

HYBRIDIZATION AMONG BUTEOS: SWAINSON'S HAWKS
(*BUTEO SWAINSONI*) × RED-TAILED HAWKS
(*BUTEO JAMAICENSIS*)

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ABSTRACT.—We report three cases of hybridization between wild Swainson's Hawks (*Buteo swainsoni*) and Red-tailed Hawks (*B. jamaicensis*) identified by using nuclear and mitochondrial DNA markers. The hybrid individuals were field-identified as Swainson's Hawks and sampled from Alberta, Canada ($n = 2$) and Utah, USA ($n = 1$). Nineteen nuclear microsatellite loci were used in a factorial correspondence analysis to create genotypic clusters of 468 Red-tailed and 357 Swainson's hawks. Three suspected hybrids were identified by an intermediate genetic position between the genotypic clusters of the two species, indicating a hybridogenetic composition. We examined mitochondrial control region sequence data to identify the maternal background of the putative hybrids; two of the hybrid specimens had Red-tailed Hawk mtDNA haplotypes and the third a Swainson's Hawk haplotype. These results suggest that hybridization between these two species may occur in their shared breeding range and can result in reciprocal hybrid offspring, barring any social, behavioral, or biological isolating mechanisms. Received 30 October 2006. Accepted 23 February 2007.

Hybridization is relatively common among birds with documented cases in ~9% of avian species (Grant and Grant 1992). The frequency of hybridization varies greatly among Orders. Hybrids are often artificially generated among Falconiformes for falconry (Bunnell 1986, Weaver and Cade 1991, Fox and Sherrod 1999), particularly within the genus *Falco* where Peregrine Falcon (*F. peregrinus*) × Gyrfalcon (*F. rusticolis*) hybrids are especially popular. Hybridization among wild raptors is less common, especially within the genus *Buteo* (Clark and Witt 2006).

Clark and Witt (2006) reported the first conclusive example of hybridization between native North American raptors of the genus *Buteo*: a Swainson's Hawk (*B. swainsoni*) × Rough-legged Hawk (*B. lagopus*) hybrid collected in Louisiana. We estimate sequence di-

vergence between these two species based on the mitochondrial control region to range from 4.4 to 6.3% (mean 4.8%). Hybridization between these two species spans several well-supported species clades within the genus *Buteo* (Riesing et al. 2003). Hybridization between raptors has been infrequently reported and difficulty in identifying hybrid offspring in the field may result in underestimation of the extent of hybridization in the wild. We present molecular genetic evidence of three cases of hybridization between free-ranging Swainson's and Red-tailed hawks (*B. jamaicensis*) based on nuclear microsatellite genotypes and mitochondrial control region sequences.

METHODS

Sample Collection.—Samples of Swainson's ($n = 357$) and Red-tailed hawks ($n = 468$) were obtained from California to North Dakota, and Alberta to Texas between 2003 and 2005. White-tailed Hawks (*B. albicaudatus*, $n = 47$) were also sampled for outgroup comparison. Approximately 0.2 ml of blood was drawn via medial metatarsal venipuncture and two feathers were plucked from the breast of each bird. Blood samples were stored in 1.2 ml of Longmire's lysis buffer (100 mM Tris pH 8.0, 100 mM EDTA, 10 mM NaCl, 0.5% SDS) at ambient temperature until delivered to laboratory facilities where they were preserved at -80°C . Feather samples were

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stored in paper envelopes and kept cool and dry. Samples were collected from adults, juveniles, and pre-fledge young in nests by licensed raptor biologists, as well as from juveniles and adults treated at several wildlife rehabilitation facilities. All wild birds were leg-banded with U.S. Geological Survey bands and either released or returned to nests. Only one individual per presumed family group (nestlings and parents) was included in our sampling. Genomic DNA was isolated from 25 μ l of the blood/buffer solution and from feathers using QIAGEN DNeasy kits (QIAGEN Inc., Valencia, CA, USA). DNA was stored at 4° C while in use and transferred to -20° C upon completion of genetic work.

Data Collection.—Each individual was genotyped at 19 microsatellite loci (BswA110w, BswD122w, BswA204w, BswA317w, BswD210w, BswD220w, BswA303w, BswB111aw, BswD234w, BswD310w, BswD313w, BswB220w, BswB221w, BswD327w, BswA302w, BswA312w, BswD107w, BswD127w, and BswD324w; Hull et al. 2007) in six multiplex PCRs following the conditions of Hull et al. (2007). PCR products were electrophoresed with a 3730 DNA Analyzer (Applied Biosystems Inc., Palo Alto, CA, USA). PCR products were visualized and scored with STRand (Version 2.3.69; Toonen and Hughes 2001).

Four-hundred sixteen base pairs of domain I of the mitochondrial control region were amplified via PCR in three putative hybrid samples (following amplification conditions in Kimball et al. 1999), a subset of Red-tailed ($n = 5$) and Swainson's hawks ($n = 279$), and one White-tailed Hawk using primer sets 16065F (Kimball et al. 1999) and H15414 (Bollmer et al. 2005). A recent phylogenetic study of the genus *Buteo* indicated strong nodal support for the monophyly of these three species based on two mitochondrial regions (ND6 and pseudo-control region) (Riesing et al. 2003). PCR products were cleaned of unincorporated primers and dNTPs using Ultra-clean purification kits (MoBio, Carlsbad, CA, USA) and sequenced at the University of California Davis DNA Sequencing Facility using primers 14965F (J. M. Hull, unpubl. data) and H15414. Sequences were examined and aligned using SEQUENCHER (Version 4.5;

Gene Codes Corporation, Ann Arbor, MI, USA).

Microsatellite Data Analysis.—Genotypic clusters were created using a factorial correspondence analysis (FCA) in the program GENETIX 4.05.2 (Belkhir et al. 2000). We used FCA to describe genotypic groups based on microsatellite allele frequencies, using the ordination of samples along varying factorial axes to visualize genetic similarity of the species clusters in two-dimensional space. Familial relationships among hybrid individuals from similar locations were assessed using a maximum likelihood approach as implemented in ML-RELATE (Kalinowski et al. 2006).

Control Region Sequence Analysis.—We used PAUP* (Version 4.0b10; Swofford 2003) for phylogenetic analyses using maximum parsimony (MP), maximum likelihood (ML), and distance analyses with *B. albicaudatus* designated as the outgroup. We used MRMODELTEST (Version 2.1; Nylander 2004) to choose the model of DNA substitution and parameter estimation for heuristic ML and distance analyses that best fit our control region sequence data. The product for hierarchical likelihood ratio (HKY + G) and Akaike information criterion (HKY + I) tests differed slightly. Both models were used in parameter settings, but the results did not differ in tree topologies or support. Nodal support was estimated for MP and ML trees using bootstrap searches (500 pseudoreplicates) and Bayesian posterior probabilities (10 million generations, 1 million burn-in, MRBAYES Version 3.1.2; Huelsenbeck and Ronquist 2001, Ronquist and Huelsenbeck 2003).

RESULTS

We obtained multilocus genotypes for 468 Red-tailed, 357 Swainson's, and 47 White-tailed hawks. Factorial correspondence analysis displayed distinct genotypic clusters for Red-tailed Hawks, Swainson's Hawks, and White-tailed Hawks with three individuals occurring intermediate to Red-tailed and Swainson's hawks (Fig. 1) indicating hybridogenetic genotypes. Two of these hybrid individuals were from the same geographic region. Maximum likelihood estimation of familial relationship indicated these two individuals have a probability 11.7 times greater for being unrelated than for being siblings.

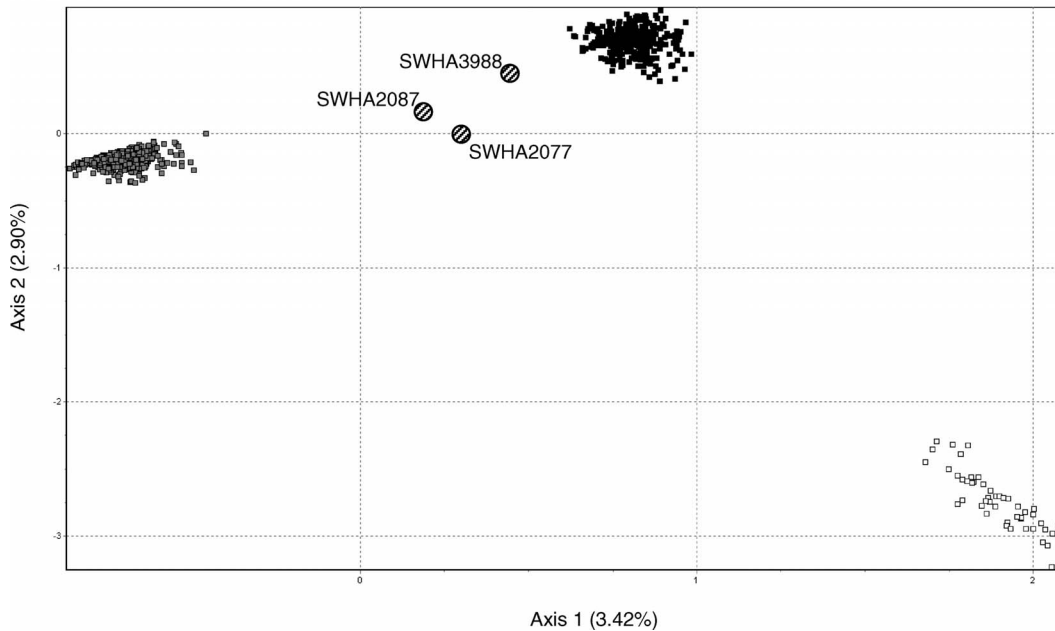


FIG. 1. Two-dimensional plot of the factorial correspondence analysis of multilocus genotypes for 357 Swainson's Hawks (black squares), 468 Red-tailed Hawks (gray squares), 47 White-tailed Hawks (white squares), and three Red-tailed Hawk \times Swainson's Hawk hybrids (shaded circles).

Five Red-tailed, 279 Swainson's (where sufficient sample allowed microsatellite and mitochondrial sequence analysis), and one White-tailed hawk were sequenced at a portion of the control region. All sequences were archived in GenBank (accession numbers pending). We found no evidence of nuclear copies of the control region as replicate DNA sequences extracted from feathers and blood yielded identical sequences. There were no indels, double peaks or heteroplasmy observed in the electropherograms, and sequences aligned with previously published raptor control region sequences. We identified 33 haplotypes for Swainson's Hawks, one single haplotype for the five Red-tailed Hawk sequences, and one haplotype for the White-tailed Hawk among the 285 sequences. Fifty-seven of the 416 bp used in the analyses were variable and 39 were phylogenetically informative. Uncorrected sequence divergences between White-tailed Hawks and in-group taxa ranged from 7.21 to 8.17% (mean = 7.8%), and between Swainson's and Red-tailed hawks from 5.53 to 7.21% (mean = 6.49%). Uncorrected sequence divergences among

Swainson's Hawk haplotypes ranged from 0.24 to 2.64% (mean = 1.14%).

Maximum parsimony, maximum likelihood, and distance analyses produced concordant tree topologies with insignificant differences at tip relationships within Swainson's Hawks (Fig. 2). Bootstrap analyses of phylogenetic trees suggested strong support for reciprocal monophyly of the Swainson's, Red-tailed, and White-tailed hawk clades (Fig. 2). Within each clade, however, there was little or no support for intraspecific geographic/genetic structure. Two individuals with intermediate microsatellite FCA assignments nested with Red-tailed Hawk haplotypes in a well-supported clade, and a third hybridogenetic individual nested within a monophyletic Swainson's Hawk clade (Fig. 2).

DISCUSSION

Microsatellite and mitochondrial data indicate that hybridization occurs between wild populations of Red-tailed and Swainson's hawks, and spans well-supported phylogenetic distances in the genus *Buteo*. Two of these samples were from juveniles at the Calgary

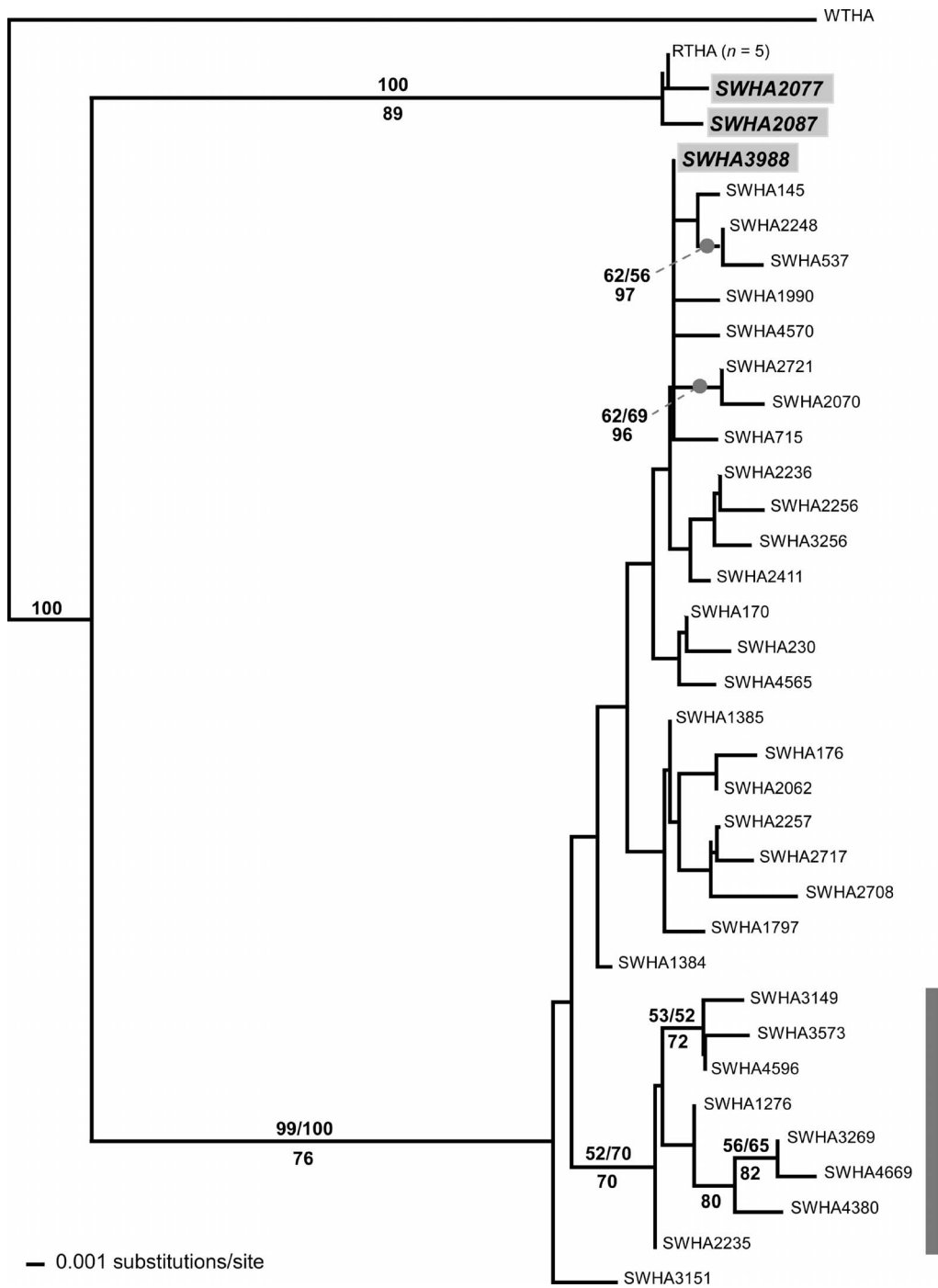


FIG. 2. Maximum-likelihood phylogram for 33 Swainson's Hawk (SWHA) mtDNA haplotypes, one Red-tailed Hawk (RTHA) haplotype, one White-tailed Hawk (WTHA) haplotype, and three haplotypes from hybrid individuals (bolded text and shaded). Two of the hybrids (SWHA2077 and SWHA2087) are nested in a well-supported clade with Red-tailed Hawks, and the third hybrid (SWHA3988) is deeply nested within Swainson's Hawks. Numbers above branches are parsimony/likelihood bootstrap values (500 pseudoreplicates); numbers below branches are Bayesian posterior probabilities. Marginal likelihood bootstrap values support some substructure within SWHA (shaded vertical bar).

Wildlife Rehabilitation Society in Alberta, while the third was from a nestling in northwestern Utah. The two samples from Alberta appear unrelated indicating this data set reflects three separate hybridization events. The mitochondrial control region sequences identify the mother in two of these cases as a Red-tailed Hawk (Alberta) and, in the third case, the mother was a Swainson's Hawk (Utah), demonstrating that hybridization is possible for both species-gender combinations. We report a small sample size of hybrid individuals, but the results suggest that intrinsic pre- and post-zygotic reproductive isolating barriers may not be uniformly present between Swainson's and Red-tailed hawks. These findings also imply courtship and mating behavior differences may not prevent interspecific breeding in all cases, nor does morphology. The average sizes of males and females of both species are comparable (Preston and Beane 1993, England et al. 1997), and resulting pairings would retain the natural intraspecific reverse sexual size dimorphism.

Nest-site observations in Utah confirm our genetic evidence of hybridization. Early in the nesting season, HawkWatch International crews observed a Swainson's Hawk and a Red-tailed Hawk switching incubation duties at the nest. The chick was banded several weeks later, during which time the Swainson's Hawk was flushed from the nest and defended the territory along with a Red-tailed Hawk, presumably its mate. Upon fledging, the hybrid offspring appeared phenotypically similar to a Swainson's Hawk with no obvious Red-tailed Hawk characteristics. In contrast, both hybrids from Alberta were phenotypically similar to Red-tailed Hawks.

Clark and Witt (2006), following Short (1969), speculated that limited overlap in the distribution of breeding Rough-legged and Swainson's hawks may facilitate hybridization through the absence of behavioral barriers to reproduction that exist among species with broad range overlap. The breeding range of Swainson's Hawks is overlapped entirely by that of Red-tailed Hawks (Preston and Beane 1993, England et al. 1997). Two of the hybrid samples were collected from the northern periphery of Swainson's Hawk range and the third was in the geographic center of the ranges for both species. Ecological or behavioral

circumstances that promoted Swainson's Hawk \times Red-tailed Hawk pairings remain unknown.

It is not known if hybrid offspring of Red-tailed and Swainson's Hawks are fertile or if other barriers exist to introgression. We do not know the lineage/genetic composition of the hybrids nor their parents, which may be of pure or mixed ancestry. It is unlikely that widespread hybridization is ongoing given the low frequency found in this study; among the sample of 825 individuals (468 Red-tailed Hawks, 357 Swainson's Hawks) only 0.004% were hybrids. The low number of hybrids may indicate mating preferences for conspecifics, or it may be that hybrids are less fit than the parental species. Given the markedly different migratory behaviors of Red-tailed Hawks (partial, moderate-distance migrant) and Swainson's Hawks (complete, long-distance migrant), hybrids with intermediate migratory behaviors may not reach optimal wintering and breeding areas and may be at a selective disadvantage. This would be particularly true from a Swainson's Hawk perspective as the vast majority of the species winters in central Argentina.

The extent of hybridization between Swainson's and Red-tailed hawks at the population level, and its causes, is unknown. The particular natural or anthropogenic conditions that promote hybridization also remain unidentified. If hybridization and introgression continue to occur at the low levels observed in this study, genetic diversity and distinctiveness may not be severely impacted. However, if environmental conditions promoting extensive Swainson's Hawk \times Red-tailed Hawk hybridization become common, genetic introgression may become an important conservation concern. Currently, the presence of distinct genotypic clusters in the FCA suggests that hybridization and introgression are not resulting in a deterioration of genetic distinctiveness between Swainson's Hawks and Red-tailed Hawks.

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