tudies in Avian Biology No. 22:51–60, 2001.

MOLECULAR SYSTEMATICS AND BIOGEOGRAPHY OF THE HAWAIIAN AVIFAUNA

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Abstract. The Hawaiian avifauna is exceptional for its high proportion of endemic taxa, its spectacular adaptive radiations, and its level of human induced extinction. Little has been known about the phylogenetic relationships, geographical origins, and timing of colonization of individual avian lineages until recently. Here we review the results of molecular studies that address these topics. Molecular data (mostly mitochondrial DNA sequences) are available for 14 of the 21 or more lineages of Hawaiian birds. We briefly review results of phylogenetic analyses of these data for lineages that have experienced major and minor radiations, and for single differentiated species and probable recent colonists. When possible, we determine the mainland species that are genetically most closely related. We find evidence that roughly half of the >21 lineages colonized from North America; not even a quarter appear to have come from South Pacific Islands. Our data also provide little evidence that Hawaiian bird lineages predate the formation of the current set of main islands (i.e., >5 Ma), as has been found for Hawaiian *Drosophila* and lobeliads.

Key Words: adaptive radiation; biogeography; Hawaiian avifauna; mitochondrial DNA; molecular systematics.

In 1943 Ernst Mayr published a short paper in *The Condor* summarizing his hypotheses about the geographic origins and closest living relatives of each known lineage in the Hawaiian avifauna. Mayr (1943) concluded that half of 14 hypothesized colonizations were of American origin and only two lineages arose from Polynesia. Therefore, although Hawai'i is considered part of the "Polynesian Region" because most of its biota and its human inhabitants had Polynesian ancestors, in terms of its birds Hawai'i is in the Nearctic Region. Since Mayr's paper, other authors have posited similar systematic hypotheses and biogeographic scenarios based on morphological, ecological, and distributional data (e.g., Amadon 1950, Pratt 1979, Berger 1981). Paleontology has offered only minor resolution of the relationships of ancestral lineages or the timing of speciation events; although there is an excellent Holocene fossil record in Hawai'i (Olson and James 1982a, 1991; James and Olson 1991), the pre-Holocene record is extremely limited (though one excellent fauna dates to >0.12 Ma ago; James 1987).

In recent years, molecular methods have proven extremely useful for inferring evolutionary relationships among taxa and the relative time frames during which taxa evolved (Avise 1994, Hillis et al. 1996). Inference from molecular data may be the best available way to reconstruct phylogenetic relationships and determine geographical origins and evolutionary time frames for Hawaiian taxa. In part this is because morphological or behavioral changes are often adaptive responses subject to natural or sexual selection (i.e., as part of the process of adaptive radiation), and they do not usually show constancy in their rates of change. Thus they can potentially mislead on issues of common ancestry via homoplasy. DNA sequences, on the other hand, while obviously not evolving in a perfect clocklike fashion (see below), do change over time, and evolve more continuously than morphology. Also, with the exception of a relatively few nonsynonymous changes within protein sequences, they generally evolve via mutation and drift (Nei 1987, Avise 1994), and are not as subject to homoplasy via convergence or stasis as are morphological or other characters. Thus major adaptive shifts in, for example, the bills of Hawaiian honeycreepers, may occur within some lineages (e.g., to thin and decurved in the nectarivorous 'I'iwi, *Vestiaria coccinea*), while not in others (e.g., conical and finchlike in the Laysan Finch, *Telespiza cantans*), in spite of an identical amount of time since evolving from their putatively "finch-billed" common ancestor. There are methods for detecting symplesiomorphic versus synapomorphic characters in phylogenetic analysis, but the higher variance in rates of change of morphological characters remains a problem for phylogenetic reconstruction (Hillis et al. 1996).

While there have been significant molecular investigations of particular Hawaiian plant and invertebrate taxa (especially *Drosophila*; e.g., Hunt and Carson 1983, DeSalle and Hunt 1987, DeSalle 1992), few molecular studies detailing evolutionary histories of the Hawaiian avifauna have been made until recently (e.g., Tarr and Fleischer 1993, 1995; Feldman 1994, Cooper et al. 1996; Fleischer et al. 1998, 2000, *this volume*; Paxinos 1998, Sorenson et al. 1999, Fleischer et al. in press, Rhymer *this volume*; C. Tarr, E. Paxinos, B. Slikas, H. James, S. Olson, A. Cooper, and R. Fleischer, unpubl. data).

TABLE 1. THE ELEMENTS OF THE HAWAIIAN AVIFAUNA

Taxon	Family	No. of species[a]	Geographic origin[b]	Comments[c]
Non-passeriformes:				
Ibises	Plataleidae	≥2	N.A.	minor radiation, flightless, *Apteribis*†
Night Heron	Ardeidae	1	N.A.	recent colonist, *Nycticorax nycticorax*
Moa-nalos	Anatidae	≥4	W. Hemisphere	minor radiation?, 3 flightless duck genera†
True Geese	Anatidae	≥3	N.A.	minor radiation, *Branta*,†**e**
Modern Ducks	Anatidae	2	N.A. and Asia	1 ± differentiated, 1 recent colonist, *Anas* **e**
Porzana Rails	Rallidae	≥12	Pacific/unknown	major radiation?, ≥2 colonizations†
Large rallids	Rallidae	2	N.A.?	recent colonists?, coot and moorhen
Black-necked Stilt	Recurvirostridae	1	N.A.	recent colonist, *Himantopus knudseni* **e**
Eagle	Acciptridae	1	Asia	recent colonist, *Haliaeetus leucophrys*†
Buteo	Acciptridae	1	N.A.	differentiated, *Buteo solitarius* **e**
Harrier	Acciptridae	1	Unknown	differentiated, *Circus dossenus*†
Long-legged Owls	Strigidae	4	Unknown	minor radiation, *Grallistrix* spp. 4†
Short-eared Owl	Strigidae	1	Unknown	recent colonist, *Asio flammeus sandwichensis*
Passeriformes:				
Crows	Corvidae	≥4	Unknown	minor radiation?, *Corvus* spp., 3†, 1 **e**
Millerbird	Sylviidae	1	South Pacific	differentiated, *Acrocephalus familiaris* **e**
ʻElepaio	Myiagridae	≥1	Australasia	differentiated, *Chasiempis sandwichensis*
Thrushes	Muscicapidae	5	W. Hemisphere	minor radiation, *Myadestes* spp., 3†, 1 **e**
Honeyeaters	Meliphagidae	≥6	South Pacific	minor radiation, *Moho* spp., *Chaetoptila*, all†
Honeycreepers	Fringillidae	≥50	Asia or N.A.?	major radiation, drepanidines, most† or **e**
>21 lineages	*13 families*	*≥102 species*		

[a] Number of species within each lineage/family, based on James and Olson (1991), Olson and James (1991), and H. James (pers. comm.).
[b] N.A. = North America; W = West.
[c] † denotes at least some members extinct; **e** denotes at least some members endangered.

Components of the Hawaiian avifauna vary greatly in the degrees to which they have speciated and become modified morphologically and ecologically (Table 1). For example, the Hawaiian drepanidines (Hawaiian finches or honeycreepers) have evolved incredible morphological, ecological, and behavioral diversity across more than 50 species and are one of the most often cited cases of adaptive radiation (Rothschild 1893–1900, Perkins 1903, Amadon 1950, Raikow 1977, Freed et al. 1987a, James and Olson 1991, Tarr and Fleischer 1995, Fleischer et al. 1998). Several species of extinct, large, flightless waterfowl (moa-nalos) show extreme morphological modification in their apparent shift into a ratite/grazing mammal/tortoise niche (Olson and James 1991; Sorenson et al. 1999). Other avian lineages have not speciated and have changed morphologically little or not at all from putative mainland relatives (e.g., Black-crowned Night Heron, *Nycticorax nycticorax hoactli*; Short-eared Owl or Pueo, *Asio flammeus sandwichensis*). Is this variance in levels of speciation and phenotypic differentiation related merely to the lengths of time that lineages have been evolving in the islands (Simon 1987, Car-

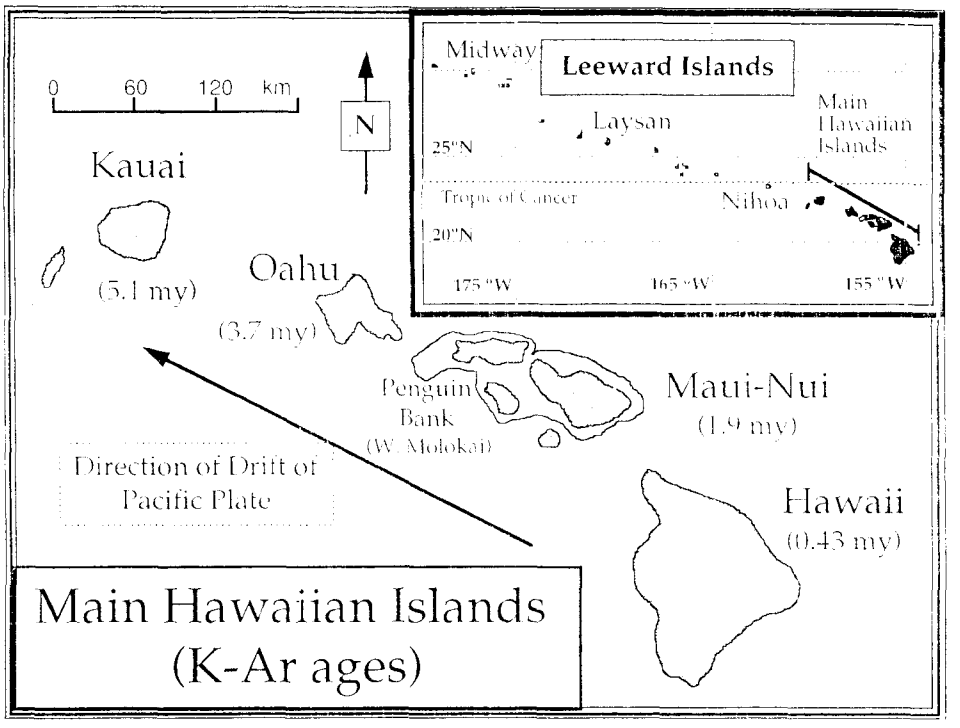

FIGURE 1. Map of the main Hawaiian Islands (plus inset map of main and leeward Hawaiian Islands). Ages of the oldest rocks from the main islands based on K-Ar dating are noted. Maui-Nui is composed of the islands of Maui, Lāna'i, Kaho'olawe, and Moloka'i, all of which were connected until about 0.3–0.4 Ma ago and again during more recent periods of low sea level.

son and Clague 1995)? Or are there other factors that have promoted stasis in some lineages and change in others, regardless of length of time in the islands? As noted above, the fossil record provides little resolution of this question. Thus, estimates of the age of separation from ancestors outside of the Hawaiian Archipelago, or the age of a radiation within the islands, can only be inferred from molecular data.

The Hawaiian Islands and its avifauna are extremely isolated from continental and other Pacific island avifaunas. This is likely the primary reason for the relatively low number of independent taxonomic avian lineages that occur in the islands (Mayr 1943, Pratt 1979). While the total number of such lineages has been increased (and continues to increase) from recent fossil findings (Olson and James 1982a, 1991; James and Olson 1991), the islands still appear to have far fewer independent avian lineages than one might expect for a tropical archipelago of this size and topographic diversity, and there may be additional factors involved that limit the primary diversity of the avifauna.

Here we summarize molecular and other data relevant to systematics and biogeography of the Hawaiian aviafauna. We first provide a brief overview of the geological history of the Hawaiian Archipelago and its utility for calibrating rates of molecular evolution (Tarr and Fleischer 1993, Fleischer et al. 1998). We then consider the origins and phylogenetic histories of each lineage within the avifauna, addressing extensive and minor radiations, well-differentiated single species, and undifferentiated (and likely recent) colonists. We also apply a molecular clock approach to obtain rough estimates of the maximum period of time that a lineage could have existed in the Hawaiian Islands.

GEOLOGICAL HISTORY AND THE CALIBRATION OF MOLECULAR EVOLUTIONARY RATES

The Hawaiian Islands have an unusual geological history (Clague and Dalrymple 1987, Walker 1990, Carson and Clague 1995; Fig. 1). They form as the Pacific Plate drifts northwest over a "hot spot" where magma extrudes from the earth's mantle through the crust to build huge shield volcanos (often to >4 km above sea level). The extreme weight of a new island, combined with the cooling of the crust as it moves away from the hot spot, causes a relatively rapid subsidence in island elevation and area. Subsidence continues slowly beyond this point, as does erosion, and islands shrink to become small coral and sand atolls and ultimately undersea mounts (Fig. 1).

The Hawaiian Islands are ordered by age in a linear pattern, with the oldest main island in the

northwest (Kaua'i at 5.1 Ma) and the youngest in the southeast (Hawai'i at 0.43 Ma; Fig. 1). This volcanic conveyor belt provides an exceptional system for evolutionary studies, as it sets up a temporal framework that can be used to estimate the timing of evolutionary events and rates of evolution. The age of an island is the maximum age for a population inhabiting the island. These ages can be used to calibrate rates of molecular change if phylogenies reveal that the pattern of cladogenesis parallels the timing of island formation, and if populations colonize near to the time of island emergence (Bishop and Hunt 1988, Tarr and Fleischer 1993, Givnish et al. 1995, Fleischer et al. 1998).

We used this rationale to calibrate part of the mitochondrial cytochrome *b* (cyt *b*) gene in Hawaiian drepanidines (Fleischer et al. 1998). The overall rate of cyt *b* divergence, corrected for minor saturation, transition bias, rate variation among sites, and potential lineage sorting is 1.6% sequence divergence/Ma. This value is similar to a rate we estimated for overall restriction site divergence in mitochondrial DNA (mtDNA) in drepanidines (~2%/Ma; Tarr and Fleischer 1993). Note that rates calibrated using this approach are based on a time period of divergence up to only about 4 Ma. Recently, Moore et al. (in press) showed through simulation modeling that cyt *b* sequence divergence is accurate as a predictor of time of divergence only to about 5 Ma (i.e., about 10% overall sequence divergence). Predictions of dates older than 5 Ma are generally underestimated. Nonlinearity of sequence divergence due to saturation and rate variation among sites appears to become problematic above about 10% overall sequence divergence for birds (Krajewski and King 1996, Randi 1996, Moore and DeFilippis 1997). Thus the drepanidine or other cyt *b* rates are not likely to be applicable to events that happened appreciably earlier than 5 Ma, and caution must be exercised when making predictions or calibrations from cyt *b* sequence divergences over 10%.

Our drepanidine rates (Tarr and Fleischer 1993, Fleischer et al. 1998) are within the range of estimates for avian and mammalian taxa based on calibrations derived from relatively recent fossil evidence of cladogenesis. This is true for both restriction fragment length polymorphisms (RFLPs) in total mtDNA and sequence divergence in the cyt *b* gene. Examples of avian rates include RFLP variation in geese at ~2%/Ma (Shields and Wilson 1987); cyt *b* sequences in partridges versus *Gallus* at 2.0%/Ma (Randi 1996; however, Arbogast and Slowinski [1998], corrected the divergences using an HKY [Hasegawa et al. 1985] model with a Γ-correction to obtain a rate of about 5.0%/Ma); RFLP variation in New World quail at 2.0%/Ma (reported in Klicka and Zink 1997); woodpecker cyt *b* at 2.0%/Ma (Moore et al. in press); cyt *b* in cranes at 0.7%/Ma for Balearicines versus Gruines (old split) and up to 1.7%/Ma for comparisons within the Gruines (Krajewski and King 1996); and cyt *b* in albatross at 0.65%/Ma (Nunn et al. 1996, recalculated for total sequence change in Klicka and Zink 1997). In the crane and albatross studies the slower rates could be caused by the longer generation times in these species, or perhaps by reduced metabolic rates in these larger-bodied taxa (Martin and Palumbi 1993, Rand 1994, Bromham et al. 1996, Nunn and Stanley 1998). Alternatively, the difference may relate to the fossil dates used for calibration: for both studies these dates are older than 10 Ma, whereas for all but the partridge/*Gallus* comparison (Randi 1996) the dates are before 5 Ma. Both studies attempt to correct for saturation (Krajewski and King 1996, Nunn et al. 1996), but may severely underestimate divergence (Arbogast and Slowinski 1998). This could be considered an inverse prediction of the findings of Moore et al. (in press): using dates older than 5 Ma to calibrate may result in an underestimate of the rate. Supporting this is a negative correlation between divergence times and divergence rates (Spearman rho = −0.51, P = 0.042) from Table 2 of Martin and Palumbi (1993). Avian rates are similar to most mtDNA/cyt *b* rates calculated for mammal taxa (e.g., ~2%/Ma; Brown et al. 1979, Irwin et al. 1991, Stanley et al. 1994, Janacek et al. 1996).

In general, then, calibrated rates of mtDNA protein coding sequence divergence in birds and mammals do not appear to vary greatly from about 2%/Ma. Most rate variation appears to be correlated with variation in body size and its correlates (i.e., metabolic rate, generation time; Martin and Palumbi 1993, Rand 1994), although some of the variation may be due to differing selective constraints on proteins in different lineages or to fluctuations in population size (Ohta 1976). In summary, with the exception of the very rapidly evolving control region (which in some sections may be evolving an order of magnitude faster than the average for mtDNA; e.g., Quinn 1992), most avian and mammalian rate calibrations based on corrected mtDNA divergence and dates before 5 Ma ago reveal rates at about, or above, 2% divergence/Ma. Based on the rather detailed rationale described above we feel that mtDNA (RFLP or cyt *b*) sequence divergence between a Hawaiian taxon and its closest non-Hawaiian relatives that is below about 10% would indicate an origin near the time of or after the formation of the island of Kaua'i.

ORIGINS AND EVOLUTION OF THE HAWAIIAN AVIFAUNA

There were more than 102 species of native breeding land- or waterbirds (i.e., non-seabirds) in the Hawaiian Islands (Table 1; constructed from James and Olson 1991, Olson and James 1991; and H. James, pers. comm.). These 102 species sort into six songbird families (Passeriformes) and seven non-songbird families (Table 1). Some families have a relatively large number of species (i.e., >4) and, in some cases, it is fairly clear that each group of species in a family represents an in situ radiation from a single colonization (e.g., drepanidines, thrushes). It is clear that in some families (e.g., anatids, rallids) there has been more than a single colonization event, while for others (e.g., corvids, meliphagids) it is difficult to determine how many independent colonization events have occurred.

Avian biologists working in the islands have been fortunate to have an excellent Holocene fossil record (Olson and James 1982a, 1991; James and Olson 1991). Without this record, we would be missing a tremendous amount of information about distributions, phylogeny, biogeography, and ecology of these birds. Even so, additional fossil taxa continue to be discovered and, thus, our knowledge remains incomplete. The advent of genetic studies employing the polymerase chain reaction (PCR) has opened a new and exciting avenue for study of these fossils. Our laboratory has had considerable success amplifying mtDNA sequences from these subfossil remains. Here we summarize what has been learned about the evolution of Hawaiian birds from phylogenetic analyses of mtDNA sequences from a number of extinct and extant taxa.

EXTENSIVE RADIATIONS

The drepanidines (Hawaiian finches or honeycreepers) are by far the most speciose group in Hawai'i, with 33 species known from historical collections and more than 17 known from subfossil remains (totaling over 50 species; James and Olson 1991; H. James, pers. comm.). The drepanidine radiation is remarkable for its extreme morphological, ecological, and behavioral diversity (Rothschild 1893–1900, Perkins 1903, Amadon 1950, Baldwin 1953, Raikow 1977, Pratt 1979, Freed et al. 1987a, James and Olson 1991). However, major adaptive shifts appear to have modified many characters traditionally used for phylogenetic reconstruction, while others less subject to selection have been conserved and provide little or no phylogenetic information. The somewhat chimeric associations of morphological traits in the group have even led to the suggestion that the drepanidines are not monophyletic (Pratt 1992a,b). Molecular data may prove especially useful for assessing evolutionary relationships in this group, and they do support a cardueline ancestry and, thus far, monophyly of the drepanidines (Fleischer et al. 1998; Fig. 2c).

Molecular data may also be effective in estimating a time frame for the drepanidine radiation. The radiation of the drepanidines would seem quite deep based on their relative degree of phenotypic diversity. Molecular evolutionary rate estimates based on DNA-DNA hybridization data (Sibley and Ahlquist 1982) are in support of this prediction with an estimated split of drepanidines from a cardueline outgroup of about 15–20 Ma. Molecular rate estimates from both allozyme (Johnson et al. 1989, Fleischer et al. 1998) and mtDNA data (Tarr and Fleischer 1993, 1995; Fleischer et al. 1998), however, strongly contradict the results of Sibley and Ahlquist (1982) and suggest a basal split that began about 4 Ma ago and a separation from a mainland cardueline ancestor (not necessarily the closest outgroup; Fig. 2c) of <5–6 Ma ago. These mtDNA results are based on several internal rate calibrations estimated as outlined above for cyt *b*. Sibley and Ahlquist's (1982) results may be biased by their use of continental biogeographic points in their calibration (Quinn et al. 1991) or by use of too distant outgroups for comparison.

No other avian radiation in Hawai'i is so diverse in morphology or number of lineages as the drepanidines. Extinct flightless rails, classified as *Porzana* (Olson and James 1991), included perhaps more than 12 species, with as many as three species on each major island. Until recently it has not been clear whether these species comprise a single highly radiated clade, or represent a number of independent colonizations from mainland or other Pacific island sources. Molecular phylogenetic analyses (B. Slikas, S. Olson, R. Fleischer, unpubl. data) indicate that each of the two historically collected *Porzana* species resulted from independent colonizations. For *Porzana palmeri* the Kimura 2-parameter corrected distance (Kimura 1980; distance and SE calculated in MEGA, Kumar et al. 1993) for 197 base pairs (bp) of ATPase8 was 2.1 ± 1.1% distant from its closest non-Hawaiian *Porzana* relative. For *P. sandwichensis* the ATPase8 Kimura 2-parameter corrected distance was 5.9 ± 1.8% to its closest non-Hawaiian *Porzana* relative. Molecular analyses of *Porzana* taxa known only from subfossil remains are underway.

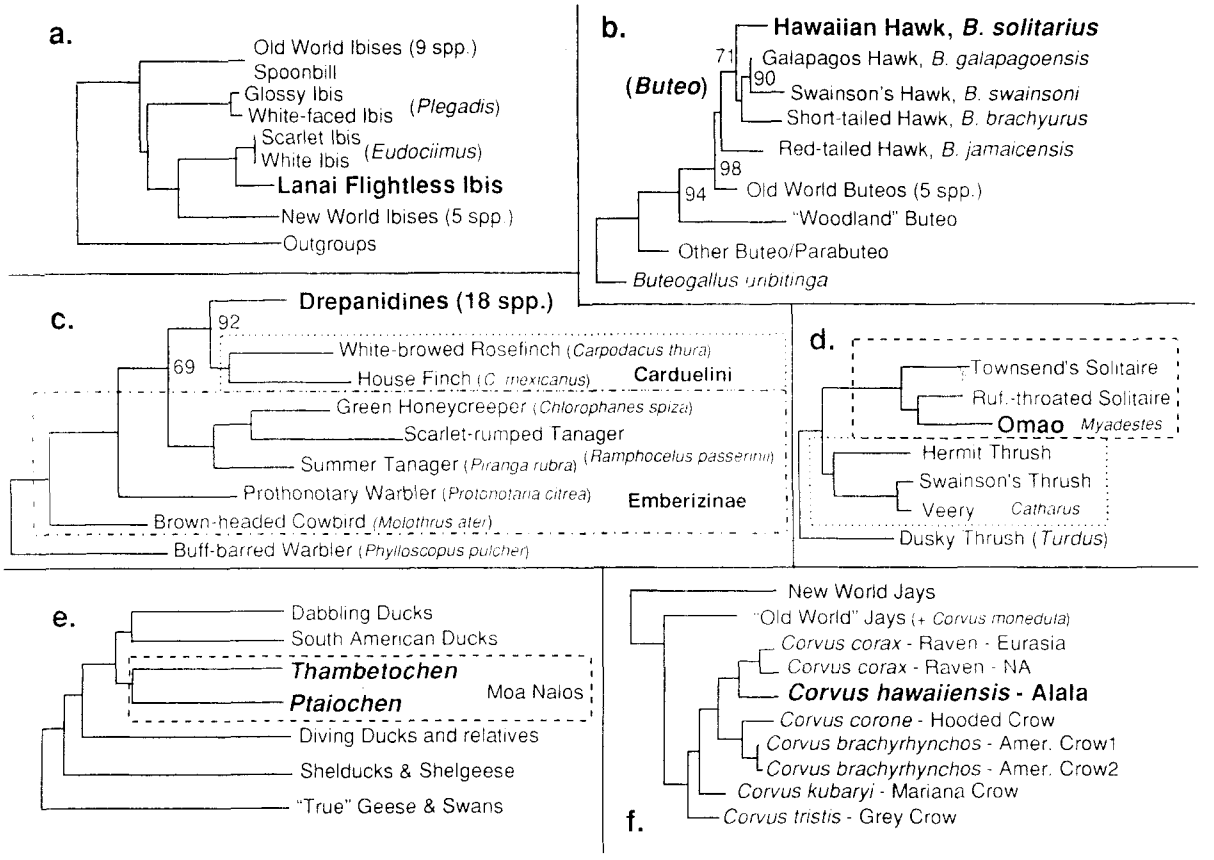

FIGURE 2. Abbreviated phylogenetic reconstructions for six Hawaiian taxa. a. Summarized maximum parsimony tree based on 407 nucleotide sites of 12s ribosomal RNA (A. Cooper, S. Olson, H. James, R. Fleischer, unpubl. data). b. Summarized parsimony phylogram based on preliminary analysis of over 1500 bp of mtDNA sequence (ATPase8, ND2, cyt *b*, and COI) in *Buteo* and related taxa (R. Fleischer, P. Cordero, C. McIntosh, I. Jones, and A. Helbig, unpublished). c. Summary of relationships of outgroups and drepanidines based on parsimony analysis of 675 bp of cyt *b* sequence. d. Parsimony phylogram constructed from 700 bp of cyt *b* sequence from two *Myadestes* and three *Catharus* taxa with 'Ōma'o and *Turdus* outgroup. e. Parsimony tree of two moa-nalo genera and a wide sampling of other waterfowl taxa showing two moa-nalo genera to be sister taxa and related to dabbling ducks. Tree is summarized from Sorenson et al. (1999), and based on over 1200 bp of mtDNA sequence. f. Parsimony phylogram showing summary of jay relationships to *Corvus* and a sampling of *Corvus* taxa based on 1008 bp of cyt *b*. The 'Alalā is most closely related to the Common Raven.

MINOR RADIATIONS

Seven other Hawaiian avian groups have undergone what appear to be minor radiations, each with fewer than six species (Table 1). These include thrushes (genus *Myadestes*), honeyeaters (genera *Moho* and *Chaetoptila*), a lineage of owls (genus *Grallistrix*), several crows (genus *Corvus*), flightless ibises (genus *Apteribis*), and two waterfowl (Anatidae) lineages: true geese (genus *Branta*) and the highly modified dabbling duck relatives called "moa-nalos" (genera *Chelychelynechen, Ptaiochen,* and *Thambetochen*).

The five species of thrushes were placed originally in their own genus, *Phaeornis,* but were considered aligned with solitaires (*Myadestes*; Stejneger 1887, Amadon 1950), robins (*Turdus*) or nightingale-thrushes (*Catharus*; Ripley 1962). Most of the morphological and other evidence (e.g., Kepler and Kepler 1983) clearly favors placement of thrushes in *Myadestes* (Pratt 1982). We analyzed variation in about 700 bp of the cyt *b* gene of mtDNA (C. McIntosh and R. Fleischer, unpubl. data), for the Hawai'i Thrush (or 'Ōma'o, *M. obscura*), three *Catharus,* two American *Myadestes* and a *Turdus* species, along with outgroup taxa. The resulting trees clearly place the 'Ōma'o within the *Myadestes* clade, regardless of the tree building algorithm (i.e., maximum parsimony, Fig. 2d; maximum likelihood or minimum evolution). We could not resolve with certainty using this data set whether the 'Ōma'o is more closely related to *M. genibarbis,* a Caribbean solitaire, or *M. townsendi* of western North America. The Kimura 2-parameter corrected distance between the 'Ōma'o and the solitaires is 6.7% for the 700 bp.

The meliphagid genera *Chaetoptila* (Kioea; 2 spp.) and *Moho* (the 'Ō'ōs; 4 spp.) may represent independent colonizations from south Pacific meliphagids (Perkins 1903), although Mayr (1943) considers both genera derived from a single colonist. One species of *Moho* occurs on each of Kaua'i, O'ahu, Maui Nui (Maui, Lāna'i, Moloka'i, and Kaho'olewa), and Hawai'i, and this well-differentiated lineage (Pratt 1979) may provide an opportunity to estimate a rate calibration. The closest sister groups for the Hawaiian meliphagids are unknown, with some authors suggesting *Gymnomyza* of Fiji and Samoa (e.g., Mayr 1943) and others favoring *Foulehaio* of Samoa or the New Zealand tui's (*Prosthemadera*; e.g., Munro 1944, Pratt 1979). Molecular studies are underway to address the origin and monophyly of the Hawaiian forms and the possibility of a rate calibration from the four *Moho* species. A calibration could be used to estimate the date of separation from the most recent common ancestor. This date is important because we estimate from our drepanidine calibrations that nectarivorous drepanidines evolved only 2–3 Ma ago, while Givnish et al. (1995) used a calibration of chloroplast DNA restriction fragment variation to estimate that bird-pollinated flowering lobeliads (genus *Cyanea*) evolved 8–17 Ma ago. Thus it is highly unlikely that drepanidines "coevolved" with these plants in the islands (as was suggested by Givnish et al. 1995). The meliphagids are the only other known native, obligate nectarivores in the islands and, if they are older, could be the coevolved taxon.

At least four crows (*Corvus*) occurred in the islands (James and Olson 1991; H. James, pers. comm.). Three of these are known only from subfossils; two of which have been described and the fourth is the highly endangered Hawaiian Crow (*Corvus hawaiiensis*), hereafter referred to as 'Alalā. It is unclear at present whether these represent a single colonization and subsequent radiation, or multiple colonizations by the same or different ancestral taxa (James and Olson 1991). Preliminary phylogenetic analyses of the 'Alalā and seven other *Corvus* taxa indicate that it is more closely related to the Common Raven (*Corvus corax*) than to more typical crows, including two South Pacific island crows (R. Fleischer and C. McIntosh, unpubl. data; Fig. 2f). The Kimura 2-parameter corrected sequence divergence for 1,008 bp of cyt *b* between 'Alalā and North American Common Raven is about 8.4 ± 1.0%.

Subfossil bones and owl pellets are all that remain of four species of long-legged owls (*Grallistrix*) that apparently were morphologically adapted to feeding on birds. While no DNA analyses have yet been made on this group, it appears likely that they represent the results of a single colonization and subsequent minor radiation.

At least four lineages of waterfowl have colonized the Hawaiian Islands. Of these, only two, the moa-nalos (Olson and James 1991, Sorenson et al. 1999) and the modern geese (*Branta*; Olson and James 1991, Paxinos 1998; E. Paxinos et al. unpubl. data), have speciated beyond a single endemic species. All of the moa-nalos evolved to very large size, flightlessness, and highly modified cranial morphology. They have become convergent in morphology to ratites in terms of postcranial morphology, and one species in particular has converged to tortoise-like cranial morphology. Like the moas of New Zealand (Darwin 1859), the moa-nalos occupied a grazing mammal or tortoise niche (Olson and James 1991). One genus and species (*Chelychelynechen quassus,* the Turtlejawed Goose) is restricted to Kaua'i and one (*Ptaiochen*) to Maui, but *Thambetochen* is found on both Maui Nui and O'ahu, suggesting the genus may have originated on O'ahu and later walked across the Penguin Bank land bridge (Fig. 1) to Moloka'i. No moa-nalo is known from the young island of Hawai'i (but see below).

Olson and James (1991) suggested that the moa-nalos were related to either dabbling ducks or shelducks (tadornines) on the basis of skeletal characters, primarily the presence and shape of their syringeal bullae. Livezey (1996) tentatively concluded from a cladistic analysis of morphology that the moa-nalos were sister to a "true" geese and swan clade, and not to anatids. Mitochrondrial DNA analyses for two of the three genera (*Thambetochen* and *Ptaiochen*; Sorenson et al. 1999) have provided a phylogenetic hypothesis and estimates of minimum genetic divergence from anatid outgroups. The two genera form a well-supported clade that is itself sister to the "dabbling" ducks, although perhaps somewhat more similar to several South American *Anas* or *Anas* relatives than to North American dabblers (Fig. 2e). Molecular data do not support a close relationship with either tadornines or true geese. The distance between the moa-nalos and their closest anatid outgroup, based on 1,009 mtDNA sites, is 6.9 ± 0.5%.

The Nēnē or Hawaiian Goose (*B. sandvicensis*) is the only extant representative of what appears to be a minor radiation of *Branta* in the islands (Olson and James 1991, Paxinos 1998; E. Paxinos et al., unpubl data.). Nēnē are clearly derived from Canada Geese (*B. canadensis*; Quinn et al. 1991), and distances based on mtDNA restriction fragment and cyt *b* sequence data suggest that the two taxa shared a common

ancestor sometime within the past 1 Ma (Quinn et al. 1991). At least two, and probably more than three additional *Branta* species existed in the islands (Olson and James 1991, Paxinos 1998; E. Paxinos et al., unpubl. data). One of these, the "very large Hawai'i goose" is the largest land vertebrate known from Hawai'i and is restricted in distribution to the island of Hawai'i (Giffin 1993). The species is highly modified morphologically with a massive body, short, stout wings (it was flightless, but may have used its wings for fighting; S. Olson, pers. comm.); and cranially quite similar to the moa-nalos. In fact, it appears to be a superb example of convergent evolution to the moa-nalos. Mitochrondrial DNA sequence analyses (Paxinos 1998) strongly support placement of the very large Hawaiian goose *Branta* and also indicate a sister taxon relationship with the Nēnē and its close, larger relative, *B. hylobadistes*.

Two species of ibis (*Apteribis*) have been described from subfossil material (Olson and Wetmore 1976, Olson and James 1991). *Apteribis* had stouter legs and shorter wings than other ibises and were flightless. The two or more species were limited to Maui Nui, and the disconnection of Maui, Lāna'i, and Moloka'i 0.3–0.4 Ma ago may have initiated the speciation event(s). Analyses of mitochondrial 12S ribosomal DNA sequences of *Apteribis* and 21 other ibis species (Fig. 2a; A. Cooper, S. Olson, H. James and R. Fleischer, unpubl. data) indicate that the closest sister taxon to *Apteribis* is the New World White Ibis (*Eudocimus albus*). The Kimura 2-parameter pairwise distance between the two taxa for 407 bp of 12S rRNA sequence is 3.2 ± 1.0%.

SINGLE DIFFERENTIATED SPECIES

Two raptors, a duck, and two songbirds represent single differentiated species. These taxa apparently colonized the islands and differentiated considerably from their ancestors but did not undergo subsequent speciation. The two raptors are the endangered Hawaiian Hawk or 'Io (*Buteo solitarius*) and an extinct accipiter-like harrier (*Circus dossenus*). The 'Io is currently restricted to the island of Hawai'i but has been found in fossil form on other islands (Olson and James 1991; S. Olson, pers. comm.). Like many other species of *Buteo,* the 'Io exhibits a light and a dark color morph. Preliminary phylogenetic analyses of more than 1,500 bp of mtDNA sequence in 18 species of *Buteo* (R. Fleischer, P. Cordero, C. McIntosh, I. Jones, and A. Helbig, unpubl. data) provides weak support for a clade containing the 'Io, the North American Short-tailed Hawk (*Buteo brachyurus*; to which it is least divergent; Fig. 2b), the North American Swainson's Hawk (*Buteo swainsoni*; as suggested by Mayr 1943), and the endemic Galápagos Hawk (*Buteo galapagoensis*). The 'Io does not have a close relationship with any Old World *Buteo* we assessed. The Kimura 2-parameter (Kimura 1980) corrected sequence divergence from *Buteo brachyurus* is only 1.4 ± 0.8% for part of cyt *b*. We have no molecular data for the extinct and highly modified *Circus*.

The Laysan Duck (*Anas laysanensis*) is a relatively differentiated, small duck whose very small and vulnerable wild population inhabits only the tiny leeward island of Laysan. It has been consistently classified as either a subspecies of the Hawaiian Duck (*Anas wyvilliana*), hereafter referred to as Koloa, or of the Mallard (*Anas platyrhynchos*) on the basis of morphology and allozyme data (see Amadon 1950, Livezey 1991, Browne et al. 1993). Recent DNA analyses (Cooper et al. 1996; J. Rhymer, unpubl. data), however, have strongly countered the above scenarios, indicating instead that the Laysan Duck is differentiated from the Koloa and Mallard and may be more closely aligned with the South Pacific Black Duck (*Anas superciliosa*) clade. The Koloa, on the other hand, does cluster closely with the North American Mallard or Mottled Duck (*Anas fulvigula*) clades. Analyses of mitochondrial control region sequences of subfossil bones (Cooper et al. 1996) have also revealed that the Laysan Duck occurred in the main Hawaiian Islands well into the period of Polynesian settlement, and in forested habitats and higher elevations (>1,500 m) not considered typical for a dabbling duck. The level of mitochondrial control region sequence divergence between the Laysan Duck and its closest outgroup taxon is about 10%; overall mtDNA divergence is lower than this (J. Rhymer, unpubl. data).

The fourth "nonradiating" species, the 'Elepaio (*Chasiempis sandwichensis*), is polytypic at the subspecies level and occurs on the islands of Kaua'i, O'ahu, and Hawai'i (enigmatically, no fossils have been found of this species on Maui Nui; James and Olson 1991). The 'Elepaio is likely related to Polynesian flycatchers in the genus *Monarcha* (Mayr 1943, Amadon 1950) and is one of the few species for which differentiated subspecies have been identified on a single small island (Hawai'i; Pratt 1980). Molecular analyses of each island subspecies may, however, reveal differentiation sufficient to elevate them to species level.

PROBABLE RECENT COLONIZATIONS

Several taxa show little phenotypic divergence from mainland outgroups, suggestive of a very recent colonization (Table 1). These in-

clude the Black-necked Stilt (*Himantopus mexicanus knudseni*), Hawaiian Coot (*Fulica alai*), Common Moorhen (*Gallinula chloropus sandvicensis*), Koloa, Black-crowned Night Heron (*Nycticorax nycticorax hoactii*), an eagle (*Haliaeetus*), and the Short-eared Owl. Of these, only the Black-crowned Night Heron is not currently considered to be distinct from mainland forms at the subspecies or species levels, but the Short-eared Owl, in spite of its subspecific designation, is thought to be a post-Polynesian colonist (Olson and James 1991).

The Common Moorhen, Hawaiian Coot, Black-crowned Night Heron, and Short-eared Owl are extremely similar morphologically to outgroup relatives (Amadon 1950), but no DNA data currently exist with which to assess the age of their splits. As noted above, the Koloa is a very close relative of the Mottled Duck and Mallard (<3% mitochondrial control region divergence; Cooper et al. 1996). The endemic subspecies of the Black-necked Stilt differs from North American Black-necked Stilts (*H. m. mexicanus*) by only about 1.5 + 0.6% sequence divergence in 447 bp of mtDNA control region (R. Fleischer et al., unpubl. data). The North American Black-necked Stilts are considered to be the closest mainland relatives on the basis of morphology. Cyt *b* and 12S rRNA sequences from a subfossil bone of the extinct eagle (*Haliaeetus* sp.; Fleischer et al. 2000) are not different from the Old World White-tailed Eagle (*H. albicilla*), and the two species differ by 1.5% for the ATPase8 gene. Skeletal characteristics could not differentiate the Hawaiian eagle bones from either White-tailed Eagle or Bald Eagle (*H. leucocephalus*; Olson and James 1991). Thus, for at least three of these seven taxa the supposition of a recent split from a mainland ancestor and recent arrival in the islands is supported by the molecular data.

SUMMARY: GEOGRAPHIC ORIGINS AND TEMPORAL FRAMEWORK

Above we summarize recent molecular systematic studies of the Hawaiian avifauna. We use these data to infer, if possible, the closest living relatives and the geographic origins of the Hawaiian taxa we sampled. Our biogeographic analyses indicate (Table 1) that at least 9 or 10 of the ≥ 21 independent lineages appear to be of North American or at least Western Hemisphere origin, 4 appear to be of South Pacific or Australasian origin, 2 or 3 are of Asian origin, and 5 are of currently unknown geographic origin. Thus Mayr's (1943) conclusion that about half the Hawaiian avifauna is of American origin is still supported by our molecular data.

We found a relatively low level of molecular divergence between the Hawaiian taxa and their closest non-Hawaiian (mostly mainland) relatives (i.e., from zero to 10.3% sequence divergence for 14 lineages). Based on these results, none of these Hawaiian lineages split from mainland ancestors earlier than about 6.4 Ma. In fact, most of our estimates, although rough and lacking meaningful standard errors, fall well within the period of formation of the current set of main islands (i.e., Kaua'i at 5.1 Ma and later, Fig. 1). Only the drepanidines (10.3%), the corvids (8.4%), and perhaps the moa-nalos (6.9%) and the thrushes (6.7%) have Kimura 2-parameter sequence divergences from mainland relatives that suggest colonization prior to even the formation of O'ahu (3.7 Ma), and in each of these cases we may not have obtained sequence for the closest mainland outgroup (which we may not have sampled or it might be extinct). The overall picture suggests that while native Hawaiian *Drosophila* (Beverley and Wilson 1985, Thomas and Hunt 1991, DeSalle 1992, Russo et al. 1995) and lobeliads (Givnish et al. 1995) may have colonized the archipelago well before the formation of Kaua'i, thus far we have little evidence that any bird lineages have done so.

These findings lead us to consider factors beyond simple isolation by distance and the anthropogenically induced Holocene extinction that may help to explain Hawai'i's low primary avian diversity. First, the unique geology of the islands (Carson and Clague 1995) results in a situation in which individual islands have a limited "lifespan" (~5–7 Ma) as a high island. Lineages that have colonized older islands, but for some reason cannot succeed onto younger islands, will be ultimately lost as their island disappears into the sea (this may be especially true for forms that have evolved to be flightless). There may be reduced chance for taxonomic diversity to build up over long evolutionary periods relative to archipelagos with longer surviving islands. Secondarily, what secondary enrichment of avifaunal lineages by speciation that does occur in the islands may allow "niches" to be filled (perhaps by now locally adapted taxa) such that they are no longer available for occupation by new (and not locally adapted) colonists from elsewhere. Thus, primary diversity could be reduced by competitive exclusion. Continued paleontological research in the islands combined with studies of DNA sequence variation should help us to address these hypotheses. We hope these new fossils and sequences will continue to shed light on the systematics, biogeography, and timescale of avian evolution on the Hawaiian conveyor belt.

ACKNOWLEDGMENTS

We thank C. Tarr, S. Olson, H. James, E. Paxinos, A. Cooper, B. Slikas, J. Rhymer, B. Arbogast, S. Conant, M. Sorenson, T. Quinn, A. Helbig, I. Jones, A. Driskell, and T. Pratt for information concerning and discussion of many of the topics covered in this paper, and C. Tarr, B. Slikas, J. M. Scott, reviewer #1, and especially H. James for comments on an earlier draft of the manuscript. Samples for many of our analyses were provided by museum or field collections of tissues and we gratefully acknowledge the cooperation of C. Kishinami and A. Allison (B. P. Bishop Museum), S. Conant (University of Hawai'i), F. Sheldon (Louisiana State University), M. Robbins and B. Slikas (Academy of Natural Sciences-Philadelphia), P. Bruner (Brigham Young University-Hawai'i), E. Bermingham (Smithsonian Tropical Research Institute), S. Olson, P. Angle, and M. Braun (U.S. National Museum), R. Cann (University of Hawai'i), S. Rowher and S. Edwards (Burke Museum), and C. Cicero (Museum of Vertebrate Zoology-Berkeley). We greatly appreciate permission from Dave Swofford to use PAUP* (a gem of a program). Funding for many of the results presented above was provided by the Smithsonian Institution Scholarly Studies Program, Friends of the National Zoo, U.S. National Science Foundation, U.S. Fish and Wildlife Service, the National Geographic Society, and the Biological Resources Division of the U.S. Geological Survey.