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Convergent Evolution in Hawaiian Honeyeaters

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Report

Convergent Evolution of Hawaiian and Australo-Pacific Honeyeaters from Distant Songbird Ancestors

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Summary

The Hawaiian "honeyeaters," five endemic species of recently extinct, nectar-feeding songbirds in the genera *Moho* and *Chaetoptila*, looked and acted like Australasian honeyeaters (Meliphagidae), and no taxonomist since their discovery on James Cook's third voyage has classified them as anything else [1–8]. We obtained DNA sequences from museum specimens of *Moho* and *Chaetoptila* collected in Hawaii 115–158 years ago. Phylogenetic analysis of these sequences supports monophyly of the two Hawaiian genera but, surprisingly, reveals that neither taxon is a meliphagid honeyeater, nor even in the same part of the songbird radiation as meliphagids. Instead, the Hawaiian species are divergent members of a passeridan group that includes deceptively dissimilar families of songbirds (Holarctic waxwings, neotropical silky flycatchers, and palm chats). Here we designate them as a new family, the Mohoidae. A nuclear-DNA rate calibration [9] suggests that mohoids diverged from their closest living ancestor 14–17 mya, coincident with the estimated earliest arrival in Hawaii of a bird-pollinated plant lineage [10]. Convergent evolution, the evolution of similar traits in distantly related taxa because of common selective pressures, is illustrated well by nectar-feeding birds [11], but the morphological, behavioral, and ecological similarity of the mohoids to the Australasian honeyeaters makes them a particularly striking example of the phenomenon.

Results and Discussion

The Australasian honeyeaters (Meliphagidae) are a group of songbirds that branch off within the passeriform (perching bird) phylogeny basal to both the "core Corvoidea" and the Passerida [9]. They have classical adaptations for nectarivory, including scroll-edged, forked, brush-tipped tongues (Figure 1) and long, often decurved, bills (Figure 2). The 182 species of Meliphagidae occur south of Wallace's line in New Guinea and Australia, with a few genera such as *Myzomela*, *Foulehaio*, and *Gymnomyza* spilling out onto the islands of Micronesia and Polynesia. Also traditionally included in the Meliphagidae were the Hawaiian *Moho* (four species of 'o'o, each found on a different island; Figures 2A and 2E) and the rather differently appearing *Chaetoptila angustipluma* (the kioea; Figure 2C). All five species were nectarivores with meliphagid-like tongues (Figure 1). Taxonomists have never doubted that *Moho* and *Chaetoptila* were meliphagids, and have only expressed uncertainty about whether they arose from a single colonization of the Hawaiian Islands (i.e., are monophyletic) and which particular meliphagid taxa might be their closest relatives ([4–8]; summarized in Supplemental Data available online).

The five historically known Hawaiian "honeyeaters" unfortunately all became extinct between the 1850s and the 1980s, and molecular analysis is limited to DNA from relatively old museum specimens. Here we evaluate the phylogenetic position of *Moho* and *Chaetoptila* within the order of perching birds, by using up to 1923 bp of nuclear and 717 bp of mitochondrial DNA sequences obtained from multiple specimens of *Moho* and *Chaetoptila* collected during the 1800s (Table S1). Although our phylogenetic analyses of mtDNA sequences provide strong support for the monophyly of all of the Hawaiian taxa (Figure 3A), we were surprised to find no support for the placement of this group within the family Meliphagidae on the basis of mtDNA (Figure 3A), nuclear *RAG-1* (Figure 3B), or nuclear intron sequences (Figure 3C). Nor was there support for including them within the basal oscine songbird clade [9] that contains meliphagids along with related families of fairy wrens, chats, and pardalotes. Instead, there was strong support (Figure 3) for including *Moho* and *Chaetoptila* in another great and secondary radiation of songbirds, the Passerida, and more specifically, within an unusual passeridan clade containing three avian families: waxwings (Bombycillidae), New World silky flycatchers (Ptiligonatidae), and the monotypic palm chat of Hispaniola (Dulidae). All of these species are frugivores or insectivores, and are not nectarivores like the two Hawaiian genera. In addition to the high bootstrap and Bayesian support for the relationship (Figure 3), we found that *RAG-1* trees constrained to include the Hawaiian taxa within the Meliphagidae were significantly less likely by Shimodaira-Hasegawa test ($p < 0.0001$; Supplemental Data) than the unconstrained maximum likelihood (ML) tree as shown in Figure 3B.

These DNA results prompted us to re-evaluate the morphological characteristics of *Moho* and *Chaetoptila* in relation to Australasian honeyeaters and other songbirds. Many of the traits that prompted systematists to place them in the Meliphagidae are adaptive trophic structures: long tarsi and strong perching feet for reaching flowers, long decurved bills and extendable tongues to probe floral nectaries, tubular or semitubular brush-tipped tongues that use capillary attraction to move nectar up into the throat (Figure 1), and an operculum over the nares to protect the nasal cavity from pollen. The Hawaiian and Australasian nectarivores also display parallels in plumage (Figure 2), behavior, and song [4–8] that indicate an even broader convergence in their life histories as part of defending ephemeral or widely spaced nectar sources. This convergence is so pervasive that, without the molecular sequence data, it would probably never have been possible to recognize the closest relatives of the Hawaiian lineage as being the waxwings and allies.

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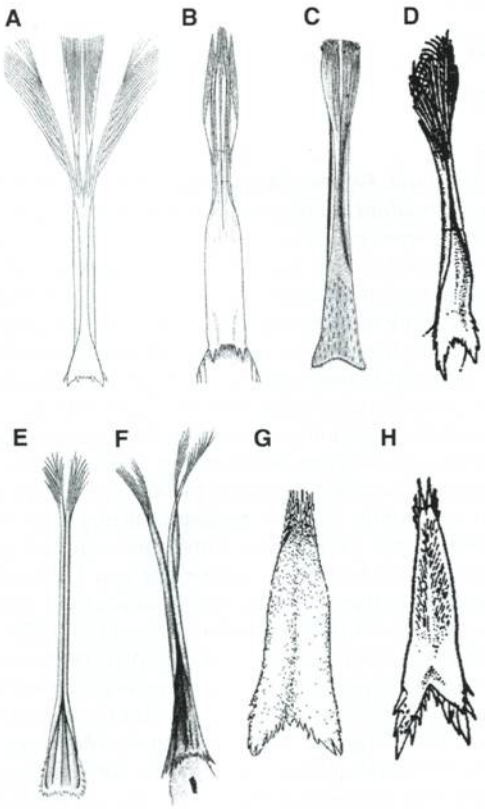

Figure 1. Tongues of Meliphagids, *Moho*, and Relatives of *Moho* and *Chaetoptila*

Shown are illustrations of tongues of meliphagids (A–D), two species of *Moho* (E and F), and relatives of *Moho* and *Chaetoptila* on the basis of our results (G and H). This suggests convergence of the tongues of *Moho* from ancestral tongues like (G) and (H) to tongues like (A)–(D). The following are shown: (A), *Meliphaga fasciogularis*; (B) *Myzomela sclateri*; (C), *Anthornis melanura*; (D), *Philemon buceroides*; (E), *Moho nobilis*; (F), *Moho braccatus*; (G), *Dulus dominicus*; and (H), *Phainopepla nitens*. Illustrations (A), (C), and (E) are from Dorst [21]; (B) is from Scharnke [22]; (D) and (H) are from Beecher [23]; (F) is from Gadow [24]; and (G) is from Gardner [25]. Tongue illustrations are reproduced with permission from the British Ornithologists' Union, American Ornithologists' Union, Société Ornithologique de France, and the Journal of Ornithology.

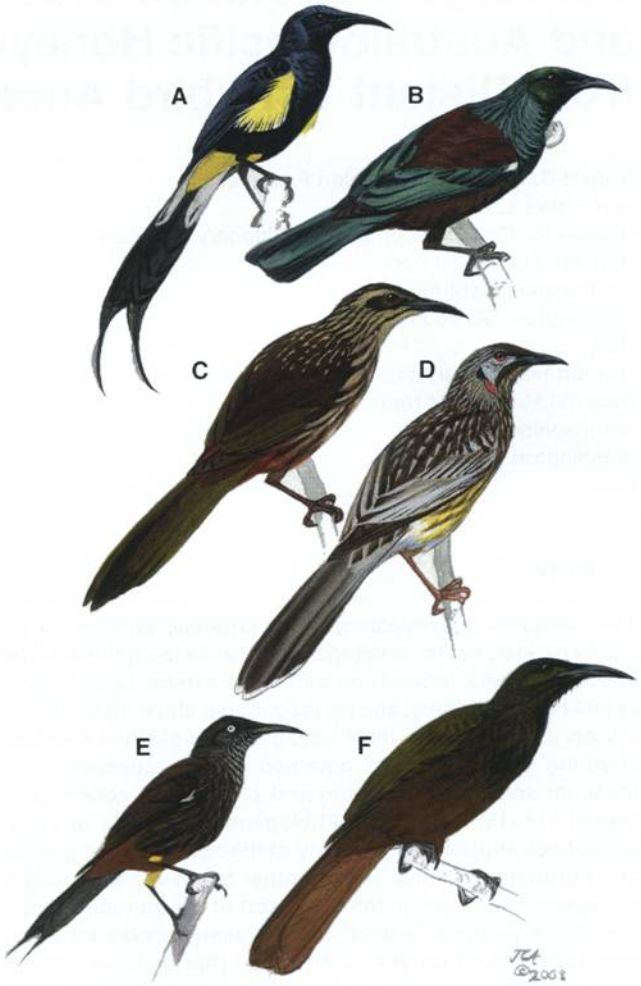

Figure 2. Illustrations of Three of the Five Species of Hawaiian "Honeyeaters" and Three Representative Meliphagid Honeyeaters

The three Hawaiian taxa represent the three primary morphological types found in Hawaiian "honeyeaters" (Mohoidae: [A], *Moho nobilis*; [C], *Chaetoptila angustipluma*; and [E], *Moho braccatus*). The three meliphagids include one from New Zealand ([B], *Prosthemadera novaeseelandiae*), one from Australia ([D], *Anthochaera carunculata*), and one from Samoa ([F], *Gymnomyza samoensis*). Paintings are by John Anderton and are used here with permission.

Our results indicate that the Hawaiian birds were derived from Holarctic or Neotropical, and not South Pacific, ancestors. This further strengthens Mayr's contention that the Hawaiian avifauna is more American than otherwise [12, 13]. Our molecular analyses also show that *Moho* and *Chaetoptila* are unique taxonomically and relatively divergent from any of their closest relatives (Table 1), necessitating the recognition of a new family-level rank.

Mohoidae, New Family

Type genus: *Moho* Lesson, 1831.
Included genera: *Moho*, *Chaetoptila* Gray, 1869.
Diagnosis: Passerida with the nectar-feeding adaptations mentioned above, and a single pneumotricipital fossa of the humerus with a large pneumatic opening.

The Mohoidae present one of the most deceptive cases of convergent evolution in birds. Their closest relatives, and presumably their common ancestor, look nothing like meliphagids, yet *Chaetoptila* and *Moho* have such typical meliphagid characteristics (e.g., Figure 1) that they fooled generations of taxonomists into placing them in the Meliphagidae without equivocation [1–8]. New Zealand's endemic stitchbird (*Notiomystis cincta*) is another "honeyeater" that does not fall within the Meliphagidae on the basis of nuclear and mtDNA sequence analysis [14, 15]. It represents another deceptive case of convergent evolution; but this species is placed among the basal songbird lineages, along with the meliphagids, as opposed to the Hawaiian taxa, which are placed deep within the Passerida. In addition, whereas the stitchbird does have meliphagid characteristics, other aspects of its morphology and biology had led taxonomists to question its placement in Meliphagidae prior to the molecular analyses [16]. Also, the convergence we report is not limited to a single mohoid and a single meliphagid morphotype; instead, at least three distinct morphotypes in the Mohoidae are also represented in the Meliphagidae, suggesting parallel adaptations across two independent radiations (Figure 2).

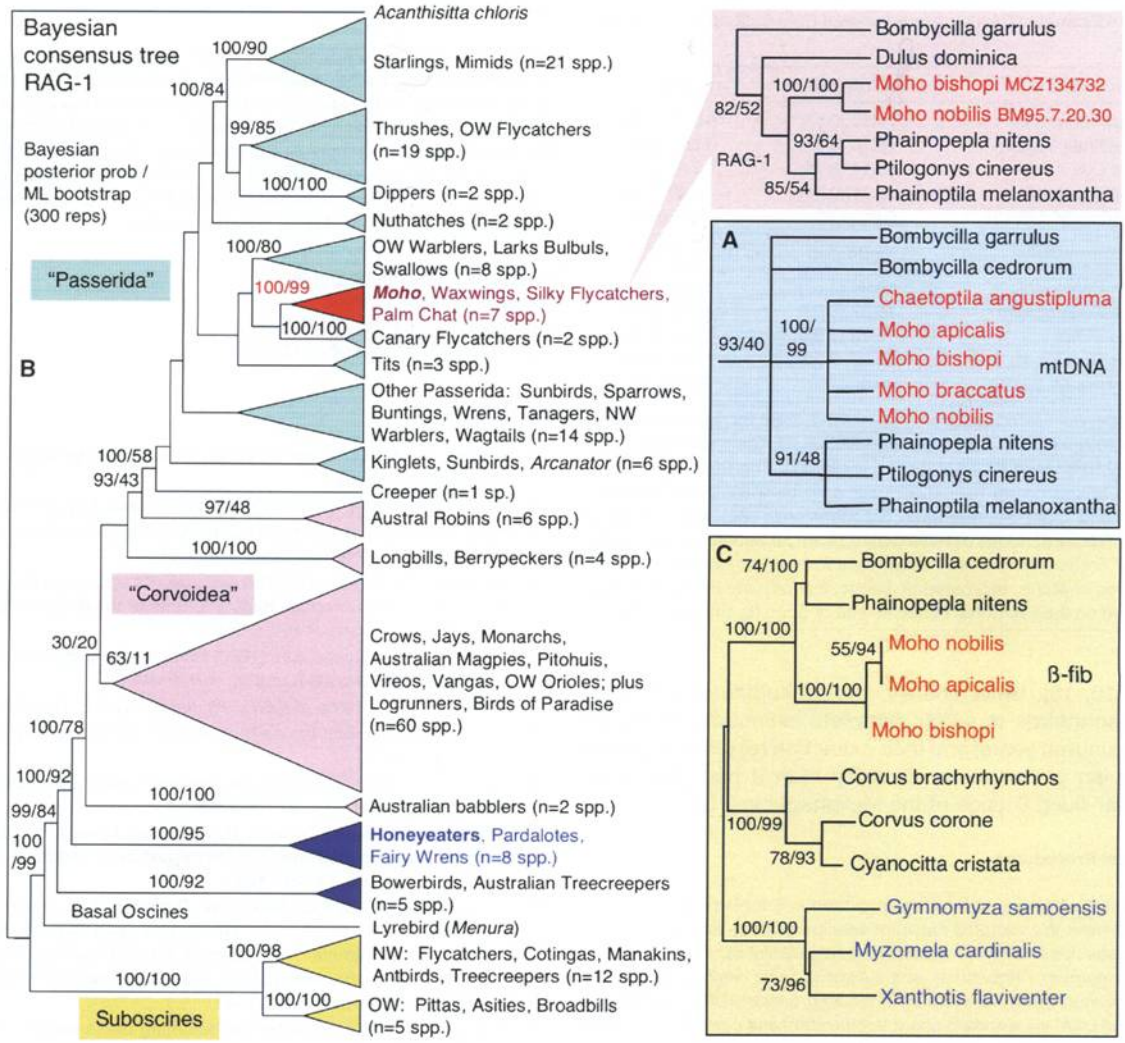

Figure 3. Phylogeny Reconstructions for Hawaiian Mohoids and Outgroups with Different Data Partitions

(A) Section of a ML tree constructed from up to 717 nucleotide sites of mtDNA sequence for the five species of Mohoidae and 43 additional songbird taxa. The tree shows strong support for monophyly of the Mohoidae and also supports placement of the Mohoidae within the waxwing and silky flycatcher clade and Passerida. Relationships among species within the Mohoidae are not well resolved. Bayesian posterior probabilities and ML bootstrap support values are provided at relevant nodes.

(B) Schematic of a phylogenetic tree constructed with Bayesian inference from 190 sequences of up to 1544 bp of the *RAG-1* gene [9, 19]. Taxa are merged into triangles indicating major, supported clades that generally match the topology found by Barker et al. [9] with a larger data set. Sequences from *Moho nobilis* and *Moho bishopi* fall within the red clade, rather than, as expected, within the basal honeyeater clade (dark blue); the expanded clade shown at upper right reveals the position of these taxa within the clade containing waxwings, silky flycatchers, and the palm chat. This tree includes only the two *Moho* species for which more than 1000 bp of *RAG-1* sequence was obtainable. Shorter *RAG-1* sequences of *Chaetoptila angustipluma* and *Moho apicalis* are nearly identical to these sequences of *Moho* in sections of overlap, and thus support these results (see Supplemental Data).

(C) Maximum likelihood phylogram constructed from analysis of up to 421 nucleotide sites of *β-fibrinogen introns 5* and *7* combined. At nodes are Bayesian posterior probabilities and ML bootstrap values (100 repetitions). The sequence data set for this tree was limited to outgroup species for which sequences of both genes were available, but analyses with considerably larger numbers of taxa (115 and 189 sequences) for each gene separately produced the same results.

Although the degree of convergence between the Mohoidae and the Meliphagidae may seem remarkable, one must take into account the amount of time since the Hawaiian lineage diverged from a mainland ancestor. On the basis of a RAG-1 external rate calibration [9] with nonparametric rate smoothing (NPRS) and penalized likelihood (PL) approaches [17], we estimated the divergence time between *Moho* and its closest mainland relatives, the silky flycatchers, to range from about 14 to 17 million years (Table 1). A divergence time based on mtDNA divergences and island age is less precise, estimating a split from silky flycatchers or the palm chat at 10–20 million years (Table 1). Either of these estimated timeframes would presumably provide ample opportunity to evolve the adaptations for nectarivory that make *Moho* and *Chaetoptila* appear so similar in gestalt to the distantly related Australasian honeyeaters. In addition, if either one of these estimated time periods corresponds to the presence of the Mohoidae in the Hawaiian Islands, they would be the oldest avian lineage in the Hawaiian Islands [13, 18], and the more recent estimates would coincide well with the earliest postulated arrival of bird-pollinated plant

Table 1. Date Estimates Based on Independent Rate Calibrations with the Program r8s

A. Comparison-*RAG-1*	NPRS (SE)	PL (SE)
Moho versus *Phainoptila*	14.35 (0.58)	16.01 (0.50)
Moho versus *Phainopepla*	16.16 (0.55)	17.27 (0.42)
Moho versus *Dulus*	16.17 (0.75)	16.89 (0.57)
Moho versus *Bombycilla*	18.11 (0.84)	19.95 (0.63)
M. nobilis versus *M. bishopi*	0.88 (0.03)	0.56 (0.02)
B. Comparison-mtDNA	**NPRS (SE)**	**PL (SE)**
Moho versus *Phainoptila*	19.91 (3.07)	12.28 (3.20)
Moho versus *Phainopepla*	19.67 (3.07)	12.25 (3.20)
Moho versus *Dulus*	18.13 (3.30)	10.62 (2.35)
Moho versus *Bombycilla*	21.28 (3.22)	13.72 (4.35)
M. nobilis versus *M. bishopi*	2.44 (0.25)	2.13 (0.19)

(A) Date estimates of nodes from the *RAG-1* tree for comparisons of different close relatives of *Moho* (Ptilogonatidae, Dulidae, Bombycillidae), estimated by NPRS and PL approaches in r8s [17]. The RAG1 calibration, as in [9], is based on an 82 million year split between New Zealand's *Acanthisitta* and other passeriforms. Standard error (SE) was calculated from mean of dates at nodes of trees derived from 50 bootstrap repetitions. (B) Dates of nodes from mtDNA sequences for comparisons of available close relatives of *Moho*, estimated as above, but with an internal rate calibration based on the estimated subaerial age of Oahu (Supplemental Data).

lineages [10, 18]. Unfortunately, the Mohoidae are the only family of songbirds to suffer complete extinction during the past few hundred years, and their extinction resulted in greater loss of avian phylogenetic diversity than if had they been merely a far-flung lineage of the Meliphagidae [15].

Experimental Procedures

Detailed experimental procedures are provided in Supplemental Data, but summarized here. We sampled museum specimens of at least one individual of each species of *Moho*, a *Chaetoptila angustipluma*, a Samoan meliphagid (*Gymnomyza samoenisis*), and a crow (*Corvus nasicus*) (Table S1). DNA was isolated from the samples in isolated ancient-DNA laboratories (in the UK and USA) via standard phenol-chloroform and centrifugal-dialysis protocols with extreme care and controls to avoid or detect contamination. Primers were designed to amplify small segments from three nuclear genes (Table S2), and existing primers were used to amplify from mtDNA *12 s RNA*, *cytochrome b*, and *ATPase6* and *ATPase8* genes. These products were sequenced, providing up to 1502 bp of *RAG-1*, 717 bp of mtDNA, 250 bp of *β-fibrinogen intron 5*, and 171 bp of *β-fibrinogen intron 7*. Comparative sequences were obtained from GenBank and relied mostly on two large RAG-1 datasets [9, 19].

Phylogenies were estimated from the data sets via maximum-parsimony, maximum-likelihood, and Bayesian approaches. Support for nodes was assessed by bootstrapping for the MP and ML trees, and by posterior probabilities for the Bayesian trees. In addition, we used Shimodaira-Hasegawa tests to test whether trees obtained through heuristic searches differed from ones constraining the position of *Moho* within the Meliphagidae. We did not combine the different sequence partitions (except for the *β-fib* sequences) because we had mostly different comparative taxa or individuals. Dates of particular nodes were estimated from the *RAG-1* and mtDNA data sets (Table 1 and Supplemental Data) via NPRS and PL methods [17] with a calibration date from Barker et al. of 82 million years for RAG1 [9]. This is the estimated date of the separation of *Acanthisitta* from the other Passeriformes, which was based on estimates of the timing of isolation of New Zealand from Antarctica. A calibration point internal to the genus *Moho* was used for the mtDNA data set and was based on the age of the island of Oahu [20].

Supplemental Data

Supplemental Data include a taxonomic summary, detailed Supplemental Experimental Procedures, Supplemental Results, and two tables and can be found with this article online at http://www.current-biology.com/supplemental/S0960-9822(08)01420-6.

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Supplemental Data

Convergent Evolution of Hawaiian and Australo-Pacific Honeyeaters from Distant Songbird Ancestors

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Supplemental taxonomic history of *Moho* and *Chaetoptila*

Early taxonomists placed these two taxa within the Meliphagidae, beginning with Lesson [S1] for *Moho* and Peale [S2] for *Chaetoptila*. All subsequent taxonomists whose references we have found have placed these taxa in Meliphagidae. For example, Rothschild noted in his classic *Aves Laysanensis* [S3] about *Chaetoptila* that this "…remarkable form is doubtless a member of the family *Meliphagidae*, and its nearest ally, as far as the external structure goes, seems to be *Acanthochaera mellivora* (Lath.) of Australia.", and about *Moho* simply that "*Moho* is a genus of the *Meliphagidae*". Wilson and Evans [S4] state that: "…while, out of the whole Hawaiian avifauna, only two genera could be referred to the Meliphagidae, namely *Acrulocercus* (*Moho* of some writer) and *Chaetoptila*, the last being presumably extinct. All the other forms which had been accounted Melaphagine presented a peculiar structure of tongue forbidding that alliance…", adding that "We have (or had) the two Meliphagine genera *Acrulocercus* and *Chaetoptila* – the latter, indeed, beyond anatomical examination, but shewing no very great external deviation from well-known Australian types; while the former undoubtedly retains the normal Meliphagine tongue." The anatomist Gadow [S4] examined fluid-preserved specimens of *Moho nobilis* and *M. braccatus* and a skin of *Chaetoptila* and detected nothing to disturb their usual placement, finding that the two species of *Moho* "belong to the family Meliphagidae" and that "they approach the subfamilies Myzomelinae and Meliphaginae proper". *Chaetoptila* was also "certainly a member of the Meliphagidae".

Munro [S5] writes: "The progenitors of the Meliphagine family in Hawaii were undoubtedly from the Australian side." And "some of their notes and actions remind me of the New Zealand tui (*Prosthemadera novae zealandia*) also a Meliphagine bird with which I am well acquainted". Perkins [S6], referring to both Hawaiian genera, wrote: "…in the Meliphagidae, of which there were certainly two immigrant ancestral species, it is most probable that the two immigrations took place at widely separated periods of time, and also that the original immigrants were themselves widely separated species." More recently, Amadon [S7] opined that: "*Chaetoptila* is a close relative of *Gymnomyza* [meliphagid] of Fiji and Samoa" and perhaps more astutely, considering our findings: "*Moho* bears some resemblance to the Tui (*Prosthemadera*) of New Zealand, both in habits and appearance (Munro, 1944, p. 831). Close comparison, however, suggests that *Moho* and *Chaetoptila* are more nearly related than might appear at first glance. Probably they are both descendants of a single invasion of *Gymnomyza*-like stock and the resemblance of *Moho* to *Prosthemadera* is only parallelism. The presence of yellow tufts of feathers in the plumage is widespread in the Meliphagidae."

Supplemental Experimental Procedures

Specimens

For the extinct Hawaiian honeyeaters we used a scalpel to sample toe pads from museum specimens of all four species of *Moho* and *Chaetoptila angustipluma* (Table S1). We also sampled old museum specimens of a Polynesian meliphagid (*Gymnomyza samoensis*) and a species of *Corvus* as controls, and the *RAG-1* sequences for these matched expectation based on their presumed phylogenetic position (Meliphagidae and Corvidae, respectively). We sequenced some DNA regions from a blood sample of a phainopepla (*Phainopepla nitens*). All other comparative sequences were obtained from Genbank; with *RAG-1* sequences mostly derived from [S8, S9]. Genbank accession numbers are available from R. C. F.

DNA methods

DNA was isolated from the sampled toe pads in dedicated ancient DNA laboratories located at the National Zoological Park, Smithsonian Institution, USA, and at the Department of Biological Sciences, Durham University, UK, using an overnight proteinase k-DTT-SDS buffer digestion, followed by phenol and chloroform extractions, and centrifugal dialysis [S10]. DNA extractions and PCR setups followed stringent standards for ancient DNA analysis; most sequences were replicated by a combination of multiple extractions, multiple PCRs, multiple individuals per species, and in independent laboratories on two continents.

Ten sets of generalized primers of the nuclear *RAG-1* gene were designed from passeriform (perching bird) *RAG-1* sequences downloaded from Genbank. The primer sets (Table S2) were designed to cover a span of 1544 bp with some minor gaps between fragments, resulting in 1502 bp of total sequence possible. *RAG-1* was chosen because an exceptionally extensive passeriform phylogeny was available for this gene [S8, S9], and the gene showed high utility for resolving passeriform relationships. As a backup [S11] primers were also designed to amplify portions (up to 421 bp) of two additional nuclear genes for which there are abundant passeriform sequences available on Genbank: ß -fibrinogen intron 5 (ß-fib5) and intron 7 (ß -fib7). In addition, mitochondrial genes were amplified from the *Moho* and *Chaetoptila* museum DNA samples, including parts of the *12s ribosomal RNA* gene (287 bp), *cytochrome b* (301 bp) and *ATP6&8* (347 bp). Primers for *ATP6*, *ATP8*, Cytb2/CytbS2H, Cytb-wow/Cytb-2rc, and 12Sa are available in supplemental table 3 in [S10]; 12Sf is 5'-AGAAAATGTAGCCCATTGCT).

Phylogenetic analysis

Initial analyses involved only *RAG-1* sequences obtained from one individual museum specimen of *Moho nobilis* (all four specimens analyzed for *RAG-1* for this species had identical sequence across the sequenced regions they had in common), one individual museum specimen of *Moho bishopi*, two other outgroup museum specimens (the meliphagid *Gymnomyza samoensis* and the crow *Corvus nasicus*), and 186 comparative passeriform sequences from Genbank derived mostly from [S8, S9]. *RAG-1* sequences were simple to align, and had no gaps in our sequences. Aligned sequences were

subjected to a range of methods to infer phylogenetic relationships, including maximum parsimony (PAUP* [S12]), maximum likelihood (RAxML [S13]), and Bayesian (Mr Bayes [S14]) approaches. For maximum parsimony, we ran 100 bootstrap replicates with heuristic searches. Maximum likelihood searches were conducted using a GTR model with γ-parameter (α = 0. 956) and invariant sites (= 0.479) estimates, empirical base frequencies, and 300 bootstrap replicates. Bayesian analysis was performed using a maximum likelihood model employing six substitution types, empirically derived base frequencies, and rate variation across sites using a γ parameter. Markov chain Monte Carlo searches were run with four chains, each for 5,000,000 generations, with trees sampled every 1000 generations and the first 25% of trees discarded as "burn-in". Trees based on *RAG-1* were rooted with *Acanthisitta chloris*; this was found to be the basal passeriform lineage in [S8]. Trees were also constructed using the same ML and Bayesian methods for each of the other sequence partitions (i.e., *β-fibrinogen introns* combined; mtDNA combined). Full trees with all sequences and Genbank numbers are available from R. C. F.

Dating analysis

As in [S8], we used as our primary calibration date for *RAG-1* sequences the estimated age of the isolation of *Acanthisitta* from the other Passeriformes, which was based on estimates of the timing of separation of New Zealand from Antarctica at 82 my. We also followed [S8] in using non-parametric rate smoothing (NPRS) and penalized likelihood (PL) approaches in r8s [S15] to estimate the ages of particular nodes from trees built from *RAG-1* and other sequences. For NPRS we used a Powell algorithm, and for PL we used the TN algorithm and a smoothing parameter of 100, estimated from a cross-validation procedure. For *RAG-1* we pared the dataset to 35 representative taxa, including *Moho*, a sampling of passerid and corvid oscines, and suboscines, to reduce the time of bootstrapping and to remove polytomies. In order to estimate confidence limits on the dates, this smaller dataset was bootstrapped 50 repetitions in Garli 0.96b8 (*S16*) and resulting trees with branch lengths were rooted and analysed in r8s for each of the two dating methods. Means and standard errors of nodal dates were calculated from the sample of bootstrapped trees for nodes between the two *Moho* species and their closest relatives (i.e., *Phainoptila*, *Phainopepla*, *Dulus*, *Bombycilla*). We repeated the dating analyses for the smaller mtDNA dataset (up to 719 bp), using the date of the split between *Moho braccatus* (Kauai) and *Moho apicalis* (Oahu) [S17]. This split is estimated to be the age of Oahu, which became subaerial about 3.5 mya ([S18], see [S17] for methods and assumptions).

Supplemental Results

DNA sequences and Phylogeny

RAG-1 results: Substantial lengths of *RAG-1* sequences were obtained from museum skin specimens of four *Moho nobilis* and one *Moho bishopi;* shorter sequences were obtained from one *Moho apicalis* and one *Chaetoptila angustipluma* (Table S1). The *Chaetoptila* sequence differed by two bp (0.7%) from *Moho nobilis* and *Moho bishopi* (which did not differ from each other or *Moho apicalis*). In addition, we obtained *RAG-1* sequences from skin specimens of *Corvus nasicus* and *Gymnomyza samoensis*. The placement of the Hawaiian taxa was well supported: high bootstrap values and posterior

probabilities (Figure 3b), and a Shimodaira-Hasegawa test in PAUP* [S12] revealed a significantly longer tree when *Moho* was constrained to the meliphagid clade ($p < 0.0001$; 27 additional steps).

ß *–fibrinogen* results: We obtained *ß –fibrinogen intron* sequences for *Moho nobilis*, *Moho apicalis*, and *Moho bishopi* (Table S1). There was no difference between *M. nobilis* and *M. apicalis*, but one substitution differed between *M. bishopi* and the other taxa. These sequences were aligned to ones downloaded from Genbank (and to a *Phainopepla nitens* sequence). Both combined (Figure 3c) and individual gene (not shown) phylogenetic analyses strongly support the placement of the Hawaiian clade within a waxwing/silky flycatcher clade and not in Meliphagidae.

mtDNA results: We obtained more than 600 bp of *Cytb*, *12S rRNA* and *ATP6&8* for two individuals of *Moho nobilis* and one *Moho bishopi*, and 350-500 bp for five additional individuals of these and the remaining mohoid species (Table S1). Sequences matched very closely among conspecifics and monophyly was supported, but they did not provide much power for resolving the topology within the clade of Hawaiian taxa (Figure 3a).

Dating

NPRS and PL methods estimated similar ages at particular nodes of interest (Table 1) for *RAG-1* sequences, but the two methods provided rather different dates based on combined mtDNA sequences. For *RAG-1* sequences, dates for *Moho* versus *Phainoptila* range from 14.35 to 16.01 my and have low standard errors (Table 1). The dates obtained for this split using the combined mtDNA sequences and internal calibration (age of Oahu at 3.5 my) varied more widely, from 12.28 (PL) to 19.91 my (NPRS). Interestingly, when we use the *RAG-1 Acanthisitta* calibration to estimate the divergence of *Moho bishopi* (Molokai/Maui) and *Moho nobilis* (Hawaii Island) the predicted dates range from 0.56 to 0.88 my (Table 1); the island of Hawaii became subaerial ~1.0 mya and its maximal shield building date was ~0. 5 mya [S18]. The estimates based on mtDNA are a bit more than twice this expected age (Table 1), but this difference may reflect a faster rate of sequence evolution for mtDNA at earlier times of divergence [S19].

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Table S1. List of museum specimens sampled, extracted and sequenced for this study, and number of base pairs obtained for each gene region listed. BM = British Museum, Tring; UMZC = Cambridge University Museum of Zoology; MCZ = Museum of Comparative Zoology, Harvard University; AMNH = American Museum of Natural History; USNM = U. S. National Museum. Year = year of collection, if known. GB# = Genbank numbers.

Species	Museum #	Year	*RAG-1*	*Bfib5*	*Bfib7*	*mtDNA	GB#
Mohoidae:							
Moho nobilis	BM 95.7.20.30	1892	1502	250	171	717	FJ378041
							FJ378048
							FJ378052
							FJ378056
							FJ378063
Moho nobilis	UMZC-27/Mel/22/d/2	1887	1027	146	92	353	FJ378066
							FJ378051
							FJ378053
							FJ378057
							FJ378064
Moho nobilis	UMZC-27/Mel/22/d/6	1888	1068	-	-	418	FJ378067
							FJ383126
							FJ392533
Moho nobilis	Liverpool-T16488	1887	512	-	-	97	FJ378068
							FJ378065
Moho nobilis	MCZ 10990	-	-	-	-	628	FJ378055
							FJ383120
							FJ383121
Moho bishopi	MCZ 134732	1893	1043	99	-	719	FJ378042
							FJ378047
							FJ378059
							FJ383124
							FJ383119
Moho apicalis	AMNH 459000	<1850	146	103	-	465	FJ378046
							FJ378049
							FJ378058
							FJ383123
Moho braccatus	UMZC-27/Mel/22/c/2	1888	-	-	-	356	FJ378060
							FJ383125
Chaetoptila Angustipluma	UMZC-27/Mel/6/2/1	1859	231	-	-	441	FJ378045
							FJ378061
							FJ383122
Meliphagidae:							
Gymnomyza samoensis	UMZC-27/Mel/13/b/4	-	1190	194	117	554	FJ378043
							FJ378050
							FJ378054
							FJ378062
Corvidae:							
Corvus nasicus	USNM 396599	1949	1378	-	-	-	FJ378044

*mtDNA sequence includes *12s rRNA*, *Cytb* and *ATP6&8* genes

Table S2. Primers for *RAG-1* (10 sets) and *β-fibrinogen intron 5* and *β-fibrinogen intron 7* (two sets each) are shown. These were designed for this study from alignments of passeriform sequences downloaded from Genbank.

RAG1-1L: 5’-TTCATCTTCTTCCTGAGGTGTTT
RAG1-1R: 5’-CATGAGGATCGCCACACTG
RAG1-2L: 5’-GGGTACAAATGTAAGTGGAACCT
RAG1-2R: 5’-TGGGTTGGACCTCCATATTT
RAG1-3L: 5’-TCACCGCCCTCTTCTTTCTC
RAG1-3R: 5’-TATGATACTGACTACAGCTGAGAAA
RAG1-4L: 5’-GATTCGGATGGCCAGACAG
RAG1-4R: 5’-ACATTTTTCAGGGGAGGTTTC
RAG1-5L: 5’-CACTCAAACGGGTGGTAACC
RAG1-5R: 5’-GCCTTCCAAGATCTCCTCCT
RAG1-6L: 5’-TATCGCTCCAGATTTTCAGC
RAG1-6R: 5’-CTTCTTCCTGAGGTGTTTGTCA
RAG1-7L: 5’-TCCTCCATGTCCTTTAAGGC
RAG1-7R: 5’-TGTGAAAGAAAAGCGAACAGC
RAG1-8L: 5’-ACAGCAGGCCCACTTCCA
RAG1-8R: 5’-TCTGATTCATCAGCCAGCAT
RAG1-9L: 5’-GCACAAGGGCTTGCAACAC
RAG1-9R: 5’-GGGTTGCATCACACAGGGTA
RAG1-10L: 5’-ACACCGGCTTCATCTTCAGATA
RAG1-10R: 5’-TTTCGATGATTTCAGGAACATGAG
βfib5-1L: 5’-GGAAACAGATAATGGAGGTTAGTG
βfib5-1R: 5’-CATCAGCAGATGACCTCAACA
βfib5-2L: 5’-TCGTTCAGGGAAGTCTTGTTG
βfib5-2R: 5’-CTTGTCTGCCCACCTACACA
βfib7-2L: 5’-TTAGTGACAGTCCATAACCAAGTAAAA
βfib7-2R: 5’-GTGTCCTAAGCACTGCTGCTG
βfib7-3L: 5’-CAGGGACTGACAGCAGCA
βfib7-3R: 5’-CAACTGAACTCCTGTCTTCTGAG