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The origins of the recently discovered Hispaniolan Olive-throated Parakeet: A phylogeographic perspective on a conservation conundrum

Steven C. Latta[1], Andrea K. Townsend[2,3] and Irby J. Lovette[2]

[1]*National Aviary, Allegheny Commons West, Pittsburgh, PA 15212.*
Tel. 412-323-7235 ext. 288; Fax 412-321-4364; e-mail: steven.latta@aviary.org
[2]*Fuller Evolutionary Biology Program, Cornell Laboratory of Ornithology,*
Cornell University, 159 Sapsucker Woods Road, Ithaca, NY 14850
[3]*Current address: Department of Wildlife, Fish, and Conservation Biology, University of California,*
One Shields Ave, Davis, CA 95616, USA

Abstract.—The Olive-throated Parakeet (*Aratinga nana*) occurs on the eastern slope of Middle America and the island of Jamaica. A resident population has been recently discovered in remote areas of Hispaniola, where it might represent an overlooked relict population or a recent introduction. If the Hispaniolan population of *A. nana* is native, then it would merit conservation attention. Conversely, if the Hispaniolan population is a recent non-natural introduction, the potential for competitive interactions with the threatened endemic Hispaniolan Parakeet, *Aratinga chloroptera*, should be assessed. To explore the origins of the Hispaniolan *A. nana* population, we sequenced the mitochondrially encoded ND2 gene from individuals from five mainland populations of *A. nana vicinalis* and *A. nana astec*, as well as the Jamaican *A. nana nana* and the Hispaniolan *A. nana* (subspecies unknown). Mitochondrial variation was highly structured into well differentiated island and mainland clades separated by close to 2% nucleotide divergence, but within each group there was very low ND2 haplotype variation. These results suggest that the Jamaican and Hispaniolan populations are evolutionarily distinct from the mainland populations, and they add support to the hypothesis that the Hispaniolan *A. nana* population results from a recent, human-mediated introduction.

Keywords.—*Aratinga astec*, *Aratinga nana*, Hispaniola, Jamaica, phylogeography

Introduction

The genus *Aratinga* currently comprises 20 species of moderately-large parakeets divided into three groups based primarily on size and plumage traits (Collar 1997). Recent phylogenetic studies have addressed some relationships within these species groups (Ribas and Miyaki 2004), but there has been no work on the *Aratinga pertinax* complex, which also includes *A. aurea*, *A. canicularis*, *A. cactorum*, and *A. nana* (Silveira et al. 2005). Three distinct populations of the Olive-throated Parakeet (*A. nana*) have been recognized (AOU 1998). On the mainland, *A. nana vicinialis* is found in northeastern Mexico and *A. nana astec* is found from southeastern Mexico south to western Panama. The nominate subspecies, *A. nana nana*, is common on the island of Jamaica. Variation among these *Aratinga* populations is sufficient that some authors have recognized the mainland and island forms as distinct species (Howell and Webb 1995; Bond 1940). Another population (subspecies unknown) has been recently reported in remote areas of Hispaniola (Smith 1996; Latta et al. 1997; Keith et al. 2003). Published accounts speculate whether this Hispaniolan population was founded by a recent natural or human-assisted introduction, or whether it is a previously unrecorded relict population (Smith 1996; Latta et al. 1997).

Elucidation of the genetic structure among the populations of *A. nana* is critical to determine the appropriate conservation and management strategies for the island populations. The distinction of the Jamaican *A. nana* population as a species separate from the mainland populations would promote increased monitoring and conservation efforts on Jamaica (S. Koenig, pers.

comm.). Likewise, if it is an evolutionarily distinct unit, the Hispaniolan population would also merit heightened conservation status. Conversely, if the Hispaniolan population is a recent non-natural introduction, the potential for competitive interactions with the threatened endemic Hispaniolan Parakeet, *Aratinga chloroptera*, should be assessed.

There is conflicting evidence on whether the Hispaniolan *A. nana* population is of recent, anthropogenic origin. Its rapid demographic increase is suggestive of an expanding recent introduction (Latta et al. 1997). However, the two locations where the species is resident in the Sierra de Bahoruco are fairly remote, have not until recent years been thoroughly inventoried by ornithologists, and are a considerable distance from cities which are the most likely source of escapees or human-aided introductions. The species is believed by local residents to have been present before 1970, and it is recognized as distinctive with its own local name. In addition, several other West Indian birds have affinities between only Hispaniola and Jamaica (Smith 1996; Keith et al. 2003), suggesting that natural colonization might have occurred.

Here we use molecular techniques to diagnose the origin of the Hispaniolan Olive-throated Parakeet, and to explore the relationships between the geographically distinct populations of *A. nana* across its range. Our questions include: 1) is the population on Hispaniola derived from the Jamaican population or from a continental population, or is it genetically distinct? 2) Are the Jamaican and Hispaniolan populations evolutionarily significant units and distinct from the mainland populations? And, 3) what does the pattern of mitochondrial variation suggest about species limits within the *Aratinga nana* complex?

Materials and Methods

Taxon sampling and laboratory methods. Samples were obtained from throughout the range of *A. nana* (Fig. 1, Table 1), including

Fig. 1. Distribution and sampling sites of *A. nana vicinalis* (gray), *A. nana astec* (gray), Jamaican *A. nana nana* (white); and Hispaniolan *A. nana nana* (*incertae sedis*) (white). Circles indicate sampling localities. Numbers within circles correspond to the locations in the haplotype networks (Figure 2); n = sample size at each location.

TABLE 1. Taxa sequenced for this study, tissue types, collecting localities, institutional sources, and GenBank accession numbers.

Taxon	Museum source[a] and sample number	Type[b]	Locality[c]	Site code (Fig. 1)	GenBank accession #
A. nana vicinalis	CU-3197	S	Tamesi River; Tamaulipas, MX	1	FJ361230
A. nana vicinalis	CU-29131	S	2 miles W San Andres Tuxtla; Veracruz, MX	2	FJ361231
A. nana astec	CU-30527	S	15 km S Rio Lagartos; Yucatan, MX	3	FJ361233
A. nana astec	CU-3196	S	Chichen Itza; Yucatan, MX	3	FJ361232
A. nana astec	UWBM-69978	T	Puerto Cabezas; Nicaragua	4	FJ361227
A. nana astec	USNM-B465	T	Isla San Cristobal, Bocatorito; Panama	5	FJ361228
A. nana astec	USNM-B466	T	Isla San Cristobal, Bocatorito; Panama	5	FJ361229
A. nana nana	HZ-0021	F	Jamaica; wild caught, captive birds in Hope Zoo, Kingston	6	FJ361219
A. nana nana	HZ-0022	F	Jamaica; wild caught, captive birds in Hope Zoo, Kingston	6	FJ361220
A. nana nana	HZ-0023	F	Jamaica; wild caught, captive birds in Hope Zoo, Kingston	6	FJ361221
A. nana nana	HZ-0024	F	Jamaica; wild caught, captive birds in Hope Zoo, Kingston	6	FJ361222
A. nana	SCL-001	F	Wild caught, adult bird from Aguacate, Dominican Republic	7	FJ361218
A. nana	SCL-002	F	Wild caught, adult bird from Aguacate, Dominican Republic	7	FJ361223
A. nana	SCL-003	F	Wild caught, adult bird from Aguacate, Dominican Republic	7	FJ361224
A. nana	SCL-004	F	Wild caught, adult bird from Las Mercedes, Pedernales, Dominican Republic	7	FJ361225
A. nana	SCL-005	F	Collected at nest, Aceitillar area, Dominican Republic	7	FJ361226

[a]Institutional sources of samples: CU: Cornell University Museum of Vertebrates, Ithaca, New York, USA; HZ: Hope Zoo, Kingston, Jamaica; SCL: samples collected in the field or from privately held birds by S. C. Latta in the Dominican Republic; USNM: National Museum of Natural History, Washington, D.C., USA; UWBM: University of Washington Burke Museum, Seattle, Washington, USA.

[b]Sample types: F = Feather collected from live bird; T = buffer-preserved tissue; S = toe-pad shaving from museum skin.

five mainland populations of *A. nana vicinalis* and *A. nana astec*, as well as the Jamaican *A. nana nana* and the Hispaniolan *A. nana* (subspecies unknown). Samples derived from toe-pad tissues were taken from museum skin specimens by shaving a narrow band of skin from a single toe (usually the hallux) using a sterile scalpel. These samples were placed dry into sterile 1.5 mL tubes, sealed, and transferred to our degraded-DNA laboratory. Samples from island populations were derived from feather tips (Table 1). Samples from Jamaica were collected from birds at the Hope Zoo in Kingston, whereas samples from Hispaniola were taken from nests or wild-caught birds held in captivity in private homes.

DNA was extracted from tissue samples and feather tips using DNAeasy tissue kits (Qiagen) following the manufacturers' protocols. We amplified and sequenced the mitochondrially encoded ND2 gene (1041 bp) with the primers METb and TRPc (Eberhard and Bermingham 2004). Laboratory methods for the generation of these sequences are given in Lovette and Rubenstein (2007).

All toe-pad skin samples were processed in a laboratory designed for and dedicated to degraded DNA extraction and PCR set up, with stringent controls to avoid and

TABLE 2. Optimized conditions for internal ND2 primers for DNA amplification of degraded *A. nana* samples.

Primer	Sequence	Annealing temp (°C)	$MgCl_2$ concentration (mM)
Ara341R	CAGAAGTGGAAGGGGGTTAGG	54	4
Ara265R	GCTGGGTGATGTCTCACTGTCC	56	4
Ara243F	CGGACAGTGAGACATCACCCAGC	60	4
Ara558R	GTGGGAGATGGATGAGAAGGC	55	4
Ara468F	CATGTCCATCATATCCATTGCTC	53	4
Ara763R	GGAGGCCTGCTAATGATAATAGTG	53	4
Ara682F	CCAACACTRATAACCTCCTGAACC	53	4
Ara992R	AGTGAGGTGAGTGTGGGGATTAG	54	4
Ara828F	CACAACAGCCACAGCCATCTC	55	4

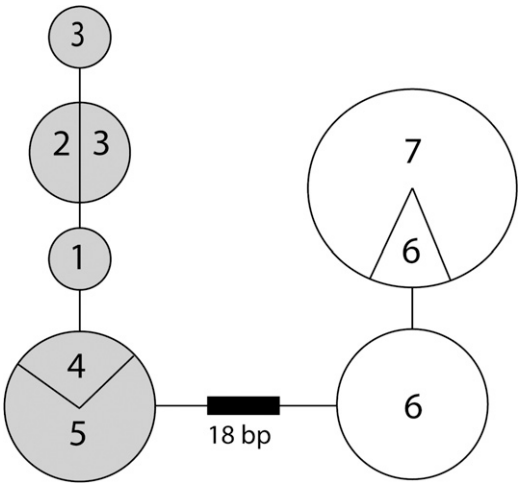

FIG. 2. Statistical parsimony haplotype networks. Each circle represents a specific haplotype. Circles increase in size with haplotype frequency. Branches represent single base-pair substitutions. The heavy black bar represents 18 substitutions. Numbers and shading correspond to the sampling localities in Figure 1.

detect amplification of contaminant DNA templates. The infrastructure and protocols for these reactions follow Lovette and Rubenstein (2007). Degraded DNA PCR amplifications targeted short (100-500 bp), overlapping regions of the ND2 gene. Primer sequences used to amplify degraded samples are given in Table 2. We found no evidence of nuclear copies in the chromatogram data or overlapping regions amplified in separate PCR reactions.

Sequences were aligned by eye in Sequencher™. We obtained ND2 gene sequences (959-1041 bp) for 16 individuals (Table 1). Sequences from all individuals have been deposited in GenBank (Table 1). We constructed statistical parsimony haplotype networks using TCS 1.21 (Clement et al. 2000) to estimate relationships among haplotypes (Fig. 2). For phylogenetic analysis, we used the neighbor-joining method, performed in MEGA 4.1 (Tamura et al. 2007), using the Tamura-Nei evolutionary model, applying complete deletion of gaps and specifying 2000 bootstrap replicates. We included *Aratinga pertinax* (voucher: National Museum of Natural History B04190; Genbank accession number EU327600; Wright et al. 2008) and *Aratinga weddelli* (voucher: Avian Molecular *Genetics and Evolution Laboratory* 2085; Genbank accession number: AY669445; Ribas et al. 2005) as an outgroup (Fig. 3).

RESULTS

Mitochondrial variation in *Aratinga nana* was highly structured into two well differentiated clades, but within each there was very low ND2 haplotype variation (Fig. 2). These clades were separated by 18 fixed nucleotide substitutions, representing 1.73-1.88% uncorrected divergence. The clusters were reciprocally monophyletic and geographically concordant: one haplotype group included all mainland samples, whereas the other group contained all island samples. There was high bootstrap support (100%) for the split (Fig. 3). ND2 sequences within each group were identical, or were separated by the low level of divergence typical of within-species comparisons among different geographic sampling locations, with

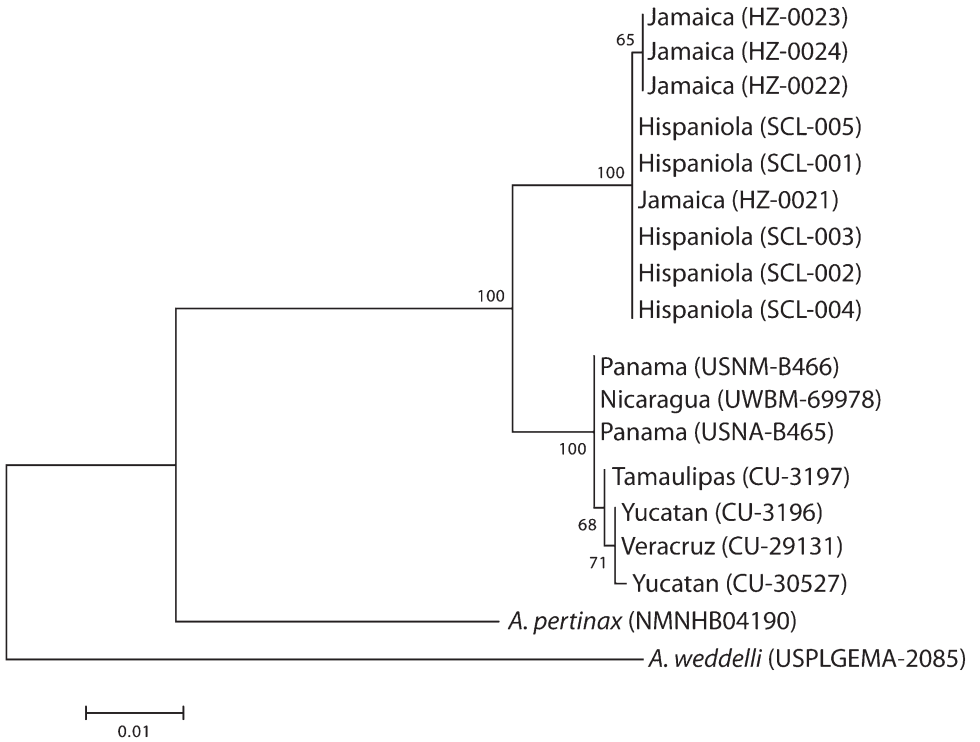

FIG. 3. Neighbor-joining tree (constructed in MEGA 4.1) showing relationships between the island and mainland populations of *A. nana*. The tree is "rooted" with two congeneric species (*A. pertinax* and *A. weddelli*). Values at nodes denote relative percent support from 2000 bootstrap iterations.

a maximum difference of four nucleotide substitutions among samples.

Discussion

Mitochondrial ND2 haplotypes in *A. nana* clustered into two reciprocally monophyletic groups, separated by 18 nucleotide substitutions (Fig. 2). One group included all birds sampled from the mainland population (Mexico, Nicaragua and Panama), whereas the other group included all birds sampled from the islands of Jamaica and Hispaniola. Our evidence for genetic divergence between these mainland and island populations is consistent with earlier observations of morphological distinctions between them. For example, Peters (1937), Bond (1940), and Howell and Webb (1995) recognized variation in plumage and mensural characters and considered these forms to be distinct species, with the Jamaican Parakeet (Bond 1940) or Olive-throated Parakeet (Howell and Webb 1995; *A. nana*, *sensu stricto*) on the islands, and the Aztec Parakeet (*A. astec*) on the mainland. The reciprocal monophyly of the two groups support their potential recognition as diagnosable phylogenetic species, and their overall magnitude of mitochondrial differentiation, although modest, is similar to that among some other *Aratinga* taxa that are recognized as full species (Ribas and Miyaki 2004).

The lack of mitochondrial differentiation between the Hispaniolan and Jamaican populations suggests that one of these populations is of recent origin. Given that the species was unreported on Hispaniola until 1996 this implies its recent colonization of Hispaniola. Whether colonization was aided by humans or was a natural event cannot be determined conclusively, but while *Aratinga* and other psittacids are thought to have dispersed over water to the Caribbean islands from Middle America, this occurred in the early Pleistocene when sea levels

were as much as 120 m lower and overwater dispersal distances much shorter than present (Keith et al. 2003). We suggest that it is unlikely that *A. nana* flew the >500 km across the Caribbean Sea from Jamaica to Hispaniola (Wiley 1993); it is more plausible that a few individual parakeets were brought to Hispaniola on ships that ran between Cabo Rojo, Dominican Republic and the south coast of Jamaica in the 1960s and 1970s, when large amounts of bauxite ore were mined in the Sierra de Bahoruco and exported to Jamaica for processing (Keith et al. 2003).

Because the rate of neutral mutation is approximately 10 times faster in mitochondrial loci than in nuclear loci (Graur and Li 2000), we suggest that mitochondrial ND2 was appropriate for this analysis of population structure in *A. nana*, given that the period of isolation between the island populations was likely to be relatively short (Zink and Barrowclough 2008). We acknowledge, however, that studies based on a single mitochondrial gene can provide only a single-locus perspective on patterns of divergence. Although ultimately it might be instructive to compare these patterns with multiple, unlinked nuclear loci (Rubinoff and Holland 2005), our use of ancient DNA would have made use of nuclear markers in the present study a challenge.

Our findings present both conservation challenges and opportunities. The broader *A. nana* complex is not considered threatened by BirdLife International (2000). Even if considered as separate species, *A. nana* and *A. astec* are unlikely to be listed as species of conservation concern, as neither group meets the thresholds of small population size or continuous decline in numbers. In Jamaica, *A. nana* is widespread and fairly common locally in lowland wooded hills (S. Koenig, pers. comm.), although it is listed in Appendix II of CITES, and its numbers are thought to have declined recently as the result of habitat loss (Wiley et al. 2004). The parakeet has achieved full legal protection in Jamaica (Wild Life Protection Act 1998), but enforcement is not comprehensive (S. Koenig, pers. comm.). Reciprocal monophyly at mitochondrial loci is one criterion for identifying evolutionarily significant units (ESUs) that merit separate conservation management (Moritz 1994). In combination with studies of phenotypic variation across populations, identification of ESUs can facilitate the conservation of adaptive phenotypic variation and its underlying genetic variation (Crandall et al. 2000). Recognition of *A. nana* as an ESU might stimulate habitat conservation measures on the island through increased attention in Environmental Impact Assessments and Jamaica's Important Bird Areas program.

On Hispaniola, the presence of *A. nana* presents a different conservation conundrum. Although currently restricted to the western Sierra de Bahoruco, *A. nana* co-occurs there with the endemic *A. chloroptera* which is considered vulnerable by BirdLife International (2000). One of the remaining major population centers of *A. chloroptera* is the Sierra de Bahoruco (Keith et al. 2003). Competition for food resources between *chloroptera* and *nana* is a potential but unstudied threat, as both species share similar habitat, and are frequently reported to join the same small foraging flocks (Keith et al. 2003; Latta et al. 2006). Competition for scarce cavity nesting sites is also possible, as natural cavities and termitaria (frequently used by *nana*) are in short supply in this region (Keith et al. 2003). Recent reports suggesting a quickly growing population of *nana* in the Bahorucos (Latta et al. 1997; Keith et al. 2003; Latta et al. 2006) is further evidence of potential detrimental impacts of *nana* on *chloroptera* populations. It is not known whether the two species are hybridizing, but this too is a possibility and should be investigated. Accelerating studies of the impact of *nana* on *chloroptera* is warranted (BirdLife International, 2000).

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