

On the origin of the Galápagos hawk: an examination of phenotypic differentiation and mitochondrial paraphyly

JOSHUA M. HULL^{1*}, WESLEY K. SAVAGE², JENNIFER L. BOLLMER³,
REBECCA T. KIMBALL⁴, PATRICIA G. PARKER³, NOAH K. WHITEMAN^{3,5} and
HOLLY B. ERNEST^{1,6}

¹Wildlife and Ecology Unit, Veterinary Genetics Laboratory, University of California, One Shields Avenue, Davis, CA 95616, USA

²Section of Evolution and Ecology, University of California, One Shields Avenue, Davis, CA 95616, USA

³Department of Biology, University of Missouri – St Louis, St. Louis, MO 63121, USA

⁴Department of Zoology, University of Florida, PO Box 118525, Gainesville, FL 32611, USA

⁵Museum of Comparative Zoology, Department of Organismic and Evolutionary Biology, Harvard University, 26 Oxford Street, Cambridge, MA 02138, USA

⁶Department of Population Health and Reproduction, School of Veterinary Medicine, University of California, One Shields Avenue, Davis, CA 95616, USA

Received 18 September 2007; accepted for publication 11 March 2008

Previous phylogenetic hypotheses suggest a sister group relationship between mainland and widespread *Buteo swainsoni* (Swainson's hawk) and the island archipelago taxon *Buteo galapagoensis* (Galápagos hawk). We further describe phylogenetic relationships of this clade using molecular data from the mitochondrial control region, and consider the role of niche expansion on phenotype using morphological data from *B. galapagoensis*, *B. swainsoni*, and related *Buteo jamaicensis* (red-tailed hawk). Among 52 unique *Buteo* haplotypes, phylogenetic analyses support a monophyletic *B. galapagoensis* clade within a clade of *B. swainsoni* haplotypes, rendering *B. swainsoni* paraphyletic with respect to *B. galapagoensis*. Mitochondrial paraphyly is likely a result of incomplete lineage sorting subsequent to a recent colonization event and exemplifies speciation of peripheral population isolates. Morphological comparisons indicate that metrics associated with prey capture differ significantly between *B. galapagoensis* and *B. swainsoni*, but are similar between *B. galapagoensis* and *B. jamaicensis*. These results suggest directional selection on *B. galapagoensis* morphology associated with feeding, possibly an outcome of decreased interspecific competition and change towards a more generalist diet shared by *B. jamaicensis*. In the *B. galapagoensis* lineage, our results suggest that genetic drift influences the neutral mitochondrial marker, whereas selection may have driven phenotypic character change. © 2008 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2008, **95**, 779–789.

ADDITIONAL KEYWORDS: *Buteo galapagoensis* – *Buteo swainsoni* – archipelago – island – morphology – niche expansion.

INTRODUCTION

Differentiation between island and mainland populations of species may result from several factors, including population size, genetic drift, selection, and the extent of ongoing gene exchange (Wright, 1931; Barton, 1998). Between sister species, these processes

may be of varying importance depending upon the character under investigation (Lynch, 1990; Clegg *et al.*, 2002). Under the neutral theory, noncoding genomic DNA, not closely linked to regions under positive selection, will be affected by stochastic processes such as genetic drift (Kimura, 1983), with no direct connection to phenotypic change (Bromham & Hendy, 2000; Bromham *et al.*, 2002, but see also Omland, 1997). Consequently, divergence in neutral

*Corresponding author. E-mail: jmhull@ucdavis.edu

molecular markers and phenotype is predicted to proceed independently.

Rapid phenotypic change from an ancestral mainland phenotype is frequently observed among island taxa (Lack, 1971; Grant, 1998) and has been attributed to several processes, including founder effects, change in selective landscape, relaxation of interspecific competition, and niche expansion (Grant, 1998; Whittaker, 1998; Rasner *et al.*, 2004). Among birds and mammals, morphological changes among island species often follow the 'island rule' (Foster, 1964; Van Valen, 1973; Lomolino, 1985; Clegg & Owens, 2002), where small-bodied taxa evolve to larger size and larger-bodied taxa evolve towards smaller size. Such phenotypic changes between island and mainland taxa can mislead assumptions of phylogenetic relationships. Phylogenetic analysis using character information from neutral molecular markers (particularly those with smaller effective population sizes), in conjunction with morphological characters, can facilitate an objective examination of the relationships among closely-related taxa and an understanding of the evolutionary processes leading to their divergence (Scott *et al.*, 2003).

Buteo swainsoni (Swainson's hawks; Bonaparte) and *Buteo galapagoensis* (Galápagos hawks; Gould) are phenotypically distinct in body size, plumage, diet, and mating system, and their geographic ranges are entirely non-overlapping and differ greatly in extent. *Buteo swainsoni* is an abundant raptor species that breeds throughout western North America and a large number of individuals migrate through central America to the grasslands of South America (England, Bechard & Houston, 1997). Limited micro-satellite population genetic structure exists across their breeding range (Hull *et al.*, 2008) and regional differences in morphology have not been documented (England *et al.*, 1997). By contrast, *B. galapagoensis* are restricted to eight (historically eleven) islands of the Galápagos archipelago (de Vries, 1975, 1976). Extremely limited dispersal occurs among the islands and most populations are genetically distinct (Bollmer *et al.*, 2005) and differ in morphology (Bollmer *et al.*, 2003). Until recently, the obvious phenotypic and behavioural differences between *B. swainsoni* and *B. galapagoensis* (de Vries, 1975, 1976) have been regarded as untested evidence for the recognition of two divergent *Buteo* species. Moreover, a sister relationship between these two species was not evident based on phenotypic evidence (de Vries, 1973). Recent mitochondrial phylogenetics of the genus *Buteo* shows a close sister relationship between *B. swainsoni* and *B. galapagoensis* (Riesing *et al.*, 2003) with an estimated Galápagos colonization 126 000 years ago (95% confidence interval = 51 000–254 000 years; Bollmer *et al.*, 2006).

Table 1. Sample size for genetic and morphological data (including the number of females and males in parentheses) from *Buteo swainsoni*, *Buteo galapagoensis*, and *Buteo jamaicensis*

Species	Genetic	Morphological
<i>Buteo swainsoni</i>	308	104 (38/66)
<i>Buteo galapagoensis</i>	122	217 (91/126)
Española	10	19 (10/9)
Isabela	20	19 (11/8)
Santa Fe	9	18 (9/9)
Santiago	21	128 (54/74)
Marchena	15	21 (4/17)
Pinta	13	12 (3/9)
Fernandina	20	0
Pinzón	10	0
Santa Cruz	4	0
<i>Buteo jamaicensis</i>	5	132 (45/87)

The present study aimed to describe the phylogenetic relationship between *B. swainsoni* and *B. galapagoensis* with fine-scale sampling, and to investigate the extent of molecular and morphological differentiation. Accordingly, we describe the phylogenetic relationship between these *Buteo* species using samples from throughout their current ranges to test the monophyly of each lineage and to evaluate the strength of morphological differentiation. We investigate phenotypic divergence by comparing five morphological characters among *B. galapagoensis*, *B. swainsoni*, and *Buteo jamaicensis* (red-tailed hawk; Gmelin). We include *B. jamaicensis* to provide a contrast between a generalist mainland *Buteo* and a generalist island taxon (*B. galapagoensis*).

MATERIAL AND METHODS

SAMPLE COLLECTION

We collected whole blood and feathers from 365 *B. swainsoni* and 122 *B. galapagoensis*, as well as five *Buteo regalis* (ferruginous hawk), three *Buteo lagopus* (rough-legged hawk), six *Buteo lineatus* (red-shouldered hawk), five *B. jamaicensis*, and one *Buteo albicaudatus* (white-tailed hawk) for outgroup comparison. Blood was stored in lysis buffer and feathers were stored dry. Three hundred and thirty-six *B. swainsoni* were sampled on breeding grounds (from June to July) in North America (for locality information, see Table 1) between 2003 and 2005, and the remaining 29 *B. swainsoni* were captured on wintering grounds in central Argentina during January 2003, as described previously (Whiteman *et al.*, 2006). Samples were collected from 122 *B. galapagoensis* (Table 1) captured with either bal-chatris or rope nooses on poles.

LABORATORY METHODS

For *B. swainsoni* samples, we isolated total cellular DNA from 25 µl of the blood/buffer solution using Qiagen DNeasy kits (QIAGEN Inc.). We extracted genomic DNA from *B. galapagoensis* samples using a modification of phenol/chloroform extraction (Sambrook, Fritsch & Maniatis, 1989) with final purification by dialysis against TNE₂. A total of 367 bp of domain I of the mitochondrial control region were amplified by the polymerase chain reaction (PCR) using primers 16065F (Kimball *et al.*, 1999) and H15414 (Bollmer *et al.*, 2006). Purification of the PCR product and sequencing reactions were conducted according to the methods of Bollmer *et al.* (2006) and Hull *et al.* (2008). For a subset of individuals, both blood and feather samples were used from the same individuals to aid in detection of nuclear-mitochondrial insertions. All sequences generated in the present study were deposited in GenBank (accession numbers: AY870866–AY870892 and EF568728–EF568773).

SEQUENCE ANALYSIS AND PHYLOGENETIC METHODS

We examined and manually aligned sequences with SEQUENCHER, version 4.5 (Gene Codes Corporation). We then identified identical sequences and reduced the dataset to single, unique haplotypes using MACCLADE, version 4.08 (Maddison & Maddison, 2005). To test for selective neutrality in control region sequences, we calculated Fu's F_s statistic (Fu, 1997) and Fu and Li's D^* and F^* statistics (Fu & Li, 1993) in DNASP, version 4.10.9 (Rozas *et al.*, 2003). Comparing F_s , D^* , and F^* permits discrimination of population expansion from background selection; a significant F_s with nonsignificant D^* and F^* supports an interpretation of population expansion (Fu, 1997). Prior to pruning the redundant haplotypes, we used statistical parsimony in TCS, version 1.21 (Clement, Posada & Crandall, 2000) and median joining in NETWORK, version 4.201 (Bandelt, Forster & Röhl, 1999) to generate parsimony and minimum spanning networks, respectively, of *B. swainsoni* and *B. galapagoensis* haplotypes.

Phylogenetic reconstruction of aligned sequences was conducted and evaluated using maximum parsimony (MP), maximum likelihood (ML), and Bayesian methods. For all methods, we assumed *B. albicaudatus* was the outgroup taxon and include four other closely-related *Buteo* species (Riesing *et al.*, 2003) but we focused on the relationship between *B. galapagoensis* and *B. swainsoni*. We conducted heuristic MP searches in PAUP*, version 4.0b10 (Swofford, 2003) [ten addition-sequence replicates, and tree bisection and reconnection (TBR) branch swapping] with equal weights applied to all nucleotide substitutions. We constructed an MP phylogeny with MEGA, version

3.1 (Kumar, Tamura & Nei, 2004) to confirm our approximation of the most parsimonious tree reconstruction (closest-neighbour-interchange with search level 3, random addition trees with 900 replications).

MODELTEST, version 3.7 (Posada & Crandall, 1998) was used to evaluate the best-fit model of nucleotide evolution and parameter estimation to the control region sequence data for the ML tree search. Hierarchical likelihood ratio tests and Akaike Information Criterion (AIC) both selected the HKY + Γ model with the following parameters: empirical base frequencies (A = 0.2184, C = 0.2316, G = 0.1842), transition/transversion ratio = 7.1092, and gamma shape parameter = 0.0155). We used this substitution model and parameter estimates in an ML analysis to test for a molecular clock using a likelihood ratio test in PAUP*. Heuristic ML searches were conducted with stepwise addition of taxa, and TBR branch swapping. Although we used several methods to construct trees, we only present the ML tree to show model-based branch lengths and tree topology. We assessed tree support by bootstrap analyses performed for MP with 200 pseudoreplicates (ten random addition-sequence replicates) in PAUP*, and for ML with 500 repetitions using GARLI, version 0.951 (Zwickl, 2006). High sequence similarity among *B. swainsoni* haplotypes resulted in computationally intensive searches, which limited the number of MP bootstrap replicates that we could perform.

We conducted Bayesian analyses to evaluate clade support using a parallel version of MRBAYES, version 3.1.2 (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003), incorporating the HKY nucleotide model selected by hierarchical likelihood ratio tests and AIC implemented in MRMODELTEST, version 2.2 (Nylander, 2004). We ran three independent analyses to adequately arrive at a global optimum tree topology, each with 10^7 generations, using random starting trees and default priors. For these analyses, ten Markov chains (nine incrementally heated chains, in the range 0.36–0.83) were sampled every 1000 generations. We assessed convergence towards stationarity by evaluating the potential scale reduction factor (Gelman & Rubin, 1992) and discarded trees sampled prior to the diagnostic approaching one as burn-in samples. We also plotted the log-likelihood scores by generations of the Markov chains as an additional assessment of convergence (Leaché & Reeder, 2002) because neither method can be used to absolutely assess convergence on the optimal tree topology.

We discarded one million trees sampled prior to convergence as burn-in samples, and used the remaining trees to construct a 50% majority-rule consensus tree of clade credibility (assessed with posterior probability values). To determine whether the inferred tree topology for *B. swainsoni* and

B. galapagoensis was statistically supported, we compared alternative topologies using the nonparametric Shimodaira–Hasegawa test (Shimodaira & Hasegawa, 1999) in PAUP*, version 4.0b10 (Swofford, 2003), with RELL bootstrapping (10 000 replicates). We compared the tree with the best likelihood score against two alternative tree topologies: one with monophyletic *B. swainsoni* and *B. galapagoensis* sister groups and one containing a monophyletic but unresolved polytomy of *B. swainsoni* and *B. galapagoensis* haplotypes.

MORPHOLOGICAL DATA COLLECTION AND ANALYSIS

We analysed morphological character data for three recognized *Buteo* species: *B. galapagoensis* (54 females, 79 males), *B. swainsoni* (38 females, 66 males), and *B. jamaicensis* (45 females, 87 males; Table 1). We used the population mean \pm SD for five morphological measurements from published material (*B. galapagoensis*: Bollmer *et al.*, 2003; *B. swainsoni*: Sarasola & Negro, 2004; *B. jamaicensis*: Pitzer *et al.*, 2008). We examined wing chord (unflattened, from tip of longest primary to carpal joint), tail length (posterior base of uropygial gland to tip of central rectrices; not available for *B. jamaicensis*), culmen length (anterior margin of cere to tip of mandible), hallux chord (from proximal to distal extent of exposed talon), and body mass. The process of taking these measurements is standardized (GGRO, 1998) and no evidence of statistical differences between observers has been detected (Pitzer *et al.*, 2008). Within *B. galapagoensis*, our sample size for statistical tests was only valid for Santiago (54 females, 74 males). We tested pairwise significance between all *Buteo* species for each available metric using Welch's approximate *t*-test. We assumed an alpha level of 0.017 after a sequential Bonferroni correction (Rice, 1989).

RESULTS

PHYLOGENETIC ANALYSIS

Our dataset includes 52 unique *Buteo* haplotypes for 367 bp of the control region, with 71 (19.3%) variable sites, of which 52 (14.2%) provide phylogenetically informative signal. We did not recover gaps in the sequence alignment. No evidence of nuclear copies of the control region was observed: replicate sequences from feathers and blood yielded identical sequences. No heteroplasmy was observed in electropherograms (Sorenson & Quinn, 1998) and sequences aligned with published *Buteo* control region sequences. A large number of sequences from within each of the focal groups (*B. swainsoni* and *B. galapagoensis*) were identical and suggest that the level of taxon sampling

sufficiently covers the range of haplotype variation; five ($N = 122$) sequences were unique *B. galapagoensis* haplotypes and 36 ($N = 365$) sequences represented unique *B. swainsoni* haplotypes (Fig. 1). Two hybrid individuals identified as *B. swainsoni* based on phenotype have *B. jamaicensis* haplotypes, and are discussed in Hull *et al.* (2007). Uncorrected *p*-distances across all taxa were in the range 0.27–10.38% (mean = 3.45%) and 3.81–10.08% between the focal group and the five outgroup species. Within the island-endemic *B. galapagoensis*, the five haplotypes differ by up to 0.82% (mean = 0.54%), whereas divergence among haplotypes from the widespread *B. swainsoni* is as much as 3.09% (mean = 1.68%). Between *B. galapagoensis* and *B. swainsoni*, we find similarly low estimates of divergence, in the range 0.27–3.54% (mean = 1.79%). Fu's F_S statistic was significant for the sample of *B. swainsoni* and *B. galapagoensis* haplotypes ($F_S = -51.4$, $P < 0.001$); however, neither Fu and Li's F^* and D^* were significant ($D^* = 1.2$, $P > 0.10$; $F^* = 0.5$, $P > 0.10$). These results suggest that neutral evolution, and not positive (diversifying) selection, is contributing to the observed pattern in control region data.

Statistical parsimony and median-joining analyses resulted in identical haplotype networks (Fig. 2). The network of control region haplotypes revealed distinct *B. swainsoni* and *B. galapagoensis* clusters separated by five nucleotide changes.

MP, ML, and Bayesian analyses generally resolved concordant tree topologies. Where encountered, differences in topologies occur at nodes which lack statistical support, particularly in tip relationships within *B. swainsoni* (Fig. 1), as well as some rearrangement of relationships between the outgroups (*B. regalis*, *B. lagopus*, *B. lineatus*, and *B. jamaicensis*) and the focal ingroup. The unresolved relationships among the outgroup taxa are evident from the lack of statistical support for nodes. Maximum parsimony analyses in PAUP* resulted in 82 576 equally parsimonious trees with a tree length of 138, and identical scores and tree topology are produced by MP searches in MEGA (CI = 0.543, RI = 0.806; consensus not shown). ML analyses result in a single, optimized tree with a likelihood score of $-\ln L = 1230.71$. Based on likelihood ratio tests under ML, a molecular clock constraint is not supported by the control region data ($P < 0.003$). Because of the equally parsimonious reconstructions, we present the single ML tree and focus on the combined support values (bootstrap and posterior probabilities) for clades consistently recovered by each phylogenetic method.

Bootstrap analyses and Bayesian posterior probabilities single out the monophyly of a well-supported clade that contains *B. swainsoni* and *B. galapagoensis*, currently recognized as separate monophyletic

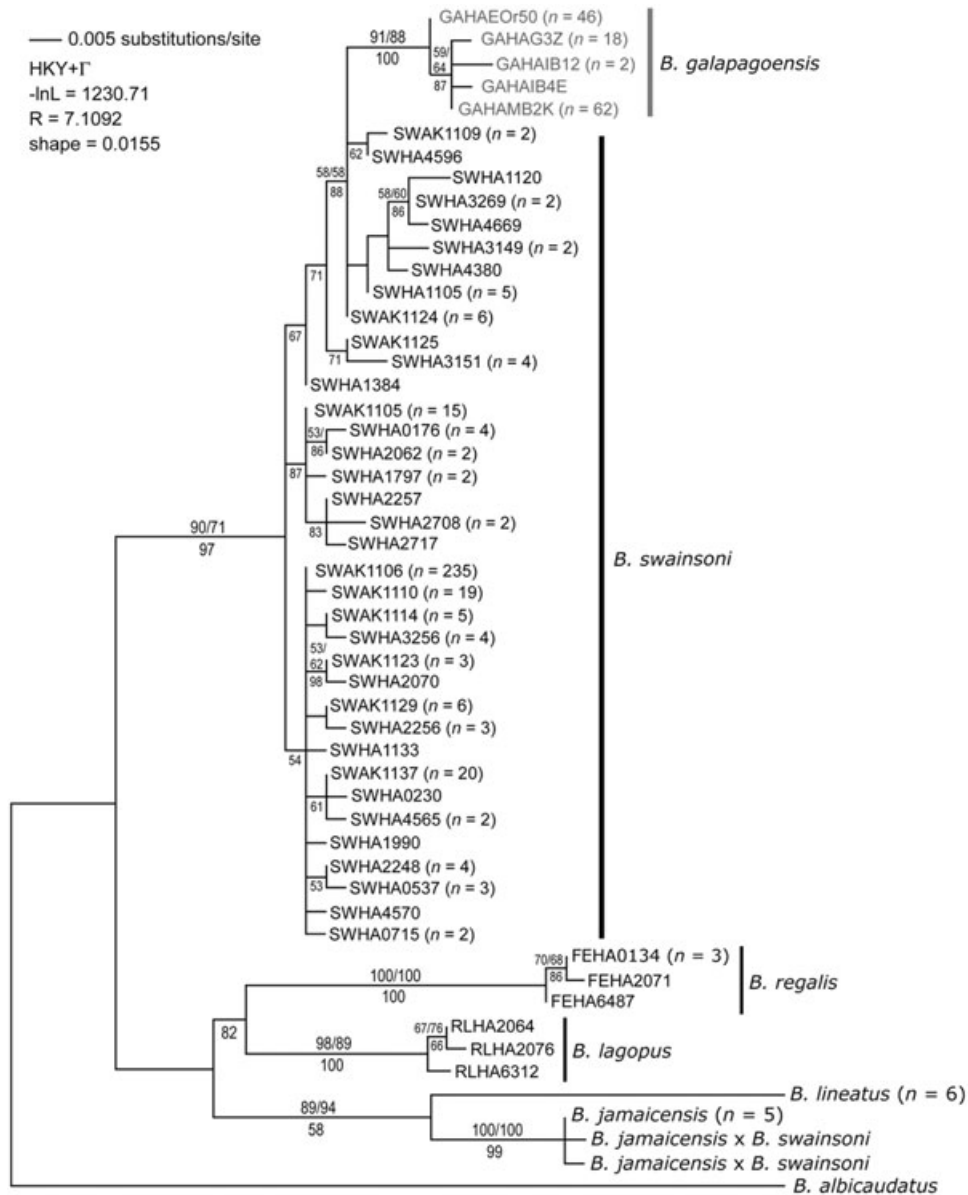


Figure 1. Phylogenetic relationship of *Buteo swainsoni* and *Buteo galapagoensis* based on mitochondrial DNA control region sequence data for 367 bases. Numbers above branches are bootstrap values (maximum parsimony/maximum likelihood) and Bayesian posterior probabilities are below. The relationships among the outgroup taxa and the *B. swainsoni* clade are less resolved. Haplotype sample sizes are next to haplotype names. The paraphyly of the *B. swainsoni* clade is indicated by statistical node support and the nested position of *B. galapagoensis* (grey text).

sister taxa (Fig. 1). Additionally, paraphyly of the *B. swainsoni*/*B. galapagoensis* clade is found in a strict consensus of the 99% set of credible trees recovered from the Bayesian analysis. Phylogenetic analysis of the control region clearly identifies a widespread but monophyletic *B. swainsoni* group, which includes a well-supported monophyletic lineage of haplotypes endemic to the Galápagos Islands (*B. galapagoensis*). Within the *B. swainsoni* group outside of the *B. galapagoensis* lineage, there is some branch support

in internodes to suggest that genetic structuring is occurring in some lineages but the overall relationships are unresolved. Based on the Shimodaira–Hasegawa test, the inferred paraphyly is significantly supported over the two alternate topologies ($P < 0.0001$, $P = 0.0002$, $\alpha = 0.05$). We consider that the Shimodaira–Hasegawa test results, in conjunction with branch support values and credible sets of trees, support the observed phylogeny of *B. swainsoni* and *B. galapagoensis*.

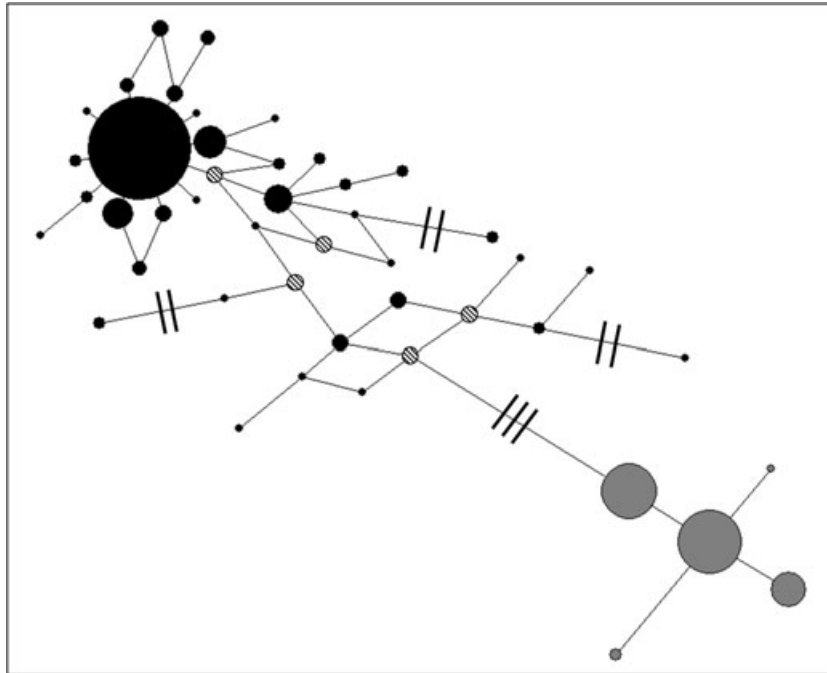


Figure 2. Minimum spanning networks depicting absolute difference between haplotypes. *Buteo swainsoni* haplotypes are shaded black; *Buteo galapagoensis* shaded grey. The relative abundance of each haplotype is indicated by the size of the circles; hash marks indicate the number of mutated positions between haplotypes and a single mutation is implied where no mark occurs; cross-hatched circles indicate missing haplotypes inferred by the analysis.

Table 2. Morphological comparisons between males and females of *Buteo swainsoni*, *Buteo galapagoensis* (from Isla Santiago only), and *Buteo jamaicensis* ($\alpha = 0.017$ following sequential Bonferroni correction; Rice, 1989)

Metric	<i>Buteo swainsoni</i> versus <i>Buteo galapagoensis</i>		<i>Buteo galapagoensis</i> versus <i>Buteo jamaicensis</i>		<i>Buteo swainsoni</i> versus <i>Buteo jamaicensis</i>	
	Female	Male	Female	Male	Female	Male
Wing length	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	NS	NS
Tail length	$P < 0.001$	$P < 0.001$	–	–	–	–
Culmen length	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$
Hallux chord	$P < 0.001$	$P < 0.001$	$P < 0.001$	NS	$P < 0.001$	$P < 0.001$
Body mass	$P < 0.001$	$P < 0.001$	NS	NS	$P < 0.001$	$P < 0.001$

NS, not significant.

MORPHOLOGICAL ANALYSIS

Buteo galapagoensis from Santiago were significantly larger than *B. swainsoni* in all measurements for both males and females (Table 2). Although significantly different, wing length appears to be the most qualitatively similar metric between *B. swainsoni* and *B. galapagoensis* from Santiago as well as from all other island populations of *B. galapagoensis* (Fig. 3). Wing length was most similar between *B. swainsoni* and *B. galapagoensis* among females from Pinta, and males from Pinta and Marchena.

Although *B. galapagoensis* is significantly larger in most comparisons (Table 2), *B. galapagoensis* and *B.*

jamaicensis are qualitatively more similar in size than *B. galapagoensis* and *B. swainsoni* (Fig. 3). We found no significant difference between *B. galapagoensis* and *B. jamaicensis* in body mass (males and females), tail length, or hallux chord in males. *Buteo swainsoni* is significantly smaller than *B. jamaicensis* in all measures except wing length (Table 2).

DISCUSSION

MITOCHONDRIAL DIFFERENTIATION

Among 52 unique *Buteo* control region haplotypes, phylogenetic analyses recover a well-supported,

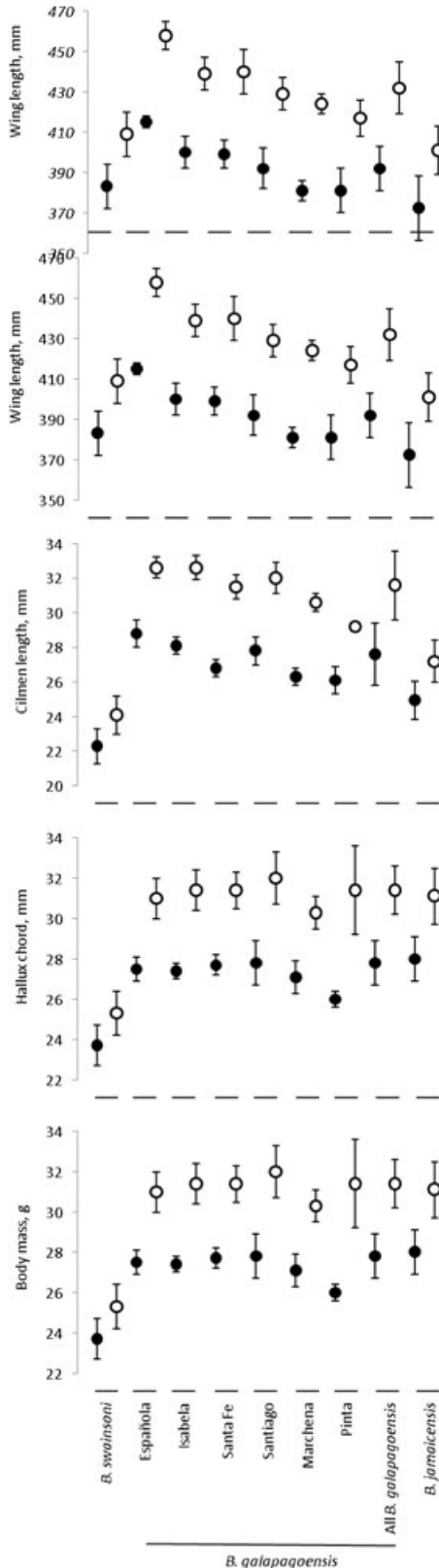


Figure 3. Mean and standard deviation for tail length, wing length, culmen length, hallux chord, and body mass for *Buteo swainsoni*, *Buteo galapagoensis* (from Española, Isabela, Santa Fe, Santiago, Marchena, and Pinta), and *Buteo jamaicensis*. Solid circles, males; open circles, females.

monophyletic clade that contains *B. swainsoni* and *B. galapagoensis*, which is distinct from the outgroup taxa. Within this clade, *B. galapagoensis* represents a well-supported monophyletic lineage separated from *B. swainsoni* by five fixed nucleotide sites, whereas *B. swainsoni* is paraphyletic with respect to *B. galapagoensis*. This is in contrast to the results of Riesing *et al.* (2003), which included three *B. swainsoni* and two *B. galapagoensis* samples, where the two taxa were found to be reciprocally monophyletic sister taxa. Although Riesing *et al.* (2003) were primarily concerned with species-level relationships within *Buteo*, our intent was to focus on a more thorough sampling of *B. swainsoni* and *B. galapagoensis*. Omland, Lanyon & Fritz (1999) suggested that dense sampling may be necessary to detect relationships among closely-related groups, which is corroborated by our study of 308 *B. swainsoni* and 122 *B. galapagoensis* samples. Outside of our focal group, the relationships among outgroup taxa are consistent with those described by Riesing *et al.* (2003), but the present study suggests that range-wide sampling can be a critical part of resolving phylogenetic relationships.

Our data illustrate a case of incomplete lineage sorting of *B. swainsoni* mitochondrial haplotypes, with *B. galapagoensis* following the pattern of budding or peripheral isolates (Harrison, 1991; Frey, 1993). New World ravens display a similar pattern with the restricted range of *Corvus cryptoleucus* (Chihuahuan raven) phylogenetically nested within the widespread *Corvus corax* (common raven). In these cases, the small population is more strongly influenced by genetic drift than the parental population, resulting in monophyletic daughter haplotypes nested within the phylogeny of the parental group (Funk & Omland, 2003). This pattern fits the natural history of a widespread *B. swainsoni* and an isolated *B. galapagoensis*. *Buteo galapagoensis* is thought to be a recent arrival to the Galápagos archipelago that diverged from *B. swainsoni* 126 000 years ago (Bollmer *et al.*, 2006). Furthermore, the population size of *B. galapagoensis* is orders of magnitude smaller than the mainland population of *B. swainsoni*. The total population of *B. galapagoensis* individuals numbers less than 1000 (Ferguson-Lees & Christie, 2001) compared with the census population

of *B. swainsoni*, which exceeds one million individuals (England *et al.*, 1997). Consequently, genetic drift is likely to have a much stronger influence on *B. galapagoensis*, which could explain the monophyly of this lineage relative to the lack of resolution among other *B. swainsoni* haplotypes.

In the continued absence of migration between mainland Americas and the Galápagos archipelago, these species will likely achieve reciprocal monophyly. Although we have no data on reproductive compatibility between *B. swainsoni* and *B. galapagoensis* (hybridization is common in birds, including occasional occurrences between *B. swainsoni* and both *B. jamaicensis* and *B. lagopus*; Clark & Witt, 2006; Hull *et al.*, 2007), the ecology and behaviour when coupled with molecular and morphological data strongly argue for the demographic independence of the Galápagos archipelago populations and retention of current American Ornithologists Union species designations for both *B. swainsoni* and *B. galapagoensis*.

MORPHOLOGICAL DIFFERENTIATION

By contrast to mitochondrial data, *B. swainsoni* and *B. galapagoensis* are quite morphologically differentiated, with *B. galapagoensis* being significantly larger in all metrics for both sexes. *Buteo swainsoni* have narrow wings with low body mass resulting in low wing loading (mass/wing area) and a high aspect ratio (wing span/wing chord) relative to many other *Buteo* species, presumably reflecting an adaptation to long distance migration (Kerlinger, 1989). Although wing length is similar between these species, the larger overall size of *B. galapagoensis* causes higher wing loading and a phenotype more suited to a sedentary life history.

Morphologic differentiation is much more apparent in tail-length, mass, culmen, and hallux. Although tail-length and mass reflect the overall larger body size of *B. galapagoensis*, the differences in culmen and hallux may be associated with differences in diet between *B. galapagoensis* and *B. swainsoni*. *Buteo swainsoni* generally takes smaller prey than other North American *Buteo* species; during breeding and nesting, their diet is composed primarily of small mammals, whereas the nonbreeding diet contains as much as 94% insects (Snyder & Wiley, 1976). During the breeding season in North America, *B. swainsoni* co-occurs with several other raptors, including *B. jamaicensis*, *B. regalis*, *B. lagopus*, and *Cathartes aura* (Turkey Vulture), all of which are potential niche competitors. Consequently, interspecific competition may limit the realized niche of *B. swainsoni*, and effect their morphology.

Within the Galápagos archipelago, *B. galapagoensis* is the only established diurnal raptor and the top

native predator on the islands where it occurs, resulting in little interspecific competition for food and therefore an expanded dietary niche. The diet of *B. galapagoensis* is much more varied than *B. swainsoni*, including many small prey items (e.g. small lizards and locusts) as well as larger species (e.g. iguanas and sea birds) and carrion (de Vries, 1976). Release from competition with mainland raptors allows a wider range of foraging opportunities, and may have resulted in selection for larger body size, particularly in the morphology associated with food capture.

An alternative explanation for larger size in *B. galapagoensis* is predominance of polyandrous mating among most island populations. High levels of competition for mates in such systems may drive evolution of larger body size in the competitive sex. Previous research investigating the association of body size and degree of polyandry among *B. galapagoensis* suggests the opposite pattern, with body size decreasing with increasing polyandry (Bollmer *et al.*, 2003).

Genetic drift may also be responsible for the observed morphological differences between *B. galapagoensis* and *B. swainsoni*. As with molecular markers, morphology may change more rapidly in small populations due to stochastic sampling. Within the Galápagos archipelago, populations of *B. galapagoensis* and their vertically transmitted ectoparasites show very limited movements between islands and display extremely high degrees of island-level differentiation at minisatellite and mitochondrial markers (Bollmer *et al.*, 2005, 2006; Whiteman, Kimball & Parker, 2007). If genetic drift were a primary factor in morphological change between *B. galapagoensis* and *B. swainsoni*, we would expect to see strong differences across isolated island populations with some populations evolving larger body size and others smaller than the ancestor of *B. galapagoensis* and *B. swainsoni*. In fact, we find that all *B. galapagoensis* populations are larger in body size than *B. swainsoni*. Individuals within some island populations are, on average, significantly larger than other island populations (Bollmer *et al.*, 2003), suggesting that drift may be responsible for these differences *within* the *B. galapagoensis* lineage but not between *B. galapagoensis* and *B. swainsoni*.

Niche expansion in *B. galapagoensis* leading to a more generalist diet and changes in morphology is supported by morphometric comparisons with *B. jamaicensis*. *Buteo jamaicensis* is a generalist North American raptor taking a wide range of prey, including reptiles, birds, amphibians, medium-sized mammals, and some carrion (Preston & Beane, 1993). Although *B. galapagoensis* is generally larger than *B. jamaicensis*, there is much more similarity in feeding morphology, particularly in hallux and culmen mea-

surements. By contrast, *B. swainsoni* have much smaller hallux and culmen dimensions than *B. jamaicensis*. The similarity in size between *B. galapagoensis* and *B. jamaicensis* suggests that a larger hallux and culmen may be selected traits in a generalist *Buteo* and indicates that competitive release and selection for a larger culmen and hallux may be primary factors in the size shift between *B. swainsoni* and *B. galapagoensis*. A pleiotropic consequence of selection for feeding on larger prey may be the evolution of larger body size in general.

TAXONOMIC CONSEQUENCES AND CONCLUSIONS

The rapidly evolving mitochondrial control region reveals a paraphyletic *B. swainsoni* with respect to *B. galapagoensis*, inclusive of a monophyletic *B. galapagoensis* lineage. This relationship is evolutionarily informative of a contemporaneous speciation event (Funk & Omland, 2003) and could provide evidence that *B. galapagoensis* recently colonized the Galápagos archipelago from a *B. swainsoni* ancestor. Basing our phylogenetic hypothesis on the mitochondrial data, the monophyletic *B. galapagoensis* clade forms a good genealogical species, but we acknowledge that the validity of basing species-level recognition on a single, nonrecombining locus, is a strongly debated topic. However, when considering the entire *B. galapagoensis/B. swainsoni* clade from a strict perspective of lineage monophyly, and following the Principle of Priority (ICZN 2000) in which the oldest name for a taxon is valid, *B. swainsoni* (1838, Bonaparte) and *B. galapagoensis* (1837, Gould) should be considered a single *B. galapagoensis* species. As discussed above, this view is not supported by behavioural or morphological characters, which clearly demonstrate significant differences in overall body size, as well as in metrics associated with prey capture, indicating ecological divergence between these two taxa. Size and feeding morphology have been found to evolve rapidly in other bird taxa (Grant, Grant & Petren, 2000; Bunce *et al.*, 2005; Zink *et al.*, 2005), and previous research suggests that there is no general association between rates of molecular and morphological evolution (Bromham *et al.*, 2002). In addition to our morphological comparison, clear differences exist between these two taxa in plumage, mating behaviours, and, importantly, their non-overlapping distributions. These differences considered together support the current recognition of both the Galápagos hawk and the Swainson's hawk as distinct species. As de Queiroz (2005) notes in his argument for a unified species concept, recognition of distinct population lineages is a common element to all species concepts. Secondary properties of lineage divergence, such as phenotypic change and reciprocal monophyly, are

each consequences of lineage divergence, each arising at different points during the process of divergence. Within the *B. galapagoensis/B. swainsoni* clade, phenotypic differences are clearly established whereas reciprocal monophyly is not yet evident, at least with respect to the mitochondrial control region. Differences in signal among these secondary properties should not overshadow recognition of *B. swainsoni* and *B. galapagoensis* as distinct lineages and therefore species.

ACKNOWLEDGEMENTS

J. Hull was supported by the UC Davis Graduate Group in Ecology, the UC Davis Veterinary Genetics Laboratory, and the UC Davis Genetic Resources Conservation Program. W. Savage was funded by an NSF Graduate Research Fellowship and the UC Davis Graduate Group in Ecology. J. Bollmer, N. Whiteman, and P. Parker's research was facilitated by support from the National Science Foundation (NSF; INT-030759 to P.G.P. and N.K.W.), the Field Research for Conservation Program (FRC) of the Saint Louis Zoo, Harris World Ecology Center (UM-St Louis), Sigma Xi, and the E. Desmond Lee Collaborative in Zoological Studies. Part of this work was carried out by using the resources of the Computational Biology Service Unit from Cornell University, which is partially funded by Microsoft Corporation. For assistance in collecting Swainson's and red-tailed hawk samples in North America, we thank the Arizona Department of Game and Fish, Calgary Wildlife Rehabilitation Society, California Department of Fish and Game, Cascades Raptor Center, Golden Gate Raptor Observatory, Hawkwatch International, Laramie Raptor Refuge, Last Chance Forever, Lindsay Wildlife Hospital, Montana Raptor Center, Rocky Mountain Raptor Project, South Plains Refuge, UC Davis Raptor Center, R. Anderson, M. Bradbury, J. Estep, R. Schlorf, the Swainson's Hawk Technical Advisory Committee, C. Boal (Texas Technical University), P. Bloom, C. Briggs (University of Nevada Reno), W. Clark, A. Hull (Golden Gate Raptor Observatory), J. Papp, and W. Mattox (Conservation Research Foundation). We thank J. H. Sarasola and J. J. Negro (Estación Biológica de Doñana, Seville, Spain), M. Beilstein (UM-St Louis) and D. Eakman (Bothel, WA), for facilitating Swainson's hawk sampling in Argentina and for collection and export permits, Dr Sandra Aliscioni (Instituto de Botánica Darwinion), the Gobierno de Provincia de Córdoba Secretaria de Agricultura, Ganadería y Recursos Naturales Dirección de Ambiente and the Ministerio de Desarrollo Social Secretaria de Ambiente y Desarrollo Sustentable, Dirección Nacional de Recursos Naturales y Conservación de la Biodiversidad, Buenos Aires, Argentina.

For Galápagos hawk samples, we thank the Charles Darwin Foundation for logistical support and the Galápagos National Park for support and collection and export permits. We also appreciate field assistance from T. De Vries, P. Sanchez, N. Gottdenker, C. McWilliams, B. Nims, V. Dosanjh, R. Campbell, J. Rabenold, and T. Walsh. Editor-in-Chief J. Allen and two anonymous reviewers provided valuable comments on previous versions of this manuscript

REFERENCES

- Bandelt HJ, Forster P, Röhl A. 1999.** Median-joining networks for inferring intraspecific phylogenies. *Molecular Biology and Evolution* **16**: 37–48.
- Barton NH. 1998.** Natural selection and random genetic drift as causes of evolution on islands. In: Brush H, Clark GA Jr, eds. *Perspectives in ornithology*. Cambridge: Cambridge University Press.
- Bollmer JL, Kimball RT, Whiteman NK, Sarasola JH, Parker PG. 2006.** Phylogeography of the Galápagos hawk (*Buteo galapagoensis*): a recent arrival to the Galápagos Islands. *Molecular Phylogenetics and Evolution* **39**: 237–247.
- Bollmer JL, Sanchez T, Cannon MD, Sanchez D, Cannon B, Bednarz JC, de Vries T, Struve MS, Parker PG. 2003.** Variation in morphology and mating system among island populations of Galápagos hawks. *The Condor* **105**: 428–438.
- Bollmer JL, Whiteman NK, Cannon MD, Bednarz JC, de Vries T, Parker PG. 2005.** Population genetics of the Galápagos hawk (*Buteo galapagoensis*): genetic monomorphism within isolated populations. *The Auk* **122**: 1210–1224.
- Bromham L, Woolfit M, Lee MSY, Rambaut A. 2002.** Testing the relationship between morphological and molecular rates of change along phylogenies. *Evolution* **56**: 1921–1930.
- Bromham LD, Hendy MD. 2000.** Can fast early rates reconcile molecular dates with the Cambrian explosion? *Proceedings of the Royal Society of London Series B, Biological Sciences* **267**: 1041–1047.
- Bunce M, Szulkin M, Lerner HRL, Barnes I, Shapiro B, Cooper A, Holdaway RN. 2005.** Ancient DNA provides new insights into the evolutionary history of New Zealand's extinct giant eagle. *PLoS Biology* **3**: 44–46.
- Clark WS, Witt CC. 2006.** First known specimen of a hybrid *Buteo*: Swainson's Hawk (*Buteo swainsoni*) x Rough-legged Hawk (*B. lagopus*) from Louisiana. *Wilson Journal of Ornithology* **118**: 42–52.
- Clegg SM, Degnan SM, Moritz C, Estoup A, Kikkawa J, Owens IPF. 2002.** Microevolution in island forms: the roles of drift and directional selection in morphological divergence of a passerine bird. *Evolution* **56**: 2090–2099.
- Clegg SM, Owens IPF. 2002.** The 'island rule' in birds: medium body size and its ecological explanation. *Proceedings of the Royal Society of London Series B, Biological Sciences* **269**: 1359–1365.
- Clement M, Posada D, Crandall KA. 2000.** TCS: a computer program to estimate gene genealogies. *Molecular Ecology* **9**: 1657–1660.
- England AS, Bechard MJ, Houston CS. 1997.** Swainson's Hawk (*Buteo swainsoni*). In: Poole A, Gill F, eds. *The birds of North America*, No. 265. Philadelphia, PA: The Academy of Natural Sciences; Washington, DC: The American Ornithologists' Union.
- Ferguson-Lees J, Christie DA. 2001.** *Raptors of the world*. Boston, MA: Houghton Mifflin Company.
- Foster JB. 1964.** The evolution of mammals on islands. *Nature* **202**: 234–235.
- Frey JK. 1993.** Modes of peripheral isolate formation and speciation. *Systematic Biology* **42**: 373–381.
- Fu Y. 1997.** Statistical tests of neutrality of mutations against population growth, hitchhiking, and background selection. *Genetics* **147**: 915–925.
- Fu Y, Li WH. 1993.** Statistical tests of neutrality of mutations. *Genetics* **133**: 693–709.
- Funk DJ, Omland KE. 2003.** Species-level paraphyly and polyphyly: frequency, causes, and consequences, with insights from animal mitochondrial DNA. *Annual Review of Ecology, Evolution, and Systematics* **34**: 397–423.
- Golden Gate Raptor Observatory. 1998.** *Golden gate raptor observatory Bander's manual*, 3rd edn. San Francisco, CA: Golden Gate Raptor Observatory.
- Gelman A, Rubin D. 1992.** Inference from Iterative Simulation Using Multiple Sequences. *Statistical Science* **7**: 457–511.
- Grant PR. 1998.** Patterns on islands and microevolution. In: Grant PR, ed. *Evolution on islands*. Oxford: Oxford University Press.
- Grant PR, Grant BR, Petren K. 2000.** The allopatric phase of speciation: the sharp-beaked ground finch (*Geospiza difficilis*) on the Galápagos islands. *Biological Journal of the Linnean Society* **69**: 287–317.
- Harrison RG. 1991.** Molecular changes at speciation. *Annual Review of Ecology and Systematics* **22**: 281–308.
- Huelsenbeck JP, Ronquist F. 2001.** MRBAYES: Bayesian inference of phylogeny. *Bioinformatics* **17**: 754–755.
- Hull JM, Anderson R, Bradbury M, Estep JA, Ernest HB. 2008.** Population structure and genetic diversity in Swainson's Hawks (*Buteo swainsoni*): implications for conservation. *Conservation Genetics* **9**: 305–316.
- Hull JM, Savage WK, Smith JP, Murphy N, Cullen L, Hutchins AC, Ernest HB. 2007.** Hybridization among *Buteos*: Swainson's hawks (*Buteo swainsoni*) x red-tailed hawks (*Buteo jamaicensis*). *Wilson Journal of Ornithology* **119**: 579–584.
- International Commission on Zoological Nomenclature. 2000.** *International code of zoological nomenclature*, 4th edn. London: The International Trust for Zoological Nomenclature.
- Kerlinger P. 1989.** *Flight strategies of migrating hawks*. Chicago, IL: University of Chicago Press.
- Kimball RT, Braun EL, Zwartjes PW, Crowe TM, Ligon JD. 1999.** A molecular phylogeny of the pheasants and partridges suggests that these lineages are not monophyletic. *Molecular Phylogenetics and Evolution* **11**: 38–54.

- Kimura M. 1983.** *The neutral theory of molecular evolution*. Cambridge: Cambridge University Press.
- Kumar S, Tamura K, Nei M. 2004.** MEGA3: integrated software for molecular evolutionary genetics analysis and sequence alignment. *Briefings in Bioinformatics* **5**: 150–163.
- Lack D. 1971.** *Ecological isolation in birds*. Oxford: Blackwell Scientific.
- Leaché AD, Reeder TW. 2002.** Molecular systematics of the eastern fence lizard (*Sceloporus undulatus*): a comparison of parsimony, likelihood, and Bayesian approaches. *Systematic Biology* **51**: 44–68.
- Lomolino MV. 1985.** Body size of mammals on islands: the island rule re-examined. *The American Naturalist*, **125**: 310–316.
- Lynch M. 1990.** The rate of morphological evolution in mammals from the standpoint of the neutral expectation. *The American Naturalist* **136**: 727–741.
- Maddison DR, Maddison WP. 2005.** *MACCLADE*, Version 4.08. Sunderland, MA: Sinauer Associates.
- Nylander JAA. 2004.** *MRMODELTEST*, version 2.2. Program distributed by the author. Uppsala: Evolutionary Biology Centre, Uppsala University.
- Omland KE. 1997.** Correlated rates of molecular and morphological evolution. *Evolution* **51**: 1381–1393.
- Omland KE, Lanyon SM, Fritz SJ. 1999.** A molecular phylogeny of the New World orioles (*Icterus*): the importance of dense taxon sampling. *Molecular Phylogenetics and Evolution* **12**: 224–239.
- Pitzer SE, Hull JM, Ernest HB, Hull AC. 2008.** Sex determination of three raptor species using morphology and molecular techniques. *Journal of Field Ornithology* **79**: 71–79.
- Posada D, Crandall KA. 1998.** MODELTEST: testing the model of DNA substitution. *Bioinformatics* **14**: 817–818.
- Preston CR, Beane RD. 1993.** Red-tailed Hawk (*Buteo jamaicensis*). In: Poole A, Gill F, eds. *The birds of North America*, No. 52. Washington, DC: The Academy of Natural Sciences; Philadelphia, PA: The American Ornithologists' Union.
- de Queiroz K. 2005.** Different species problems and their resolution. *Bioessays* **27**: 1263–1269.
- Rasner CA, Yeh P, Eggert S, Hunt KE, Woodruff DS, Price TD. 2004.** Genetic and morphological evolution following a founder event in the dark-eyed junco, *Junco hyemalis thurberi*. *Molecular Ecology* **13**: 671–681.
- Rice WR. 1989.** Analyzing tables of statistical tests. *Evolution* **43**: 223–225.
- Riesing MJ, Kruckenhauser L, Gamauf A, Haring E. 2003.** Molecular phylogeny of the genus *Buteo* (Aves: Accipitridae) based on mitochondrial marker sequence. *Molecular Phylogenetics and Evolution* **27**: 328–342.
- Ronquist F, Huelsenbeck JP. 2003.** MRBAYES version 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19**: 1572–1574.
- Rozas J, Sánchez-DelBarrio JC, Messegyer X, Rozas R. 2003.** DnaSP, DNA polymorphism analyses by the coalescent and other methods. *Bioinformatics* **19**: 2496–2497.
- Sambrook J, Fritsch EF, Maniatis T. 1989.** *Molecular cloning*. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.
- Sarasola JH, Negro JJ. 2004.** Gender determination in the Swainson's Hawk (*Buteo swainsoni*) using molecular procedures and discriminant function analysis. *Journal of Raptor Research* **38**: 357–361.
- Scott SN, Clegg SM, Blomberg SP, Kikkawa J, Owens IPF. 2003.** Morphological shifts in island-dwelling birds: the roles of generalist foraging and niche expansion. *Evolution* **57**: 2147–2156.
- Shimodaira H, Hasegawa M. 1999.** Multiple comparisons of log-likelihood with applications to phylogenetic inference. *Molecular Biology and Evolution* **16**: 1114–1116.
- Snyder NFR, Wiley JW. 1976.** Sexual size dimorphism in hawks and owls of North America. *Ornithological monographs*, No. 20. [Battle Creek, Mich.]: Washington, DC: American Ornithologists' Union.
- Sorenson MD, Quinn TW. 1998.** Numts: A challenge for avian systematic and population biology. *Auk* **115**: 214–221.
- Swofford DL. 2003.** *PAUP*: phylogenetic analysis using parsimony (*and other methods)*, Version 4. Sunderland, MA: Sinauer Associates.
- Van Valen L. 1973.** Body size and numbers of plants and animals. *Evolution* **27**: 27–35.
- de Vries T. 1973.** The Galápagos Hawk, an ecogeographical study with special reference to its systematic position. DPhil Thesis, Vrije University.
- de Vries T. 1975.** The breeding biology of the Galapagos Hawk, *Buteo galapagoensis*. *Le Gerfaut* **65**: 29–57.
- de Vries T. 1976.** Prey selection and hunting methods of the Galapagos Hawk, *Buteo galapagoensis*. *Le Gerfaut* **66**: 3–43.
- Whiteman NK, Kimball RT, Parker PG. 2007.** Co-phylogeography and comparative population genetics of the Galápagos Hawk and three ectoparasite species: ecology shapes population histories within a parasite community. *Molecular Ecology* **16**: 4759–4773.
- Whiteman NK, Matson KD, Bollmer JL, Parker PG. 2006.** Disease ecology in the Galápagos Hawk (*Buteo galapagoensis*): host genetic diversity, parasites, and natural antibodies. *Proceedings of the Royal Society of London Series B, Biological Sciences* **273**: 797–804.
- Whittaker RJ. 1998.** *Island biogeography: ecology, evolution and conservation*. Oxford: Oxford University Press.
- Wright S. 1931.** Evolution in Mendelian populations. *Genetics* **16**: 97–159.
- Zink RM, Rising JD, Mockford S, Horn AG, Wright JM, Leonard M, Westberg MC. 2005.** Mitochondrial DNA variation, species limits, and rapid evolution of plumage coloration and size in the savannah sparrow. *The Condor* **107**: 21–28.
- Zwickl DJ. 2006.** Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion. DPhil Thesis, University of Texas at Austin.