. squamata* B	0.764	0.750	0.742	0.720	0.752	0.644	0.106
13. *Colinus virginianus* A	0.770	0.732	0.741	0.741	0.753	0.687	0.262
14. *C. virginianus* B	0.772	0.729	0.739	0.738	0.751	0.687	0.262
15. *Oreortyx pictus*	0.808	0.764	0.751	0.744	0.760	0.649	0.348
16. *Cyrtonyx montezumae* A	0.744	0.731	0.746	0.737	0.754	0.684	0.497
17. *C. montezumae* B	0.735	0.727	0.742	0.734	0.751	0.680	0.492

TABLE 1. Continued.

Species									
8	9	10	11	12	13	14	15	16	17
1.495	1.495	1.475	1.493	1.476	1.487	1.495	1.668	1.364	1.335
1.627	1.646	1.617	1.430	1.419	1.342	1.331	1.476	1.334	1.326
1.340	1.343	1.330	1.448	1.429	1.393	1.389	1.450	1.416	1.407
1.204	1.216	1.192	1.339	1.345	1.423	1.413	1.433	1.399	1.390
1.327	1.335	1.312	1.467	1.449	1.427	1.421	1.468	1.435	1.425
0.967	0.966	0.947	1.071	1.054	1.184	1.189	1.056	1.170	1.158
0.005	0.007	0.008	0.089	0.082	0.295	0.295	0.410	0.671	0.653
—	0.000	0.000	0.115	0.100	0.301	0.298	0.418	0.640	0.624
0.013	—	0.000	0.121	0.102	0.306	0.304	0.421	0.634	0.617
0.019	0.015	—	0.120	0.103	0.308	0.306	0.431	0.641	0.623
0.129	0.130	0.131	—	0.003	0.258	0.262	0.491	0.664	0.645
0.125	0.117	0.126	0.022	—	0.241	0.244	0.473	0.613	0.593
0.274	0.276	0.280	0.244	0.229	—	−0.002	0.538	0.478	0.462
0.273	0.277	0.283	0.252	0.237	0.015	—	0.540	0.463	0.448
0.355	0.354	0.367	0.400	0.391	0.424	0.427	—	0.746	0.744
0.479	0.476	0.479	0.493	0.473	0.395	0.385	0.533	—	0.001
0.476	0.472	0.472	0.483	0.463	0.389	0.383	0.533	0.018	—

have been affected by prolonged captivity. The average proportion of polymorphic loci for the wild species is 14.5% (range 0–29.6%). $\bar{H}$ is 2.6% and ranges from 0 to 5.1% (Appendix 2). The values of $\bar{H}$, percentage polymorphic loci, and number of alleles per polymorphic locus are similar to but lower than those reported for other groups of birds (Barrowclough and Corbin 1978, Avise et al. 1980a, Zink 1982).

Interspecific genetic distance.—The matrix of genetic distances between taxa is given in Table 1. A summary (Table 2) of genetic distance as a function of various taxonomic groupings shows that $\bar{D}$ increases as taxonomic groupings become more inclusive, at least to the subfamily level. This suggests that the taxonomic groupings are "biologically real," based on our genetic analysis. Levels of $\bar{D}$ for other avian taxa are also shown in Table 2 for comparison with the galliform $\bar{D}$'s.

At the local population level, the galliforms sampled here show less differentiation ($\bar{D}$ =

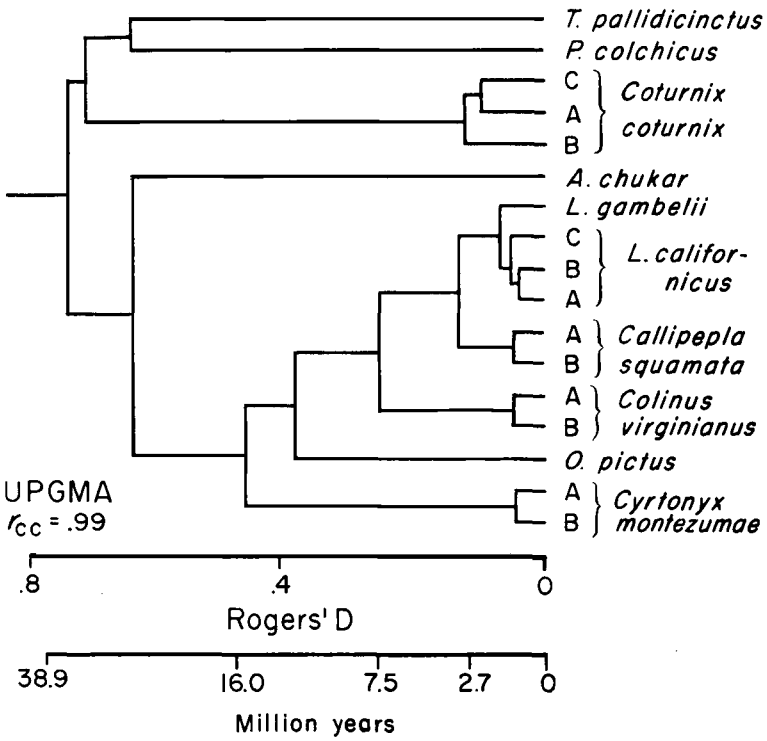

Fig. 1. UPGMA phenogram derived from the matrix of Rogers' D-values (Table 1). Geographic localities given in Appendix 1. Time scale determined from the formula $t = 26.3 \times 10^6 D$; see text.

0.0007) than other birds, although this may be due to the close proximity of some of the samples (Appendix 1). The $\bar{D}$-value between *L. gambelii* and *L. californicus*, 0.0067, is similar to that observed between subspecies of other birds [0.0048 ± 0.0049 (Barrowclough 1980)], but it is only slightly below the range given by Barrowclough, 0.0078–0.1267, for congeneric species. The $\bar{D}$ between noncongeneric species in the Odontophorinae, 0.412, is considerably less than the $\bar{D}$ between these and the other phasianids, 1.32. Both of these values are greater

TABLE 2. Mean genetic distance (Nei 1978) as a function of taxonomic rank in some galliform birds. Taxa included in each taxonomic level are given in Appendix 1. $\bar{D}$-values from Table 1. Also given are $\bar{D}$-values at comparable taxonomic levels for other birds (from Barrowclough 1980).

Taxonomic level	Number of comparisons	$\bar{D}$ ± SD	Range	Comparable data for other birds
Local population	6	0.0007 ± 0.0013	−0.00151 to 0.00331	0.0024 ± 0.0028
Congeneric species (*Lophortyx*)	3	0.0067 ± 0.0014	0.00507 to 0.00775	0.0440 ± 0.0220
Noncongeneric species in Odontophorinae	46	0.4116 ± 0.2021	0.0824 to 0.7460	0.2136 ± 0.1659
Species in Odontophorinae vs. *Phasianus colchicus*, *Coturnix coturnix*, and *Alectoris chukar*	62	1.3210 ± 0.1640	0.962 to 1.654	not available
Tympanuchus pallidicinctus (Tetraonidae) vs. all other species (Phasianidae)	16	1.4000 ± 0.1500	1.041 to 1.668	0.6829[a] ± 0.1970

[a] Based on study by Barrowclough et al. (1981) of some procellariiform birds.

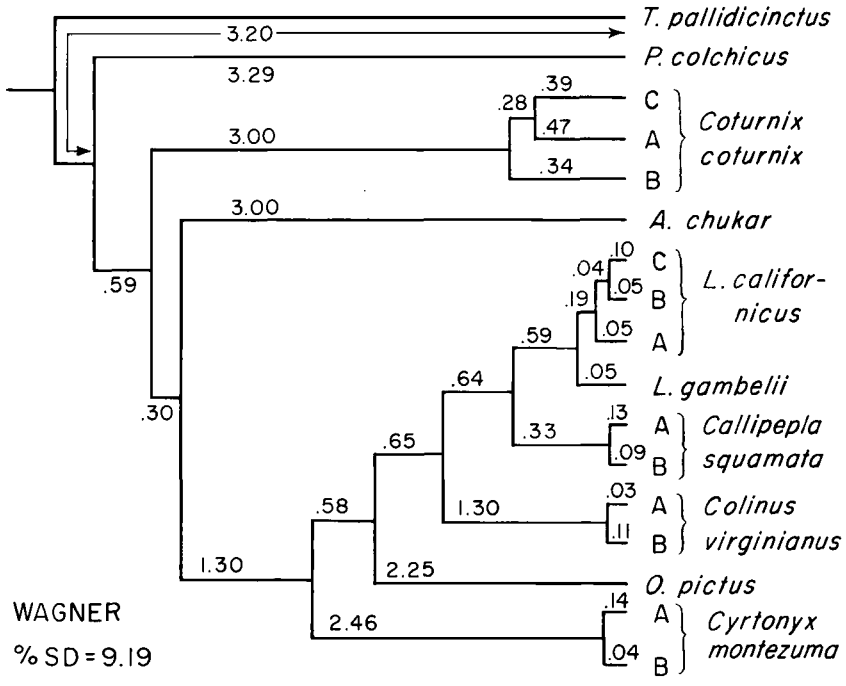

Fig. 2. Wagner tree based on Rogers' D-values. Branch lengths in units of Rogers' D (×10). The tree is "rooted" at *T. pallidicinctus* (Farris 1972).

than that given by Barrowclough (1980) for other avian confamilial but noncongeneric comparisons ($\bar{D}$ = 0.2136), suggesting that the Odontophorinae is a distinct group. At the family level, the $\bar{D}$ observed between *T. pallidicinctus* (Tetraonidae) and the other species (all in Phasianidae), 1.400, is approximately twice the $\bar{D}$ reported for interfamilial comparisons of other birds (0.6829).

Genic relationships among species.—For two reasons, phenetic (UPGMA and WPGMA) and cladistic (Wagner and F-M) procedures were used to construct branching diagrams (Figs. 1–3). First, we wanted to determine whether or not the branching structure was dependent on which methods were used (see Presch 1979). Second, the methods for constructing trees, as opposed to phenograms, are independent of the assumption of homogeneity of evolutionary rates and, hence, provide estimates of the amount of genic change along branches; Felsenstein (1978) discusses these assumptions for Wagner trees.

The UPGMA and WPGMA phenograms had equal r_{cc}'s (0.99) and were topologically similar; therefore, only the UPGMA phenogram is shown (Fig. 1). The Wagner (Fig. 2) and F-M (Fig. 3) trees resemble Fig. 1 in terms of the overall relationships suggested. Differences among these three analyses suggest to us that the branching sequence of the subfamilies, while themselves genically distinct in all analyses, is unresolved by this analysis. That is, we do not believe that a clear hypothesis of the branching order of the subfamilies emerges from our data. The level of differentiation and the branching diagrams show considerable divergence, at the structural gene level, among these galliform birds. The branching sequence of taxa within the Odontophorinae was identical in all branching diagrams; therefore, we feel that it is a robust result.

The branching diagrams show that there is considerable divergence among taxa within the Ondontophorinae, and we now discuss relationships in that group. Given the patterns in Figs. 1–3, it seems unlikely that the level of divergence among local populations would be sufficient to alter among-species patterns established here. Thus, we doubt that an analysis of geographic variation, not addressed here, would alter our conclusions, which follow. *Lo-*

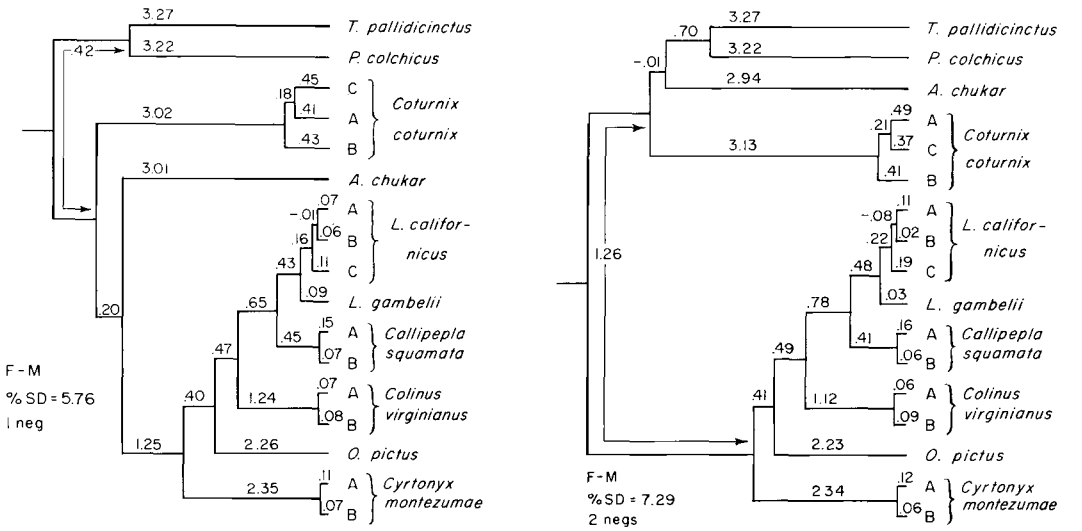

Fig. 3. Fitch-Margoliash (F-M) trees based on Rogers' *D*-values. Branch lengths in Rogers' *D* (×10). These two trees, of four examined, best summarized the original matrix, as judged by the values of the percentage of standard deviation and the number of negative branches. These trees, as well as those in Figs. 1 and 2, show that alternative hypotheses exist regarding the branching sequence of the five subfamilies.

phortyx gambelii and *L. californicus*, the only congeners in our study, consistently cluster together—the only genetic differences we found between them are minor gene frequency differences (Appendix 2). *Lophortyx* and *Callipepla* are the most similar pair of genera in our sample of Odontophorinae. The other genera in the subfamily, *Colinus*, *Oreortyx*, and *Cyrtonyx*, are each genically distinct, as evidenced by the level of separation on phenograms and branch lengths on F-M and Wagner trees. At αGpd-1, all members of the Odontophorinae are fixed for the same, apparently derived allele. Various groups of taxa in the subfamily are fixed for alleles not found in any non-Odontophorine taxon at Udh, Pept-1, Pept-2, and Est-2. The relationships among *P. colchicus*, *C. coturnix*, *C. virginianus*, *L. gambelii*, and *L. californicus* are similar to those suggested by Jolles et al. (1979) based on lysozyme sequence data.

DISCUSSION

Levels of genetic differentiation.—It is fairly well established that genetic differentiation among passerine taxa is low relative to other vertebrates (Avise et al. 1980c, Barrowclough et al. 1981). The reasons for this are unclear (see Sibley and Ahlquist 1982, Avise et al. 1980c). We believe that the most reasonable comparisons involve congeneric, interspecific levels of differentiation, when comparing *across* vertebrate classes (Zink 1982). Avian species limits are usually clearly defined, whereas higher taxonomic categories are far more arbitrary, especially across vertebrate classes. Thus, it is important to note that passerine congeners show little genic differentiation. Research should address this problem rather than differences between intergeneric or familial levels of genic divergence across vertebrate classes. Unfortunately, our only congeneric comparison, *L. gambelii-L. californicus*, is between probable sibling species.

Within a major vertebrate classes, comparisons of equivalent taxonomic levels above the species level may be more appropriate. The demonstration (Barrowclough and Corbin 1978, Barrowclough et al. 1981, Zink 1982, this study) that avian genetic distances increase as the taxonomic unit compared is more inclusive suggests that the taxonomic hierarchy reflects biological, or phylogenetic, units. We found that, at the generic and family levels, the galliforms are considerably more differentiated than passerine taxa (Table 2). We clearly require more comparisons of nonpasserine taxa

before we will understand whether or not nonpasserines present a different pattern from that of passerines. For example, the genetic distances we observed may be among the highest found in nonpasserines, but this remains to be documented.

Rates and dates of divergence.—The branch lengths of the Wagner (Fig. 2) and F-M (Fig. 3) trees can be interpreted as rough estimates of "rates" of genic divergence, thereby indicating lineages that have changed faster or slower relative to other lineages. Rates are averages across loci. The branch lengths (rates) in Figs. 2 and 3 appear homogeneous, although with missing extant taxa this is difficult to judge, because additional taxa might have evolved at different rates than the taxa we sampled. The branch lengths (Fig. 2) from the "most recent common ancestor" to members of the Odontophorinae range from 2.29 (to *C. squamata*) to 2.83 (to *O. pictus*), and the mean (±SD) equals 2.59 ± 0.17; thus, we suggest that evidence of rate heterogeneity is lacking among these taxa.

Nei's measure of genetic distance can be converted into approximate dates of divergence between taxa (Nei 1975). Nei suggested a "theoretical" conversion of $t = 5 \times 10^6 D$, where t is time since divergence from a common ancestor, and D is Nei's D-value. Yang and Patton (1981) used this conversion to estimate divergence dates among Galapagos finches. As Yang and Patton and Avise et al. (1980c) noted, other attempts to calibrate D-values (e.g. Sarich 1977) suggest that Nei's calibration may be low (i.e. too rapid) by a factor of four. Workers with other vertebrate groups (e.g. Maxson and Maxson 1979) have used the fossil record and independent estimates from microcomplement fixation studies to calibrate electrophoretic distances and divergence times, and such studies have tended to support Sarich (1977).

The fossil record of the galliforms allows what amounts to the first independent calibration of avian genetic distances. Several assumptions are made in our calibration. First, based on our reading of Holman's (1961, 1964) extensive osteological analyses, we assume that the Odontophorinae is a monophyletic group consisting of two subgroups: the *Odontophorus* group (consisting of *Odontophorus, Dactylortyx, Cyrtonyx,* and *Rhynchortyx*) and the *Dendrortyx* group (containing *Dendrortyx, Philortyx, Oreortyx, Colinus, Callipepla,* and *Lophortyx*). Derived character states (synapomorphies) support the monophyly of the Odontophorinae and each of the two subgroups.

Of interest here is *Cyrtonyx cooki,* an extinct species from the mid-Miocene [16 million yr before present (MYBP)] of Nebraska (Brodkorb 1964) and a congener of a species examined by us (*C. montezumae*). We assume here that this fossil belongs to the monophyletic lineage *Cyrtonyx,* and it is neither from the Odontophorine stock that pre-dated the *Dendrortyx-Odontophorus* split, nor is it a primitive (pleisomorph) early member of the *Dendrortyx* group. Thus, the age of *C. cooki* can be taken as a conservative estimate of the age of the *Cyrtonyx* lineage, represented in our study by *C. montezumae.* The average of D-values from *C. montezumae* to its sister taxa in the Odontophorinae (Fig. 1, Table 1), 0.609, is assumed to represent minimally 16 million yr (MY). This results in the following conversion: $t = 26.3 \times 10^6 D$. We use this calibration to indicate possible dates of divergence among the taxa shown in Fig. 1. We stress that this is a rough estimate (but probably conservative), owing to the variance of genetic distances between taxa. It is the first such approximation for an avian taxon.

Holman (1961) discussed a fossil, *Lophortyx shotwelli,* from Umatilla County, Oregon. This specimen is associated with mammalian remains from the Hemphillian stage, dated at 6 MYBP (Savage pers. comm.). Holman noted that this specimen possessed several characters unlike modern *Lophortyx,* and therefore its position in the evolutionary history of *Callipepla* and *Lophortyx* is uncertain. If this fossil is a "good" member of either *Callipepla* or *Lophortyx,* it would greatly alter our calibration of genetic distances. For instance, our estimated date of the divergence of *Callipepla* and *Lophortyx,* 2.8 MYBP, would necessarily be ≥6 MYBP, or twice our present estimate. Prager et al. (1974) suggested that phasianoid transferrins evolved at a rate of 0.97/MY. Prager and Wilson (1976) gave a transferrin immunological distance of 65 for *P. colchicus-C. virginianus,* or a divergence date of 63 MYBP. Our data ($D = 1.34$) suggest a divergence date of 35 MYBP. Unfortunately, this is the only comparison in common between our study and Prager and Wilson's (1976). It is of interest that their estimate of the divergence date for these two taxa is twice ours and in the same direction as our estimate would be if *L. shotwelli* was a valid *Lophortyx.* Clearly,

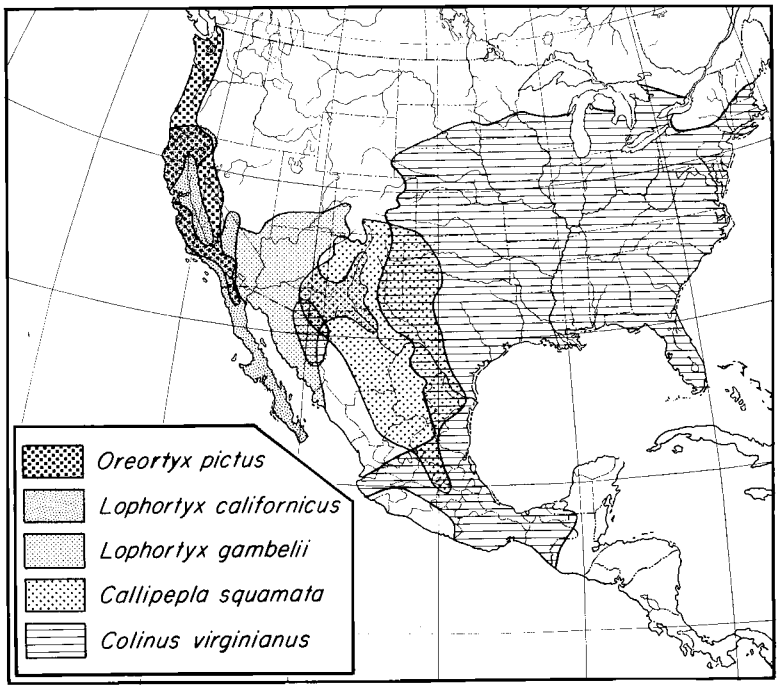

Fig. 4. Approximate breeding distributions (excluding introductions) of *Oreortyx pictus, Colinus virginianus, Callipepla squamata, Lophortyx gambelii,* and *L. californicus.* These species are all representatives of the subfamily Odontophorinae. Ranges taken from Leopold et al. (1981).

the discrepancy in our estimates deserves future attention, as does the phylogenetic position of *L. shotwelli.* We conclude at this time that Nei's (1975) conversion factor given above is probably low by a factor of five for the galliforms studied here. We note also that many assumptions, such as phylogenetic hypotheses of fossil and recent forms, need to be tested.

Biogeography of the Dendrortyx *group of the Odontophorinae.*—As mentioned above, the *Dendrortyx* group of the Odontophorinae consists of the genera *Dendrortyx, Philortyx, Oreortyx, Colinus, Callipepla,* and *Lophortyx.* Although we lack *Dendrortyx* and *Philortyx* in our genetic analysis, we will use the phylogeny of the remaining taxa and our approximate dating of cladogenetic events (Fig. 1) to develop an evolutionary perspective of the biogeography of these taxa. Without an objective estimate of phylogenetic relationships, it would be difficult to evaluate historical evolutionary patterns among these New World quail given simply a map of their current distributions (Fig. 4).

The earliest known fossil Odontophorinae are from the early Oligocene of Saskatchewan, the middle Oligocene of Colorado, and the lower Miocene (approximately 20 MYBP) of South Dakota (Brodkorb 1964). This suggests a fairly widespread distribution of ancestral Odontophorine stock. Therefore we will apply methods from vicariance biogeography (Nelson and Platnick 1981) to generate our biogeographic hypothesis. We assume first that the *Dendrortyx* and *Odontophorus* groups are distinct, monophyletic lineages (discussed above) that diverged at least 16 MYBP (middle Miocene). Next, we assume that *Dendrortyx* and *Philortyx* are primitive and/or have not affected the distribution of the remaining species. The restricted distribution of *Dendrortyx* (México to Costa Rica) and *Philortyx* (México) suggests relict status and, therefore, "primitiveness." Based on their skeletal morphology, Holman (1961) concluded that these two genera were the most primitive members of the *Dendrortyx* group. These assumptions obviously need testing.

The remaining steps in our biogeographic hypothesis are outlined *diagrammatically* in Fig. 5. The genetic data indicate that *O. pictus* orig-

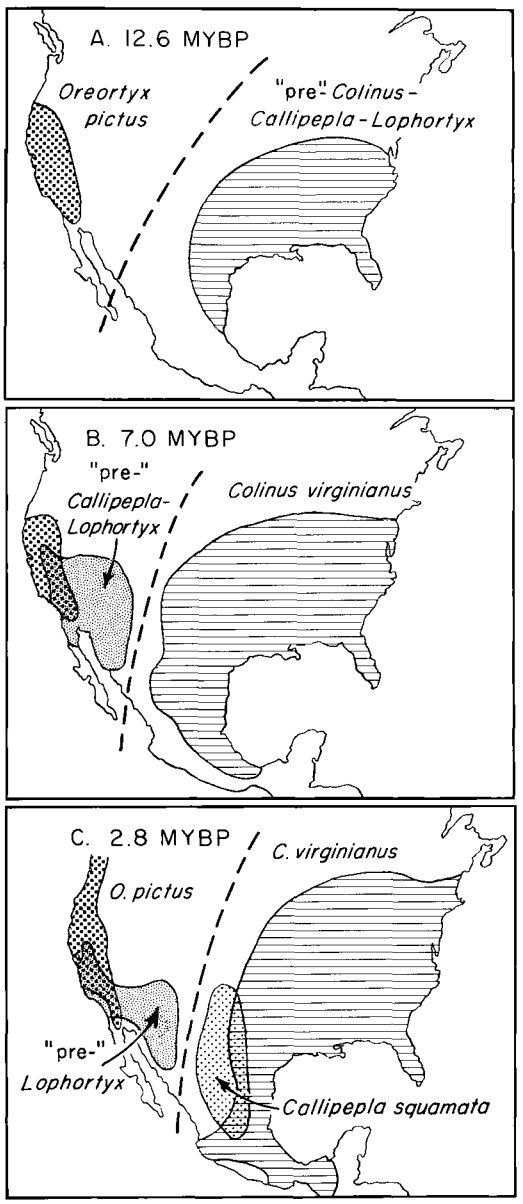

Fig. 5. Diagrammatic representation of hypothetical stages in the evolution of species shown in Fig. 4. Because historical ranges are unknown, ranges should not be interpreted strictly. The primary information we wish to convey is the isolation events, which are indicated by the dashed lines. The exact position of the dashed lines is speculative, however. Therefore, whether differentiation among these taxa was parapatric or allopatric and the extent of any gaps between taxa during their evolution are unknown. After each isolation event, dispersal probably occurred across these boundaries. A. Separation of *Oreortyx pictus*, 12.6 MYBP, presumably in areas consisting of evergreen chaparral, evergreen-broadleaf forest, or coniferous forest with shrub understory in western North America. B. Origin of *Colinus virginianus*, 7.0 MYBP. Present habitat is essentially weedy fields bordered by brush or woodlots, although in tropical lowlands it occurs in wetter conditions. The exact location of the origin of *C. virginianus* is uncertain. Note that the range of "pre" *Callipepla-Lophortyx* could have extended further east if *C. virginianus* was either isolated to the south or north. C. Divergence of *Callipepla squamata* and *Lophortyx*, 2.8 MYBP, probably concurrent with late Pliocene disjunct patches of arid and semi-arid desert. *C. squamata* and *L. gambelii* are typically found in desert scrub or arid grasslands, while *L. californicus* occurs in oak woodland, chaparral, and brushy foothills.

inated about 12.6 MYBP (Fig. 5a) and probably evolved in western North America, based on its current distribution (Fig. 4). Perhaps drying trends at this time (Axelrod 1979) resulted in the invasion of more mesic environments of higher elevation by *Oreortyx*. The southward displacement of land west of the San Andreas Fault at this time might also have isolated this taxon (see Wenner and Johnson 1980). Another east-west split of an ancestral taxon resulted in the divergence of *Colinus* (Fig. 5b), estimated by our data at 7.0 MYBP. The earliest known fossils of *Colinus* (Holman 1961, Brodkorb 1964) are from the upper Pliocene of Kansas. Because our estimated divergence date is well before upper Pliocene, the sites of fossil *Colinus* do not permit identification of the area of origin of *Colinus*.

Penultimately, *Callipepla* and *Lophortyx* diverged (Fig. 5c), about 2.8 MYBP, or late Pliocene, when their ancestor was fragmented in the southwestern aridlands of North America. Axelrod (1979) noted that during the late Pliocene isolated arid and semi-arid desert patches existed in the current Sonoran and Chihuahuan desert regions, and this could have allowed the allopatric differentiation of *Callipepla*, which presently occupies such habitats (Leopold et al. 1981). Species of *Lophortyx* diverged last, resulting in the current distribution patterns (Fig. 4). It will be of value to ascertain the phylogenetic position of *L. douglasii*.

Hubbard (1973) proposed a vicariant biogeographic explanation for the evolution of species in the genera *Lophortyx* and *Callipepla* (consid-

ered by him congeneric), which involved hypothetical ancestors and their distributions and Pleistocene glaciation cycles. According to Hubbard's scenario, a trichotomous split produced *C. squamata*, *L. douglasii*, and "pre-*californicus-gambelii*" in the Illinoian glacial epoch, with *L. californicus* and *L. gambelii* differentiating in the Wisconsinian glacial period. Using the genetic distance conversion established above, we estimate the split of *L. gambelii* and *L. californicus* at 190,000 yr ago, and that of *Lophortyx* and *Callipepla* at 2.8 MYBP, or roughly late Pliocene. While Hubbard is correct in assuming that a minimum of two geologic events (e.g. glacial-interglacial cycles) is needed to account for the distribution patterns of extant *Callipepla* and *Lophortyx*, these genera probably diverged well before the times he suggested. Illinoian age glacial cycles may well have effected speciation in *Lophortyx*, however.

This biogeographic reconstruction is a hypothesis. We note that sympatry of breeding species, which is considerable among the species studied here (Fig. 4), implies dispersal (Nelson and Platnick 1981). Thus, it is difficult to determine the relative importance of vicariance or dispersal in accounting for the evolutionary patterns in this group. Also, geological and paleobotanical evidence is sufficiently fragmentary (see Axelrod 1979, Wenner and Johnson 1980) to prevent precise correlations with our phylogenetic hypothesis. That is, we lack a well corroborated "area cladogram" (Nelson and Platnick 1981).

Our phylogenetic hypothesis and dating of cladogenetic events suggest that a series of three east–west range disjunctions (Fig. 5) could explain the evolution of these genera of New World quail. These patterns need to be corroborated with studies of other avian groups, as well as other groups of vertebrates and nonvertebrates. We believe, however, that our hypothesis (Fig. 5) is an important first approximation of the evolutionary history of these quail taxa and makes objective, testable predictions that would otherwise be difficult given only the map of current breeding distributions. We also wish to demonstrate that molecular methods of inferring phylogenies can be combined with information from the fossil record to further understanding of evolutionary patterns in avian groups.

Taxonomy.—Mayr and Short (1970) suggested that *L. gambelii* and *L. californicus* are conspecific. The genetic data presented here are consistent with this idea. Wild hybrids (Henshaw 1885, Miller and Stebbins 1964) between the forms include only the F_1 generation, however, which suggests a lack of introgression and panmixia in zones of sympatry. Furthermore, hybrids have been reported for other sympatrix quail [e.g. *Colinus virginianus* × *L. californicus* (Aiken 1930); *Callipepla squamata* × *L. gambelii* (Bailey 1928); *L. californicus* × *C. squamata* (Jewett et al. 1953); *C. virginianus* × *C. squamata* (Johnsgard 1973); *O. pictus* × *L. californicus* (Peck 1911)]. Incidence of hybridization is probably not an accurate predictor of close phylogenetic relationship, because birds retain the ability to hybridize despite considerable genetic divergence (Prager and Wilson 1975). The apparent lack of prereproductive isolating barriers in sympatry, at least to F_1 hybridization, does not necessarily indicate recency of common ancestry or close phylogenetic relationships among these quail. Therefore, we reject the notion that hybrids between *L. gambelii* and *L. californicus* prove conspecific status. As only the F_1 hybrids and not a hybrid swarm have been found, these are probably distinct biological species. We also point out that the evidence supporting apparent assortative mating in sympatry overshadows our finding a *D*-value typical of subspecies between these taxa. Clearly distinct species can be genically similar (Avise et al. 1980b, c).

Several authors (e.g. Mayr and Short 1970, Johnsgard 1973, and references therein) have suggested merging *Lophortyx* and *Callipepla*. These genera are clearly similar (Fig. 1). The $\bar{D}$ between our samples of these two taxa, 0.104, is one-half that reported by Barrowclough (1980) for similar avian intergeneric comparisons; yet it is within the range (0.0126–1.214). Thus, our data provide no clear-cut answers, and we suggest that the decision regarding their taxonomic status be made on the basis of other kinds of biological evidence.

The suggestion by Phillips et al. (1964) and Mayr and Short (1970) that *Oreortyx* be merged with *Callipepla* (including *Lophortyx*) is not consistent with our molecular data (Figs. 1–3). It would also require inclusion of *Colinus*; otherwise, the new taxon would not be monophyletic. We think that merging these taxa would obscure their relatively long, independent, evolutionary histories. We recognize, how-

ever, that there can be no absolute value of genetic distance on which to base taxonomic decisions. The external phenotypic resemblance of Mountain and California quail does not, in this case, indicate a close phylogenetic relationship. Furthermore, Hudson et al. (1959, 1966) and Holman (1961) provide data on the myology and skeletal morphology, respectively, that show that *Oreortyx* is morphologically distinct from other members of the *Dendrortyx* group. Gutiérrez (1980) reported that *Oreortyx* was very different ecologically from *Lophortyx*. Thus, genetic, morphologic, and ecologic data show that *Oreortyx* is not a close relative of *Lophortyx* or *Callipepla*, and there seems to be no basis for their generic merger.

The systematic status of *C. virginianus* is uncertain. It is shown here to be a distinct clade within the Odontophorinae and more similar to *Lophortyx* and *Callipepla* than to *Oreortyx* and *Cyrtonyx*.

Some authors (Brodkorb 1964, Mayr and Short 1970, Johnsgard 1973) consider the Tetraonidae a subfamily of the Phasianidae. Our data (Table 1) show that the distance from *T. pallidicinctus* (Tetraonidae) and *P. colchicus* (Phasianidae) to all other species is similar. Thus, it might be appropriate to consider such a taxonomic scheme.

Acknowledgments

We thank the following individuals for assisting in various ways with this project: John Davis, Richard D. Sage, Vincent Sarich, Don Straney, Allan C. Wilson, and J. W. Wilson. David E. Brown, Richard Brown, Larry Caudill, Michael Erwin, Jim Gerber, Dale Jones, Al Woodard, and Tom Zapatka either helped collect specimens or provided useful locality information. Ramone Baccus, George F. Barrowclough, John E. Cadle, Kendall W. Corbin, Ned K. Johnson, Allan Larson, A. Starker Leopold, Jill A. Marten, Gareth Nelson, James L. Patton, Duke S. Rogers, Charles G. Sibley, and David W. Steadman provided helpful comments on various drafts of this paper. Julie Feder assisted in the lab, and Jeanne A. Dawson typed the paper. Gene Christman drew the figures. Dr. Donald E. Savage provided dates on fossil localities. A Ford Foundation Fellowship to the first author, the Museum of Vertebrate Zoology, and the Union Foundation Wildlife Fund furnished financial support. RMZ was supported by NSF grant DEB-7920694 to N. K. Johnson.

Literature Cited

Aiken, C. E. H. 1930. A Bobwhite × California Quail hybrid. Auk 47: 80–81.

American Ornithologists' Union. 1957. Check-list of North American Birds, fifth ed. Baltimore, Maryland, Amer. Ornithol. Union.

———. 1973. Thirty-second supplement to the American Ornithologists' Union check-list of North American birds. Auk 90: 411–419.

Avise, J. C., J. C. Patton, & C. F. Aquadro. 1980a. Evolutionary genetics of birds. I. Relationships among North American thrushes and allies. Auk 97: 135–147.

———, ———, & ———. 1980b. Evolutionary genetics of birds. II. Conservative protein evolution in North American sparrows and relatives. Syst. Zool. 29: 323–334.

———, ———, & ———. 1980c. Evolutionary genetics of birds. Comparative molecular evolution in New World warblers and rodents. J. Heredity 71: 303–310.

Axelrod, D. I. 1979. Age and origin of Sonoran desert vegetation. Occ. Papers, Cal. Acad. Sci. 134: 1–74.

Ayala, F. J. 1975. Genetic differentiation during the speciation process. Evol. Biol. 8: 1–78.

Bailey, V. 1928. A hybrid Scaled × Gambel's quail from New Mexico. Auk 45: 210.

Barrowclough, G. F. 1980. Genetic and phenotypic differentiation in a wood warbler (Genus *Dendroica*) hybrid zone. Auk 97: 655–668.

———, & K. W. Corbin. 1978. Genetic variation and differentiation in the Parulidae. Auk 95: 691–702.

———, ———, & R. M. Zink. 1981. Genetic differentiation in the Procellariiformes. Comp. Biochem. Physiol. 69B: 629–632.

Brodkorb, P. 1964. Catalog of fossil birds: part 2 (Anseriformes through Galliformes). Bull. Florida State Mus. Biol. Sci. 8: 195–335.

Corbin, K. W. 1981. Genic heterozygosity in the White-crowned Sparrow: a potential index to boundaries between subspecies. Auk 98: 669–680.

Farris, J. S. 1972. Estimating phylogenetic trees from distance matrices. Amer. Natur. 106: 645–668.

Felsenstein, J. 1978. Cases in which parsimony or compatibility methods will be positively misleading. Syst. Zool. 27: 401–410.

Fitch, W. M., & E. Margoliash. 1967. Construction of phylogenetic trees. Science 155: 279–284.

Gutiérrez, R. J. 1980. Comparative ecology of the Mountain and California quail in the Carmel Valley, California. Living Bird 18: 71–93.

Hennig, W. 1966. Phylogenetic systematics. Chicago, Illinois, Univ. Illinois Press.

Henshaw, H. W. 1885. Hybrid quail (*Lophortyx gambelii* × *L. californicus*). Auk 2: 247–249.

Holman, J. A. 1961. Osteology of living and fossil New World quails (Aves, Galliformes). Bull. Florida State Mus. Biol. Sci. 6: 131–233.

———. 1964. Osteology of gallinaceous birds. Quart. J. Florida Acad. Sci. 27: 230–252.

HUBBARD, J. P. 1973. Avian evolution in the arid lands of North America. Living Bird 12: 155–196.

HUDSON, G. E., P. J. LANZILLOTTI, & G. D. EDWARD. 1959. Muscles of the pelvic limb in galliform birds. Amer. Midl. Natur. 61: 1–67.

———, R. A. PARKER, J. VANDEN BERGE, & P. J. LANZILLOTTI. 1966. A numerical analysis of the modifications of the appendicular muscles in various genera of gallinaceous birds. Amer. Midl. Natur. 76: 1–73.

JEWETT, S. G., W. P. TAYLOR, W. T. SHAW, & J. W. ALDRICH. 1953. Birds of Washington State. Seattle, Washington, Univ. Washington Press.

JOHNSGARD, P. A. 1973. Grouse and quails of North America. Lincoln, Nebraska, Univ. Nebraska Press.

JOHNSON, M. S., & J. L. BROWN. 1980. Genetic variation among trait groups and apparent absence of close inbreeding in Grey-crowned Babblers. Behav. Ecol. Sociobiol. 7: 93–98.

JOLLÈS, J., I. M. IBRAHIMI, E. M. PRAGER, F. SCHOENTGEN, P. JOLLES, & A. C. WILSON. 1979. Amino acid sequence of pheasant lysozyme. Evolutionary change affecting processing of prelysozyme. Biochem. 13: 2744–2752.

LEOPOLD, A. S., R. J. GUTIÉRREZ, & M. T. BRONSON. 1981. North American game birds and mammals. New York, Charles Scribner's Sons.

MAXSON, L. R., & R. D. MAXSON. 1979. Comparative albumin and biochemical evolution in plethodontid salamanders. Evolution 33: 1057–1062.

MAYR, E., & L. L. SHORT. 1970. Species taxa of North American birds. Publ. Nuttall Ornithol. Club 9: 1–127.

MILLER, A. H., & R. C. STEBBINS. 1964. The lives of desert animals in Joshua Tree National Monument. Berkeley, California, Univ. Calif. Press.

NEI, M. 1975. Molecular population genetics and evolution. Amsterdam, North Holland.

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

NELSON, G., & N. G. PLATNICK. 1981. Systematics and biogeography. New York, Columbia Univ. Press.

NEVO, E. 1978. Genetic variation in natural populations: patterns and theory. Theor. Pop. Biol. 13: 121–177.

PECK, M. E. 1911. A hybrid quail. Condor 13: 149–151.

PETERS, J. L. 1934. Check-list of birds of the world, vol. 2. Cambridge, Massachusetts, Harvard Univ. Press.

PHILLIPS, A. R., J. MARSHALL, & G. MONSON. 1964. The birds of Arizona. Tucson, Arizona, Univ. Arizona Press.

PRAGER, E. M., & A. C. WILSON. 1975. Slow evolutionary loss of the potential for interspecific hybridization in birds: a manifestation of slow regulatory evolution. Proc. Natl. Acad. Sci. 72: 200–204.

———, & ———. 1976. Congruency of phylogenies derived from different proteins. J. Molec. Evol. 9: 45–57.

———, A. H. BRUSH, R. A. NOLAN, M. NAKANISHI, & A. C. WILSON. 1974. Slow evolution of transferrin and albumin in birds according to micro-complement fixation analysis. J. Molec. Evol. 3: 243–262.

PRESCH, W. 1979. Phenetic analysis of a single data set: phylogenetic implications. Syst. Zool. 28: 366–371.

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

SARICH, V. M. 1977. Rates, sample sizes, and the neutrality hypothesis for electrophoresis in evolutionary studies. Nature 265: 24–28.

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

SIBLEY, C. G., & J. E. AHLQUIST. 1982. The relationships of the Yellow-breasted Chat (*Icteria virens*) and the alleged slowdown in rate of macromolecular evolution in birds. Postilla 187: 1–18.

SNEATH, P. H. A., & R. R. SOKAL. 1973. Numerical taxonomy. San Francisco, W. H. Freeman & Co.

TEMPLETON, A. R. 1980. Modes of speciation and inferences based on genetic distances. Evolution 34: 719–729.

WAKE, D. B. 1981. The application of allozyme evidence to problems in the evolution of morphology. Pp. 257–270 *in* Evolution today (G. G. E. Scudder and J. L. Reveal, Eds.). Proc. 2nd Internatl. Congr. Syst. Evol. Biol.

WENNER, A. M., & D. L. JOHNSON. 1980. Land vertebrates on the California Channel Islands: sweepstakes or bridges: Pp. 497–530 *in* The California Islands: proceedings of a multidisciplinary symposium (D. M. Power, Ed.). Santa Barbara, California, Santa Barbara Mus. Nat. Hist.

YANG, S. Y., & J. L. PATTON. 1981. Genic variability and differentiation in the Galapagos finches. Auk 98: 230–242.

ZINK, R. M. 1982. Patterns of genic and morphologic variation among sparrows in the genera *Zonotrichia*, *Melospiza*, *Junco*, and *Passerella*. Auk 99: 632–649.

APPENDIX 1. Location, sample sizes, and sample collection dates. Different populations of the same taxon are labeled by upper case letters, which correspond to the letters used in Appendix 2, Table 1, and the figures. The taxonomic framework is from Peters (1934).

Taxon	Number of individuals	Locality and date
Tetraonidae		
Lesser Prairie Chicken (*Tympanuchus pallidicinctus*)	13	New Mexico: 8 mi E Milnesand, Roosevelt Co., December 1975.
Phasianidae		
Phasianinae		
Ring-necked Pheasant (*Phasianus colchicus*)	13	California: Webb Tract, Sacramento Delta, Sacramento Co., November 1975.
Perdicinae		
Common Quail (*Coturnix coturnix*)	30	Avian Sciences Department, University of California, Davis, California, A—Big Brown Strain (n = 10). B—Small Brown Strain (n = 10). C—Albino Strain (n = 10). A, B, and C obtained in February 1976.
Chukar (*Alectoris chukar*)	12	Avian Sci. Dept., Univ. Calif., Davis. (n = 10), February 1976; 5 mi SE Panoche, San Benito Co., California (n = 2), Jan. 1976; samples combined.
Odontophorinae		
Dendrortyx Group		
California Quail (*Lophortyx californicus*)	36	California: A—5 mi E Shandon, San Luis Obispo Co. (n = 6), December 1974. B—5 mi N Jolon, Monterey Co. (n = 13), December 1974. C—4 mi E Mercy Hot Springs, Fresno Co. (n = 9), October 1975 and (n = 2) in January 1976; 5 mi SE Panoche, San Benito Co. (n = 6), January 1976.
Gambel's Quail (*L. gambelii*)	22	New Mexico: 1 mi E Columbus, Luna Co. (n = 19), December 1975. Arizona: 10 mi E Green Valley, Pima Co. (n = 3), January 1976; samples combined.
Scaled Quail (*Callipepla squamata*)	29	New Mexico: A—8 mi E Milnesand, Roosevelt Co. (n = 7), December 1975. B—1 mi E Columbus, Luna Co. (n = 20), December 1975. Arizona: 10 mi E Green Valley, Pima Co. (n = 2), January 1976; combined with population B as there was no differentiation.
Bobwhite Quail (*Colinus virginianus*)	15	New Mexico: A—8 mi E Milnesand, Roosevelt Co. (n = 8), December 1975. B—18 mi NE Milnesand, Roosevelt Co. (n = 7), December 1975.
Mountain Quail (*Oreortyx pictus*)	16	California: 2 mi SE Jamesburg, Monterey Co. (n = 16), June–August 1975.
Odontophorus Group		
Montezuma Quail (*Cyrtonyx montezumae*)	31	Arizona: A—15 mi E Patagonia, Santa Cruz Co. (n = 23), January 1976; B—10 mi W Patagonia, Santa Cruz Co. (n = 8), January 1976.

APPENDIX 2. Allelic frequencies for 23 presumptive loci in 10 species of gallinaceous birds. Numbers in parentheses are frequencies of alleles at a locus. A single letter denotes sample fixed for that allele. Full names for loci given in Yang and Patton (1981) or below. Names of taxa and sample sizes given in Table 1.

Locus	*T.p.*	*P.c.*	*C.c.* A	*C.c.* B	*C.c.* C	*A.c.*	*L.g.*	*L.c.* A
PGI	b	c	d (0.65) e (0.35)	d (0.60) e (0.40)	e	c	b	b
ADA[a]	g	b	d (0.35) f (0.65)	f	f	e	a (0.42) c (0.58)	a (0.06) c (0.94)
PGM-1	a	c	d	d	d	d	b	b
MPI	h	c	f	e (0.60) f (0.40)	f	a	e	e (0.94) g (0.06)
IDH-1	e	d	c	c	c	d	a (0.97) f (0.03)	a
IDH-2	g	b	g	g	g	a (0.30) c (0.70)	f	f
αGPD-1	d	c	e	e	e	a	b	b
αGPD-2	c	c	d	d	d	a	b	b
ME[b]	g	d (0.12) e (0.88)	a (0.25) b (0.75)	a (0.45) b (0.55)	a (0.40) b (0.60)	f	f	e (0.03) f (0.97)
GOT-1	i	j	h	h	h	a (0.45) b (0.05) c (0.50)	g (0.97) i (0.03)	g
GOT-2	a	b	b	b	b	a	b	b
GDH[c]	a	c	b	b	b	b	b	b
UDH[d]	d	d	d	d	d	d	c	c
SDH	a	e	a	a	a	b (0.15) c (0.85)	d	d
LDH-1	a	a	c	c	c	b	b	b
LDH-2	a	a	c	c	c	b	b	b
Pept-1	g	a	b	b	b	a	c	c
Pept-2	d	d	e	e	e	a	b	b
Alb	g	c	d (0.95) f (0.05)	c (0.40) f (0.60)	c	b	e	c (0.12) e (0.88)
Est-1	a	c (0.08) d (0.92)	e	d (0.60) e (0.40)	e	f	f (0.05) g (0.95)	f (0.03) g (0.97)
Est-2	g	g (0.38) h (0.62)	f	f	f	e	b	a (0.03) b (0.97)
6PGD	d	g	f (0.85) h (0.15)	e (0.05) f (0.80) h (0.15)	f (0.75) h (0.25)	c (0.10) d (0.90)	d	b (0.03) d (0.97)
XDH[e]	g	f	a (0.30) c (0.70)	b (0.05) c (0.95)	a (0.20) c (0.80)	c	a (0.08) c (0.92)	a (0.03) c (0.97)
Percentage polymorphism	0.0	11.1	22.2	25.9	11.1	14.8	18.5	29.6
Heterozygosity	0.0	2.6	4.8	8.9	4.8	5.2	2.5	2.8
Mean number alleles per polymorphic locus	—	2.0	2.0	2.1	2.0	2.2	2.0	2.0

[a] Adenosene de-aminase.
[b] Malic enzyme (NADP dependent).
[c] Glutamate dehydrogenase.
[d] Unidentified dehydrogenase.
[e] Xanthine dehydrogenase.

APPENDIX 2. Continued

L.c. B	*L.c.* C	*C.s.* A	*C.s.* B	*C.v.* A	*C.v.* B	*O.p.*	*C.m.* A	*C.m.* B
b	b	b	b	b	b	a	b	b
c	c	a	a (0.75) c (0.25)	a (0.56) c (0.44)	a (0.50) c (0.50)	e (0.97) g (0.03)	c	c
b	b (0.96) d (0.04)	c	c	c	c	b	c	c
e (0.83) g (0.17)	e (0.92) c (0.04) g (0.04)	e	e (0.75) g (0.25)	d	d	b	g (0.91) i (0.09)	g (0.94) i (0.06)
a	a	a	a	a	a (0.93) c (0.07)	a	b	b
f	c (0.11) f (0.89)	f	f	f	f	f	d (0.04) e (0.96)	e (0.94) g (0.06)
b	b	b	b	b	b	b	b	b
b	b	b	b	b	b	b	b (0.85) d (0.15)	b (0.87) d (0.13)
f	f	f	f	b (0.06) e (0.94)	e	c	e	e
g	g (0.92) i (0.08)	g (0.71) j (0.29)	f (0.05) g (0.70) j (0.25)	d (0.06) g (0.94)	g	g	g	e (0.13) g (0.87)
b	b	b	b	b	b	b	b	b
b	b	b	b	b	b	b	b	b
c	c	c	c	c	c	b	a	a
d	d	d	d	d	d	b (0.63) d (0.37)	b	b
b	b	b	b	b	b	b	b	b
b	b	b	b	b	b	b	b	b
c	c	c	c (0.97) f (0.03)	d	d	c (0.06) f (0.94)	e	e
b	b	b	b	b	b	b	c	c
c (0.08) e (0.92)	c (0.15) e (0.85)	e	e	a	a	e	a	a
g	g	g	g	h	h	f	a (0.83) b (0.15) c (0.02)	a (0.87) b (0.13)
b	b	b	b	b	b	s	c	b (0.19) c (0.81)
d	d	d	d	d	b (0.07) d (0.93)	d	d (0.96) f (0.04)	d
c	c	a (0.07) d (0.93)	a (0.10) d (0.90)	a (0.94) e (0.06)	a (0.86) e (0.14)	f	e	e
7.4	18.5	7.4	18.5	14.8	14.8	11.1	18.5	22.2
1.8	3.4	1.6	3.7	2.8	2.6	2.1	2.4	5.1
2.0	2.2	2.0	2.2	2.0	2.0	2.0	2.2	2.0