

Convergent evolution of 'creepers' in the Hawaiian honeycreeper radiation

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Natural selection plays a fundamental role in the ecological theory of adaptive radiation. A prediction of this theory is the convergent evolution of traits in lineages experiencing similar environments. The Hawaiian honeycreepers are a spectacular example of adaptive radiation and may demonstrate convergence, but uncertainty about phylogenetic relationships within the group has made it difficult to assess such evolutionary patterns. We examine the phylogenetic relationships of the Hawaii creeper (*Oreomystis mana*), a bird that in a suite of morphological, ecological and behavioural traits closely resembles the Kauai creeper (*Oreomystis bairdi*), but whose mitochondrial DNA (mtDNA) and osteology suggest a relationship with the amakihi (*Hemignathus* in part) and akepa (*Loxops*). We analysed nuclear DNA sequence data from 11 relevant honeycreeper taxa and one outgroup to test whether the character contradiction results from historical hybridization and mtDNA introgression, or convergent evolution. We found no evidence of past hybridization, a phenomenon that remains undocumented in Hawaiian honeycreepers, and confirmed mtDNA and osteological evidence that the Hawaii creeper is the most closely related species to the amakihi and akepa. Thus, the morphological, ecological and behavioural similarities between the evolutionarily distant Hawaii and Kauai creepers represent an extreme example of convergent evolution and demonstrate how natural selection can lead to repeatable evolutionary outcomes.

Keywords: ecological convergence; convergent evolution; Hawaiian honeycreepers; mitochondrial DNA introgression; hybridization; adaptive radiation

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1. INTRODUCTION

Adaptive radiation is a fascinating evolutionary process that has generated much biodiversity. Although several mechanisms may be responsible for such diversification, the 'ecological theory' holds that it is the outcome of divergent natural selection between environments (Schluter 2000). Whether adaptive radiations result chiefly from such ecological speciation, however, remains unclear (Schluter 2001). Convergent evolution is often considered powerful evidence for the role of adaptive forces in the speciation process (Futuyma 1998), and thus documenting cases where it has occurred is important in understanding the link between natural selection and adaptive radiation.

The more than 50 species of Hawaiian honeycreepers (subfamily Drepanidinae) are a spectacular example of adaptive radiation and an interesting system to test for convergence, which has been suspected among the nuthatch-like 'creeper' ecomorph on the islands (figure 1). The Hawaii creeper (*Oreomystis mana*) has traditionally been allied with the Kauai creeper (*Oreomystis bairdi*), either as conspecific (Amadon 1950) or as a congener (Henshaw 1902; Pratt 1992). The two birds share many similarities including bill shape, foraging and mobbing behaviours, and juvenile plumage pattern (Pratt 1992; Foster *et al.* 2000; Lepson & Woodworth 2001). Their juvenile begging calls are nearly identical and distinct from those known for other honeycreepers (Pratt 2001). Both species possess a narrow, simple notch-tipped tongue very different from the tubular tongues of most other honeycreepers (Pratt 1992), including akepas (*Loxops*) and amakihi (*Hemignathus* in part). But despite these apparent synapomorphies, recent osteological and mitochondrial DNA (mtDNA) evidence (Fleischer *et al.* 2001; James 2004) suggests these two species are evolutionarily quite distant, and instead points to a close relationship between *O. mana* and the cross-billed akepa or curve-billed amakihi. Pratt (2001) remarked that if the Kauai and Hawaii creepers derived their diverse similarities by convergence, they would present the most dramatic example of convergent evolution yet discovered in birds.

As an alternative explanation for the conflicting phylogenetic evidence, Pratt (2001) suggested a past hybridization event. Under this scenario, hybrids would have involved male *Oreomystis* mating with female akepa or amakihi. If the Hawaii creeper descended from such offspring, it could have retained most of the phenotypic characteristics of *Oreomystis* but possess mtDNA from the introgressing species. Hybridization can result in a significantly different genealogy for mtDNA from that of most genes in a species, as mtDNA is more likely to introgress than nuclear DNA (nucDNA; Ballard & Whitlock 2004). Indeed, mtDNA from one taxon can completely replace that in another, with little or no evidence of nuclear introgression or morphological change (Bernatchez *et al.* 1995). No unequivocal cases of hybridization have yet been documented in Hawaiian honeycreepers. If past introgression is responsible for the incongruence between mitochondrial sequences and some striking phenotypic characters, nucDNA markers should reveal the contribution of the *Oreomystis* lineage to the genetic signal of the Hawaii creeper.

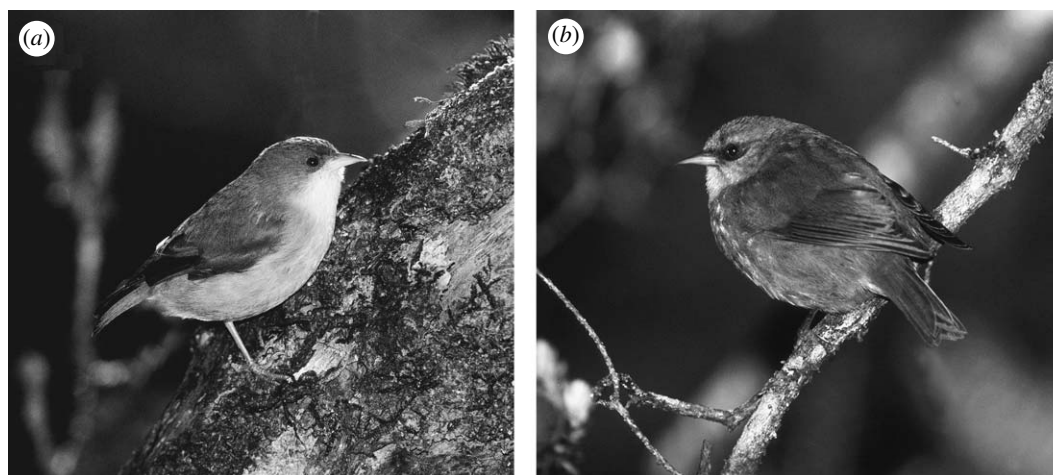


Figure 1. Photographic comparison of the (a) Kauai creeper and (b) Hawaii creeper. Photographs by Jack Jeffrey.

Here, we examine nucDNA sequences from several honeycreeper species to test whether convergence or introgression characterizes the relationship between the Hawaii and Kauai creepers.

2. MATERIAL AND METHODS

We analysed 19 individuals belonging to 11 relevant honeycreeper taxa and one outgroup. See appendix S1, electronic supplementary material, for provenance of samples. Five distinct gene regions, including four nuclear introns and one exon (see electronic supplementary material), generated *ca* 2500 BP of sequence data. We analysed the intron and exon datasets both separately and concatenated. We conducted a homogeneity partition test (ILD, Farris *et al.* 1995) with heuristic search, as implemented in the program PAUP* v. 4.0.b10 (Swofford 2002), to evaluate the congruence of phylogenetic signal between the two sequence sets. We reconstructed phylogenies using three approaches: maximum parsimony (MP); maximum likelihood (ML); and Bayesian inference (see electronic supplementary material). To evaluate the strength of the evidence for the placement of the Hawaii creeper with akepa/amakihi versus Kauai creeper, we subsequently applied constraints in the MP and ML methods to force the alternative topology, and compared the resulting ML trees with the unconstrained trees using the S–H test (Shimodaira & Hasegawa 1999; see electronic supplementary material). In addition to the nucDNA data, we obtained and analysed 1254 bp of mtDNA sequences from the same specimens (see electronic supplementary material for methods and genes used). All sequences have been deposited in GenBank (FJ266094–FJ266312).

3. RESULTS

The partition-homogeneity test was non-significant ($p=0.10$), indicating congruence between the different gene regions and justifying the concatenation of the nucDNA sequences into a single data matrix, but we also analysed the introns and exon separately. The results of the separate analyses were concordant with those from the concatenated analysis (see electronic supplementary material), so we present only the results from the combined dataset.

MP analysis of the combined dataset resulted in 120 equally parsimonious trees of length 113, and ML analysis resulted in four equally likely trees with a $-\ln$ likelihood score of 4195.1. All of the MP and ML trees placed the Hawaii creeper in a clade with the akepas and amakihis (figure 2). Bootstrap values at this node for both MP and ML analyses were moderately high (higher than 75%). Bayesian inference also produced a consensus topology similar to the MP and ML results and showed strong support

for placement of Hawaii creeper with the akepas and amakihis, as noted by a high posterior probability value of 1.0 for this clade (figure 2). Trees constructed using mtDNA sequences from the specimens in this analysis (figure S3, in the electronic supplementary material) were nearly identical to the trees from the nuclear gene analyses (figure 2) and generally matched previous trees based on more taxa, especially with regards to the position of the Hawaii creeper (Fleischer *et al.* 2001).

Constraining the topology such that the Hawaii and Kauai creepers formed a monophyletic group increased the length of the MP tree by eight steps and lowered the score of the ML tree by 28.6. Likelihood scores of the constrained trees were significantly worse ($p=0.007$, S–H test) than that of figure 2.

4. DISCUSSION

By corroborating the phylogeny obtained from mtDNA and osteology, the results from nucDNA markers indicate that creepers in the Hawaiian Islands are an astounding and strongly supported example of ecological convergence. The two species apparently evolved independently to fill similar niches on separate islands, and morphological and behavioural traits converged as a result of similar selective pressures. Although a detailed osteological analysis supports the molecular result (James 2004), most other phenotypic characters support the hypothesis of a close relationship between the Hawaii and Kauai creepers (Pratt 2001). Characters such as song and plumage, which may be subjected to natural or sexual selection, exhibit high levels of evolutionary lability and homoplasy in birds (e.g. Price *et al.* 2007). Tongue morphology may also be under selection for different foraging strategies, with a simple forked tongue being better adapted to picking up insects than the brush-tipped tubular tongue of most other honeycreepers (Richards & Bock 1973). It is, however, surprising that a behavioural trait not clearly related to foraging, such as juvenile begging calls, would be convergent. Overall, the observed level of discord between evolutionary relatedness and behavioural and morphological traits is unprecedented among Hawaiian honeycreepers, and surprising among birds generally.

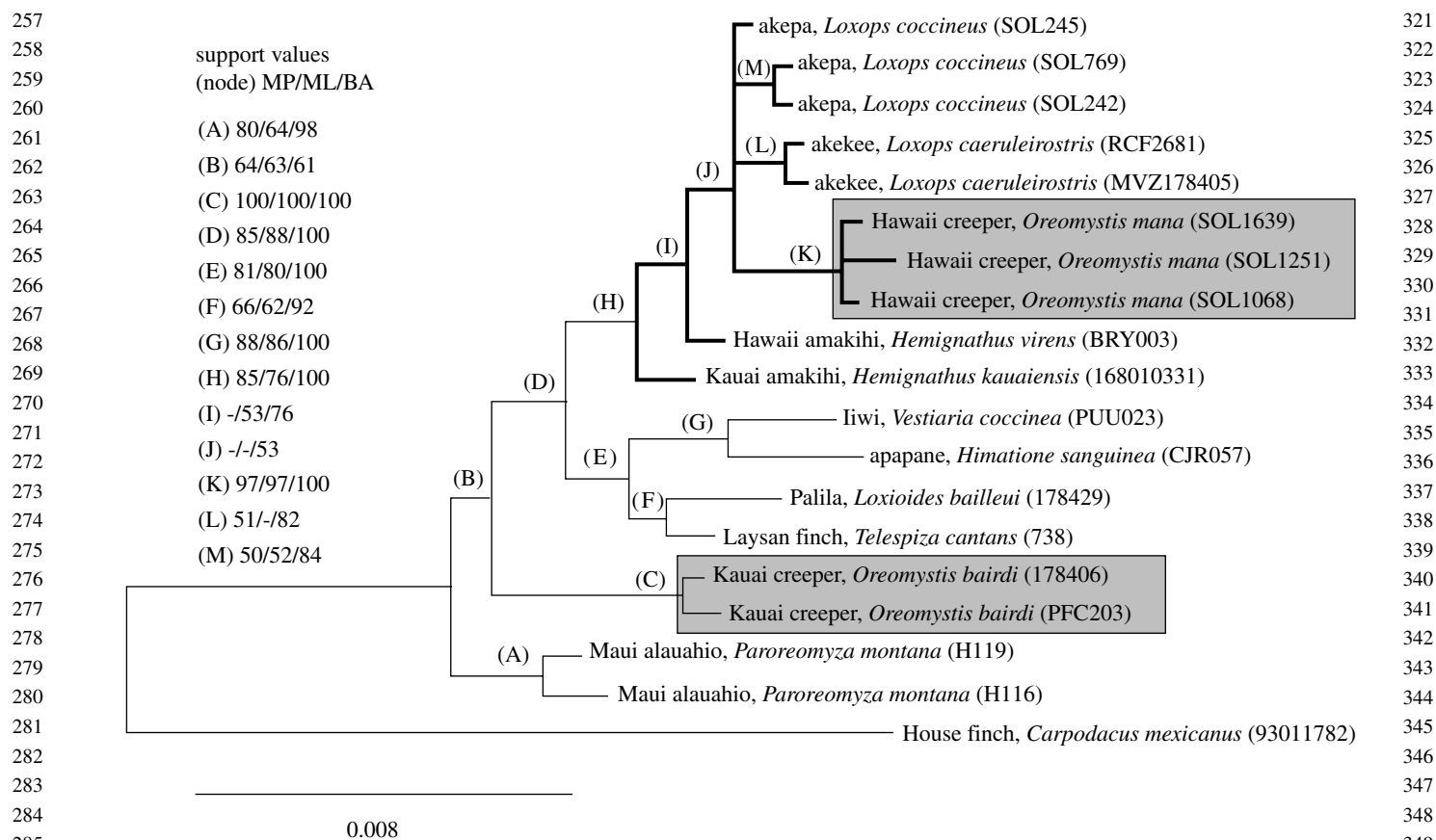


Figure 2. Bayesian consensus topology obtained from the analysis of sequences from five introns and one exon, partitioned into two parts. Bootstrap values from MP and ML analyses (1000 replicates each), and posterior probability values ($\times 100$) from Bayesian analysis (15 000 trees sampled) are provided for each node. A dash indicates less than 50% support. Collapsed nodes had low bootstrap and posterior probability values (i.e. less than 50%). The Hawaii creeper/akepa/amakihi clade (node H) is shown in bold. The scale at the bottom indicates 0.8% divergence along a branch.

Based on our and previous results, we recommend the removal of the Hawaii creeper from the genus *Oreomystis*. The statistical polytomy among the Hawaii creeper, amakihis and akepas (figure 2), however, makes the relationships among those three groups unclear. Further research will be needed before we can say whether the Hawaii creeper deserves a monotypic genus (which would require a new name) or can be classified within an existing one. But because of the rapidity of the radiation and the large number of extinctions (Fleischer *et al.* 1998; James 2004), phylogenetic study of the Hawaiian honeycreepers remains challenging.

The convergence of creepers contrasts with the pattern of the few other honeycreeper ecomorphs that have been examined, which apparently evolved only once and dispersed among the islands (Fleischer *et al.* 2001). These analyses, however, included only extant taxa. In addition, in the absence of convincing evidence of hybridization among them, Hawaiian honeycreepers contrast sharply with the other iconic avian island radiation, Darwin's finches, in which hybridization and introgression are known to play a major evolutionary role (Grant & Grant 2008). Nonetheless, the discovery of this previously unknown example of convergence in Hawaiian honeycreepers adds to the growing list of adaptive radiations demonstrating repeated evolution of similar ecomorphs in comparable environments (e.g. *Anolis*

lizards: Losos *et al.* 1998; cichlid fishes: Rüber *et al.* 1999; Hawaiian *Tetragnatha* spiders: Gillespie 2004). Such deterministic patterns of evolution underscore the important role of natural selection in shaping adaptive radiations, and call into question the view that evolution is inherently contingent and unpredictable (Gould 1989).

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