THE RELATIONSHIPS OF THE HAWAIIAN HONEYCREEPERS (DREPANININI) AS INDICATED BY DNA-DNA HYBRIDIZATION

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ABSTRACT.—Twenty-two species of Hawaiian honeycreepers (Fringillidae: Carduelinae: Drepaninini) are known. Their relationships to other groups of passerines were examined by comparing the single-copy DNA sequences of the Apapane (*Himatione sanguinea*) with those of 5 species of cardueline finches, 1 species of *Fringilla*, 15 species of New World nine-primaried oscines (Cardinalini, Emberizini, Thraupini, Parulini, Icterini), and members of 6 other families of oscines (Turdidae, Monarchidae, Dicaeidae, Sylviidae, Vireonidae, Corvidae). The DNA-DNA hybridization data support other evidence indicating that the Hawaiian honeycreepers shared a more recent common ancestor with the cardueline finches than with any of the other groups studied and indicate that this divergence occurred in the mid-Miocene, 15–20 million yr ago.

The colonization of the Hawaiian Islands by the ancestral species that radiated to produce the Hawaiian honeycreepers could have occurred at any time between 20 and 5 million yr ago. Because the honeycreepers captured so many ecological niches, however, it seems likely that their ancestor was the first passerine to become established in the islands and that it arrived there at the time of, or soon after, its separation from the cardueline lineage. If so, this colonist arrived before the present islands from Hawaii to French Frigate Shoal were formed by the volcanic "hot-spot" now under the island of Hawaii. Therefore, the ancestral drepaninine may have colonized one or more of the older Hawaiian Islands and/or Emperor Seamounts, which also were formed over the "hot-spot" and which reached their present positions as the result of tectonic crustal movement. The cardueline-drepaninine lineage probably diverged from the *Fringilla* lineage in the late Oligocene, from the New World nine-primaried oscines in the early Oligocene, and from the other oscines in the early Eocene.

During this study we also obtained evidence that the vireos (Vireonidae) are not closely related to the New World nine-primaried oscines. *Received 2 March 1981, accepted 1 July 1981.*

THE volcanic islands of the Hawaiian chain and the Galapagos archipelago are well-known as natural laboratories in which to observe the results of the processes of colonization, speciation, and adaptive radiation. Both island groups are substantial distances from the nearest continent, are composed of many islands, various distances apart, and have a variety of ecological conditions. The Galapagos archipelago includes 10 main islands and 6 smaller ones located on the equator about 900 km west of Ecuador. Thirteen species of Galapagos finches (*Geospiza, Cactospiza, Certhidea* et al.) have evolved from the original emberizine ancestor (Bowman 1961).

The Hawaiian Islands extend in a broadly linear chain for 2,500 km across the central Pacific Ocean from Hawaii in the southeast to Midway and Kure atolls at the northwest end of the Hawaiian Ridge (see Fig. 3). Hawaii is 3,000 km from California and the Aleutian Islands, and Kure Atoll is 2,500 km from the Aleutians and 3,000 km from Kamchatka. The Hawaiian Ridge makes an elbow bend and extends northward beyond the islands of the Hawaiian chain as a line of submerged volcanos, the Emperor Seamounts.

Twenty-two species of Hawaiian honeycreepers (Drepaninini of Sibley 1970: 99) are known, of which six are recently extinct (Greenway 1968, Raikow 1977b). Within historic times only eight of the 25 Hawaiian Islands have supported populations of Hawaiian honeycreepers.

The 11 genera of drepaninines are characterized by a remarkable array of bill types, from the short, finch-like bills of *Psittirostra, Melamprosops,* and *Ciridops* and the slender bills of *Viridonia* (=*Loxops*), *Himatione,* and *Palmeria,* to the parrot-like structure of *Pseudonestor* and the long, decurved bills of *Hemignathus* and *Drepanis.* This variation caused early taxonomists to distribute the Hawaiian honeycreepers among several passerine families, including the finches (Fringillidae), flower-peckers (Dicaeidae), and honeyeaters (Meliphagidae). Later, it became obvious that the Hawaiian honeycreepers are closely related to one another and that, as in the Galapagos finches, a single ancestral species had given rise to the 22 species, which adapted to the many ecological niches that were available to the first colonists in these oceanic islands (Amadon 1950, Sibley 1970, Raikow 1977b).

The identity of the ancestral taxon has produced much debate and speculation, but, because the Hawaiian honeycreepers are nine-primaried oscines, their origin must be sought among the members of that group. Gadow (1891) considered only the nectar-feeding taxa as possible ancestors and concluded that the neotropical coerebine honeycreepers are the closest relatives of the Hawaiian honeycreepers and that the tanagers (Thraupini) are also related to them. Gadow also noted, however, that the drepaninines and carduelines share characters of the horny palate. Lucas (1894) suggested that the tongues of the Hawaiian honeycreepers could have been derived from that of the New World oriole genus *Icterus* or that of the paruline genus *Dendroica.* Sushkin (1929) proposed that the cardueline finches and Hawaiian honeycreepers were most closely related, because he found similarities between the bills, skulls, and horny palates of *Psittirostra* and those of the carduelines. Amadon (1950: 232) reviewed the earlier proposals but concluded that the Coerebini or Thraupini were the more probable ancestors. Beecher (1953) found that the jaw musculature of *Psittirostra* is like that of the carduelines (e.g. *Carpodacus*), and he noted (p. 312) "the striking similarity of the Hawaiian finches to the cardueline finches in all but plumage" but concluded that the similarities are due to "parallel development from . . . thraupine stock."

Bock (1960: 477) argued that the carduelines are the most probable ancestors of the Hawaiian honeycreepers, because there are no anatomical characters that preclude the relationship and because the carduelines include species more capable of colonizing the Hawaiian Islands than do any of the other groups that have been suggested. Bock (1970) also found that the tongue apparatus of *Ciridops anna* resembles that of the carduelines, not that of the coerebines. Sibley (1970) also supported the carduelines as the ancestors of the drepaninines, based on comparisons of the electrophoretic patterns of their egg-white proteins.

Raikow studied the muscles of the hind limb (1976) and the forelimb (1977a), and reviewed and extended the morphological evidence of the origin and evolution of the Hawaiian honeycreepers (1977b). He concluded (1977b: 116) that the drepaninines "arose from a single founder species," which "was a primitive member of the Carduelines." Raikow suggested that this ancestral cardueline was a typical finch similar to the extant *Psittirostra* and that the "nectar-feeding habits arose in Hawaii and are merely convergent with similar conditions in other families of birds."

In this paper we present the results of comparisons among the homologous nucleotide sequences of the single-copy DNAs of a Hawaiian honeycreeper (*Himatione sanguinea*) and other taxa of the oscine passerines, using the technique of DNA-DNA hybridization. The DNA data provide an index to the amount of genetic divergence between taxa and to the time since that divergence began. It is therefore possible to relate the phylogeny of the birds to the geological history of the Hawaiian-Emperor island chain.

Methods

The DNA hybridization technique takes advantage of the complementary structure of the double-stranded DNA molecule. When double-stranded DNA in solution is heated to ca. 100°C, the hydrogen bonds between A-T and G-C base pairs dissociate, and the two strands separate. Under proper conditions, the two single strands will reassociate as the solution cools, because the complementary bases "recognize" one another. If the temperature is maintained at a high enough level, e.g. 60°C, complementary base pairing will occur only between long homologous sequences of nucleotides. This is because only long sequences of complementary bases will have sufficient bonding strength to form stable duplexes at that temperature, and only homologous sequences possess the necessary degree of comple-

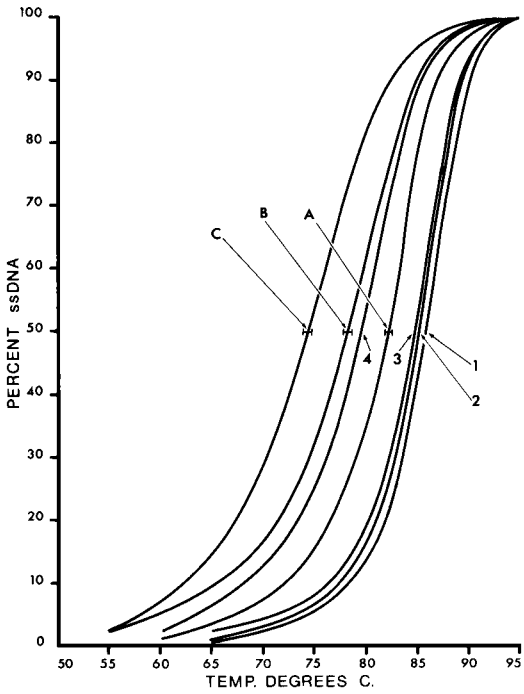

Fig. 1. Thermal dissociation curves of DNA-DNA hybrids in which the Apapane (*Himatione sanguinea*) was the labeled species. Curve number 1 is that of the homologous hybrid, *Himatione* × *Himatione*, number 2 is that of *Himatione* × *Vestiaria coccinea*, number 3 that of *Himatione* × *Viridonia* (*Loxops*) *virens*, and number 4 that of *Himatione* × *Fringilla coelebs*. The curve labeled A is the average ± one standard deviation (SD) for the DNA-DNA hybrids between *Himatione* and the 6 cardueline finches (numbers 4–9) in Table 1; the curve labeled B is the average ± one SD for *Himatione* × the 18 nine-primaried oscines (numbers 11–27) in Table 1; and the curve labeled C is the average ± one SD for *Himatione* × the 6 other oscine taxa (numbers 29–34) in Table 1.

mentarity. Thus, under appropriate conditions of temperature and salt concentration, conspecific double-stranded DNA may be thermally dissociated, and, because of their inherent properties, the single strands will reassociate only with their homologous partners.

Similarly, if the single-stranded DNAs of two different species are combined under conditions favoring reassociation, "hybrid" double-stranded molecules will form between homologous sequences. These sequences will contain mismatched bases as a result of the nucleotide sequence differences that have evolved since the two species diverged from a common ancestor. The lower bonding strength of such hybrid duplexes will cause them to dissociate at a temperature lower than that required to melt conspecific double-stranded DNA. Thus, the property of sequence recognition exhibited by homologous sequences and the decreased thermal stability of imperfectly matched hybrid sequences form the basis of the DNA-DNA hybridization technique.

The degree of nucleotide sequence homology between the reassociated single strands of any two DNAs can be determined by measuring the percentage of hybridization and the thermal stability of the reassociated duplex molecules. Following is a synopsis of the technique, which is described in more detail by Sibley and Ahlquist 1981).

DNAs were obtained from the nuclei of avian erythrocytes and purified by removing the proteins and RNA according to the procedures of Marmur (1961) and Shields and Straus (1975). The purified DNAs were sheared to an average length of ca. 500 nucleotides by sonication and sized by electrophoretic comparison with DNA fragments of known size produced by digestion of bacteriophage DNA with bacterial restriction endonucleases (Nathans and Smith 1975). Single-copy sequences were separated from repetitive sequences at an equivalent C_0t of 1,000 (Kohne 1970: 334) at 50°C in 0.48 M sodium phosphate buffer and labeled with ^{125}I (Commorford 1971, Prensky 1976). DNA-DNA hybrids were composed of one part (=250 ng) ^{125}I-labeled "tracer" DNA and 1,000 parts (=250 μg) of sheared, whole, "driver" DNA at a concentration of 2 mg/ml in 0.48 M sodium phosphate buffer. The hybrid combinations were heated to 100°C for 10 min to dissociate homoduplexes into single strands, then incubated for at least 120 h (=C_0t 16,000) at 60°C to permit the single strands to form hybrid heteroduplexes.

The hybrids were placed on hydroxyapatite columns immersed in a temperature-controlled water bath. The temperature was then raised in 2.5°C increments from 55°C to 95°C. At each of the 17 increments the single-stranded DNA was eluted in 10 ml of 0.12 M sodium phosphate buffer. This process was carried out in a custom-built apparatus that accommodates 25 hydroxyapatite columns in which the temperature is controlled to within ±0.1°C.

The radioactivity in each eluted sample was counted in a Packard Model 5220 Auto-Gamma Scintillation Spectrometer, optimized for ^{125}I. A teletype unit connected to the gamma counter printed out the data and also punched a paper tape, which is the entry to the computer program.

The computer program determined the best fit of the experimental data to one of four functions: (1) the Normal, (2) the dual-Normal, (3) the "skewed" Normal, or (4) a modified form of the Fermi-Dirac. The following parameters were then determined from the fitted distribution: (1) the modal temperature of the raw data points, and (2) the $T_{50}H$, which is the temperature above which less than 50% of the

sequences will be hybridized and below which more than 50% will be hybridized. This is, approximately, the median divergence point. The $T_{50}H$ is also the mode of a homologous hybrid and is equal to the mode of any hybrid if all single-copy sequences in the two species could form stable duplexes under the incubation conditions.

In each experimental set, the labeled taxon is hybridized with itself (=homologous hybrid) and the differences in degrees Centigrade between its parameters and those of the heterologous hybrids are the delta mode and the delta $T_{50}H$ values. The $T_{50}H$ (Bonner et al. 1981) is the same as the $T_{50}R$ of Kohne (1970).

The normalized percentage of hybridization (NPH) is calculated as the percentage of hybridization of a heterologous hybrid divided by that of the homologous hybrid × 100.

Results and Discussion

Figures 1 and 2 and Table 1 present the data from two experimental sets in which the Apapane (*Himatione sanguinea*) was the labeled species. It is apparent that the cardueline finches are the closest relatives of the Hawaiian honeycreepers and that the Old World genus *Fringilla*, the New World nine-primaried oscines, and other oscine groups are progressively more distant from them. Figure 2, based on the $T_{50}H$ values, depicts the relationships indicated by the DNA hybridization data.

It is obvious that the thermal dissociation values of the taxa in each group in Table 1 and Fig. 1 are essentially equidistant from the labeled Hawaiian honeycreeper, *Himatione sanguinea*. The five species of carduelines cluster around an average delta $T_{50}H$ of 4.3 ± 0.1 and an average delta mode of 4.1 ± 0.1 from *Himatione*. Similarly, the 15 species (17 measurements) of New World nine-primaried oscines have an average delta $T_{50}H$ of 7.3 ± 0.3 and an average delta mode of 6.7 ± 0.4. The remaining seven genera of oscines, belonging to at least six different families, have an average delta $T_{50}H$ of 11.2 ± 0.2 and an average delta mode of 10.7 ± 0.4 from *Himatione*.

This pattern of DNA hybridization values indicates that each of these clusters is a monophyletic taxon *relative* to *Himatione*. Each such cluster is also a "relative rate test" (Sarich and Wilson 1967) in which an external reference species is used to compare the rates of change in members of at least two lineages that diverged first from the external reference species and later from one another. The tight clustering

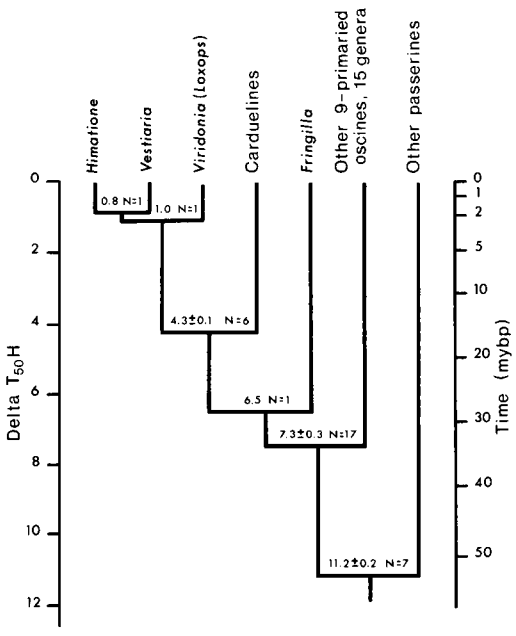

Fig. 2. The relationships of the Hawaiian honeycreepers to some other groups of oscine passerines (Passeriformes: Passeres). The averages and one SD are given for the delta $T_{50}H$ values for each group relative to *Himatione sanguinea*. Table 1 contains the data from which this diagram was constructed. Because this diagram is based upon an incomplete matrix of DNA hybridization values, it does not indicate the relationships *among* the included groups, only their average distances from the Hawaiian honeycreepers, as represented by *H. sanguinea*. The groups were defined by delta $T_{50}H$ values and morphological characters. The basis for the time scale is discussed in the text.

of the species in each group indicates that the *average* rate of nucleotide substitution has been the same, or nearly the same, in the lineages *within* each cluster since the time when the most recent common ancestor of all members of the cluster branched from the lineage that led to the external reference taxon, *Himatione*. If this were not true, we would expect to see a more scattered distribution of DNA values for the species in each group.

These data also suggest that the same *average* rate of nucleotide evolution probably occurs in *all* lineages. This is indicated by each of the clusters, especially by the values for the most distant set in Table 1, i.e. numbers 28–34, which includes members of at least six families of oscines that last shared a common

TABLE 1. DNA-DNA hybridization values.[a]

Labeled Apapane (*Himatione sanguinea*) hybridized with:	Delta $T_{50}H$	Delta mode
1. Apapane (*Himatione sanguinea*)	0	0
2. Iiwi (*Vestiaria coccinea*)	0.8	0.8
3. Amakihi (*Viridonia virens*)	1.0	1.0
4. Purple Finch (*Carpodacus purpureus*)	4.2	4.0
5. American Goldfinch (*Spinus tristis*)	4.2	4.1
6. Red Crossbill (*Loxia curvirostra*)	4.3	4.1
7. Red Crossbill (*Loxia curvirostra*)	4.4	4.1
8. Pine Grosbeak (*Pinicola enucleator*)	4.4	3.9
9. Eurasian Siskin (*Carduelis spinus*)	4.5	4.3
10. Chaffinch (*Fringilla coelebs*)	6.5	5.9
11. Cinereous Conebill (*Conirostrum cinereum*)	6.9	6.2
12. Red-winged Blackbird (*Agelaius phoeniceus*)	7.0	6.1
13. Blue Honeycreeper (*Cyanerpes cyaneus*)	7.0	6.4
14. Orchard Oriole (*Icterus spurius*)	7.1	6.4
15. Yellow-throated Tanager (*Iridosornis analis*)	7.2	6.5
16. White-sided Honeycreeper (*Diglossa albilatera*)	7.3	6.4
17. Blue Honeycreeper (*Cyanerpes cyaneus*)	7.4	6.8
18. Palm Tanager (*Thraupis palmarum*)	7.5	6.2
19. Scrub Tanager (*Tangara vitriolina*)	7.5	6.8
20. Rufous-capped Warbler (*Basileuterus rufifrons*)	7.6	6.8
21. Bananaquit (*Coereba flaveola*)	7.6	6.8
22. American Redstart (*Setophaga ruticilla*)	7.6	7.1
23. Lincoln's Sparrow (*Melospiza lincolnii*)	7.7	6.9
24. Common Yellowthroat (*Geothlypis trichas*)	7.7	7.0
25. Silver-beaked Tanager (*Ramphocelus carbo*)	7.8	7.0
26. Silver-beaked Tanager (*Ramphocelus carbo*)	7.8	7.2
27. Common Cardinal (*Cardinalis cardinalis*)	8.1	7.4
28. Common Starling (*Sturnus vulgaris*)	10.9	10.2
29. Spot-winged Monarch (*Monarcha guttula*)	11.0	10.3
30. Black Berrypecker (*Melanocharis nigra*)	11.0	10.9
31. American Robin (*Turdus migratorius*)	11.2	10.5
32. Garden Warbler (*Sylvia borin*)	11.3	11.0
33. Red-eyed Vireo (*Vireo olivaceus*)	11.3	11.1
34. Common Crow (*Corvus brachyrhynchos*)	11.6	11.0

[a] Delta $T_{50}H$ and delta mode values for DNA-DNA hybrids in which the Apapane (*Himatione sanguinea*) was the labeled species. Numbers 1–3 are Hawaiian honeycreepers (Drepaninini), numbers 4–9 are cardueline finches (Carduelini), number 10 is a fringilline finch (Fringillini), and numbers 11–27 are New World nine-primaried oscines (Emberizinae). Number 28 (*Sturnus*) is a member of the Muscicapidae: Sturninae, number 29 of the Monarchidae, number 30 of the Dicaeidae, number 31 of the Muscicapidae: Turdinae, number 32 of the Sylviidae, number 33 of the Vireonidae, and number 34 of the Corvidae. These allocations follow Sibley (1970) and Sibley and Ahlquist (1980).

ancestor with *Himatione* in the early Tertiary, 50–55 million yr ago. These six families branched from one another at various times during the Tertiary, but today, relative to *Himatione*, they differ from one another by a maximum of 0.7°C $T_{50}H$. Even these small differences are at least partly due to experimental error. The basis for the above dating is discussed below.

That DNA may evolve at the same average rate of nucleotide substitution in all of the lineages of a major group of organisms appears at first glance to violate logic as well as what we observe about the variable rates of change in morphological characters. The concept that the macromolecules (DNA and proteins) evolve in a "clocklike" manner was first proposed by Zuckerkandl and Pauling (1962), who suggested that proteins evolve at "constant" rates of amino acid replacement. Additional studies (see Fitch 1976, Wilson et al. 1977, Doolittle 1979) have demonstrated that each protein (hence each structural gene) evolves at its own rate and that the rates for different proteins vary ca. 600-fold from the slowest histone to the fastest immunoglobulin (Wilson et al. 1977: 610). An average protein of 400 amino acids is coded for by a gene composed of 1,200 nucleotides, but the avian haploid genome contains about 1.6 billion nucleotides of which ca. 60–70% are in the single-copy fraction. The DNA hybridization values are averages across this entire number; therefore, they reflect the amount of nucleotide sequence divergence due to the *average* evolutionary rate of change in about one billion nucleotides over various periods of time. Thus, the uniform average rate of nucleotide substitution in different lineages is simply a statistical phenomenon that is the product of the averaging of large numbers of

variables operating under the same constraints. Although each nucleotide, in each individual organism, may be evolving at its own rate, when *averaged* over such a large number of events the uniform average rate is the inevitable result. Sibley and Ahlquist (1981) have also discussed this problem.

Because the DNA hybridization values index the average rate of nucleotide substitution and because that average rate is the same in all lineages, the DNA values are proportional to the relative times of divergence of the taxa being compared. If the DNA values could be calibrated with reference to an external dating source, they would also provide an index to absolute time. Ideally, this would be accomplished by fossil datings of divergence times in the phylogeny of one or more groups. But, at least for birds, such dates are either lacking or unreliable. In our ratite study (Sibley and Ahlquist (1981) we used the geological dating of the rift between Africa and South America to obtain a rough estimate of the time of the interruption of the genetic contact between the African and South American populations of the common ancestor of the ostriches and the rheas. This gave a value of ca. 80 million yr before the present (mybp) for a $T_{50}H$ value of 16, or ca. 5 my/1.0 delta $T_{50}H$.

The relationship between the DNA values and time is expected to be curvilinear, with the ratio decreasing from delta 1:5 my at 80 mybp to lower ratios for more recent dichotomies. This is because of the effects of back mutations, multiple "hits" at the same site, and other phenomena that are proportional to the degree of sequence divergence between the taxa being compared. These factors make the DNA hybridization data nonlinear with time and result in proportional underestimates of the leg lengths (internodes) of trees constructed from these data. Sibley and Ahlquist (1981) have discussed this problem in greater detail.

A few dated DNA values are available for more recent divergence times, and they reflect the curvilinearity described above. They are used under the assumption that the average rate of DNA change in birds, fruit flies, and sea urchins is essentially the same, which may or may not be true.

Hunt (1980) compared the single copy DNAs of three species of Hawaiian fruit flies (*Drosophila*) and calculated that a median melting point depression (delta T_m) of 0.5°C = ca. 800,000 mybp and a delta T_m of 2.08°C = ca. 5 mybp. Angerer et al. (1976) obtained roughly dated values for comparisons among three species of sea urchins (*Strongylocentrotus*), which gave a delta T_m of 2.5°C = 10 mybp and a delta T_m of 3.5°C = 15 mybp. By using these five dated $T_{50}H$ and T_m values to calibrate the Hawaiian honeycreeper data, we estimate that the two lineages leading to the living carduelines and drepaninines last shared a common ancestor 15–20 mybp in the mid-Miocene and that their common ancestor diverged from the line leading to *Fringilla* in the late Oligocene, ca. 25–30 mybp. The common ancestor of these lineages diverged from the New World nine-primaried oscine lineage in the early Oligocene, 30–35 mybp, and the common ancestor of all the above groups separated from the line that led to the other oscine families in the early Eocene, 50–55 mybp. These dates are approximate and preliminary, but they provide the basis for an examination of the relationship between this possible history of the Hawaiian honeycreepers and the geological history of the Hawaiian Islands and the Emperor Seamounts.

There are more than 50 volcanos in the Hawaiian chain, but only 25 are currently above sea level, the others being submerged seamounts. The 10 "high" islands from Hawaii to Nihoa and Necker are mountainous; the "low" islands, from French Frigate Shoals to Kure Atoll, are the remnants of once high volcanos that have been eroded to, or near, sea level. About 1,100 km northwest of Midway, near latitude 23°N, longitude 172°E, the Hawaiian Ridge makes an abrupt bend to the north and continues as the Emperor Seamount chain of some 30 large submerged volcanos, most of which began as high, mountainous islands (Beverley 1979). The Emperor Seamounts extend northward for 2,500 km to the juncture of the Kurile and Aleutian trenches between Kamchatka and the western end of the Aleutian Islands (Fig. 3).

The more than 80 huge volcanos in the Hawaiian-Emperor chain are progressively older from the southeast to the northwest, because all were formed as the result of relative motion between the crustal slab of the Pacific plate and an area of vulcanism currently located under the island of Hawaii, where the "hot spot," or "melting spot," is indicated by the active volcanos of Mauna Loa and Kilauea. The "hot spot" is fixed in position and the Hawai-

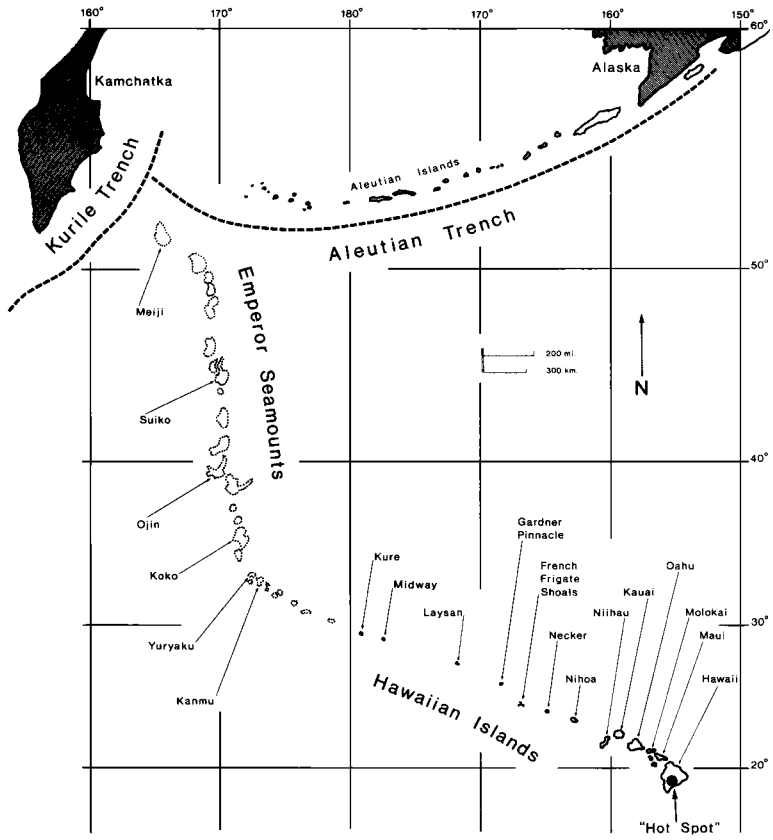

Fig. 3. The Hawaiian Islands and the Emperor Seamounts. Emergent islands are indicated by solid outlines, submerged seamounts by dotted outlines. The active volcanos of Mauna Loa and Kilauea, on the island of Hawaii, are located over the "Hot Spot."

ian-Emperor chain is the track of the average rate of about 8.5 cm/year linear movement of the Pacific plate across it (Wilson 1963, Dalrymple et al. 1974, Moberly and Larson 1975, R. L. Larson pers. comm.).

The data in Table 2 demonstrate the age progression of the Hawaiian-Emperor volcanos and the approximate dates of the rocks in the volcanic shields. The dates at which the islands were emergent and vegetated would have been somewhat later and their erosion and submergence later still. Wave action and other erosive forces can truncate a small volcano relatively quickly, but large structures, such as Kōko Seamount, required several million years to be eroded to a flat-topped "guyot." Coral reefs build up as a volcano sinks, and the resulting atoll remains emergent for additional millions of years. For example, Kōko Seamount, which was formed in the middle Eocene, ca. 46 mybp, began as an emergent island where Hawaii now is located. As it moved northwestward, it was eroded to sea level and became an atoll. Shallow water foraminifera of late Oligocene age, ca. 25–30 mybp, mixed with younger planktonic fossils, have been recovered from a site on Kōko Seamount. These were living during a time when coral reef sands were formed in water depths between 30 and 70 m (Hottinger 1975). Kōko probably remained emergent, or in shallow water as an atoll, for more than 20 million yr, and volcanic activity may have recurred long after the Eocene (Moberly and Larson 1975: 953). Other Emperor seamounts may have had similar histories. When the ancestors of the Drepaninini and the Carduelini diverged, the islands from Hawaii to French Frigate Shoal were not yet formed. Laysan Island was only 200 km from the "hotspot," near the present site of Maui, and Mid-

TABLE 2. Distances and ages of the Hawaiian-Emperor volcanos.[a]

Volcano	Island	Present distance (km) from Kilauea	K-Ar age
Kilauea	Hawaii	0	0–80,000 y
Kohala	Hawaii	100	200,000 y
Haleakala	Maui	180	800,000 y
West Maui	Maui	260	1.3 my[b]
East Molokai	Molokai	290	1.5 my
Koolau	Oahu	350	2.6 my
Waianae	Oahu	400	3.8 my
Kauai	Kauai	550	5.6 my
Nihoa	Nihoa	700	7.5 my
Necker	Necker	1,100	10 my
French Frigate Shoal	French Frigate Shoal	1,200	11 my
Laysan	Laysan	1,800	19 my
Midway	Midway	2,500	27 my
Kanmu Seamount		3,700	37–40 my
Yuryaku Seamount		3,800	42 my
Kōko Seamount		4,000	46 my
Suiko Seamount		5,500	59–64.7 my
Meiji Seamount		6,000	70 my

[a] Present distances from Kilauea and potassium-argon (K-Ar) datings for some of the Hawaiian Islands and Emperor Seamounts. The K-Ar dated samples came from various positions and depths in the volcanic shields. Data from Clague and Dalrymple (1973), Dalrymple et al. (1974), Lancelot and Larson (1975), Moberly and Larson (1975), Dalrymple et al. (1977), and Jarrard and Clague (1977).

[b] my = million years.

way was ca. 1,000 km from the "hot-spot," near the present position of Necker Island. Kōko Seamount was near the present position of Midway and Meiji Seamount was near where Kōko is today. Additional older volcanos probably existed to the north of Meiji but have been subducted into the Kurile-Aleutian trench. There is no proof that the Emperor seamounts were emergent as recently as 15–20 mybp, but some may have been atolls that could have provided a landing site for the ancestral Hawaiian honeycreeper. That this early colonist came from the north or northwest is made probable by at least two facts. First, the distance, even now, is less from the Aleutians and Kamchatka to Kure than from California to Hawaii. Second, the only records of "accidental" passerines reaching Kure and Midway atolls are for Asiatic or Aleutian taxa, namely the Barn Swallow (*Hirundo rustica gutturalis*), Skylark (*Alauda arvensis pekinenis*), Water Pipit (*Anthus spinoletta japonicus*), Red-throated Pipit (*A. cervinus*), and Snow Bunting (*Plectrophenax nivalis townsendi*) (Clapp and Woodward 1968). The prevailing westerly wind pattern further increases the probability of colonization from Asia.

Although the DNA data provide an approximate date for the cardueline-drepaninine dichotomy, they do not indicate when the ancestral Hawaiian honeycreeper colonized the islands. This event could have occurred at any time between 20 and ca. 5 mybp. The ancestor of the drepaninines must have been the first passerine to colonize the Hawaiian Islands, however; otherwise, we should expect to find many of their ecological niches occupied by species of some other group that also colonized the islands, for example, the Meliphagidae. Therefore, it seems likely that the time of colonization either coincided with or was close to the time of divergence, and we suggest that it was the colonization event that caused the dichotomy between the cardueline and drepaninine lineages.

A colonization date of 15–20 mybp may seem doubtful, because it usually has been assumed that the ancestral Hawaiian honeycreeper colonized the islands within the past 5–6 million yr, i.e. since the formation of the island of Kauai. For example, Baldwin (1953: 387) noted that "terrain capable of supporting forests may have existed for as long as five million years . . . thus ample time has apparently been available for the evolution of terrestrial birds in Hawaii." Compared with the history of the Hawaiian drosophilid fruit flies as proposed by Beverley (1980), however, the 15–20 mybp colonization date for the honeycreepers is conservative. Beverley used the technique of micro-complement fixation to compare a larval hemolymph protein among 18 species of Hawaiian and 40 species of continental drosophilids. The rate of evolution of the protein

was calibrated, and the age of the origin of the Hawaiian drosophilids was estimated at 42 mybp. If this is correct, the ancestral species must have colonized an island that is now a submerged Emperor Seamount. At 42 mybp Yuryaku Seamount, like Hawaii today, was over the "hot spot," and Meiji Seamount was near the present location of Midway. Beverley (1979) estimated the approximate original heights of the seamounts by extrapolating the undersea slopes of the truncated mountains. For example, the ancient mountain whose remnant today is Gardner Pinnacle was 2,100 m high when it was formed ca. 13 mybp, and Ojin Seamount, in the southern portion of the Emperor chain, was at least 1,500 m above sea level ca. 40 mybp. Such islands would have supported forests suitable for passerine birds as well as for drosophilid flies. Carson et al. (1970: 490–491) note that many Hawaiian honeycreepers occur today in the same habitats as do the drosophilids, and some honeycreepers feed upon the fruit flies.

Beverley proposed that, following the original colonization ca. 40 mybp, the fruit flies began the diversification that has produced the 350 species know today from the Hawaiian Islands. As the ancient islands eroded away, new islands were formed over the "hot spot," and the flies colonized them when the ecological conditions permitted. Carson (1970) and Carson et al. (1970) have demonstrated that *Drosophila* species have made at least 22 interisland colonizations among the present main Hawaiian Islands.

Beverley's dating of 42 mybp for the colonization of the Hawaiian-Emperor chain by the Drosophilidae may prove to be excessive, but any correction is unlikely to render improbable the 15–20 mybp colonization date we propose for the Hawaiian honeycreepers. Therefore, from the facts and speculations presently available, we suggest the following history for the Hawaiian honeycreepers.

Approximately in the middle Miocene, 15–20 mybp, the ancestor of the Hawaiian honeycreepers colonized an island in the Emperor-Hawaiian chain, probably less than 2,000 km from Asia. The birds spread to other islands in the chain and speciated as new islands were formed and moved up the chain. Most of the adaptive radiation that produced the 22 known species of Hawaiian honeycreepers probably occurred within the past 5 million yr on the "high" islands from Kauai to Hawaii.

The *Himatione* × *Vireo* DNA hybrid in Table 1 has a delta $T_{50}H$ of 11.3, which indicates that the vireos are not closely related to the New World nine-primaried oscines. See Sibley and Ahlquist (in press) for a more detailed study of this question.

CLASSIFICATION

The following classification, somewhat modified from Sibley (1970: 99), expresses our present understanding of the relationships among the "New World nine-primaried oscines," not all of which are confined to the New World.

Family Fringillidae
- Subfamily Fringillinae
  - Tribe Fringillini: chaffinches, bramblings.
- Subfamily Carduelinae
  - Tribe Carduelini: goldfinches, crossbills, etc.
  - Tribe Drepaninini: Hawaiian honeycreepers.
- Subfamily Emberizinae
  - Tribe Cardinalini: cardinals, grosbeaks.
  - Tribe Emberizini: buntings, etc.
  - Tribe Thraupini: tanagers, including *Tersina*.
  - Tribe Parulini: wood warblers, including *Zeledonia*.
  - Tribe Icterini: troupials, blackbirds, etc.

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