Genetic Variability in mtDNA of the Silvery Gibbon: Implications for the Conservation of a Critically Endangered Species

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Abstract: *The silvery gibbon (*Hylobates moloch*), endemic to the island of Java, relies on closed-canopy, lowland evergreen forest for its survival. Because Java has lost over 91% of its original forest, silvery gibbons currently occupy small, isolated forest fragments and are threatened with extinction. To contribute to a comprehensive conservation strategy for this species, we analyzed the mtDNA control region of 31 silvery gibbons representing most remaining populations. Our results suggest the presence of at least two genetically differentiated lineages: a "western" lineage, represented by the largest remaining natural population in Gunung Halimun National Park and a "central" lineage, consisting of smaller, more isolated populations in and around the Gunung Masigit/Simpang/Tilu complex, Gunung Gede/Pangrango, and Gunung Slamet. These two lineages, at a minimum, represent different management units that should, except in the most dire circumstances, be managed separately.*

Variabilidad Genética en el ADNm del Gibón Plateado: Implicaciones para la Conservación de una Especie Críticamente en Peligro

Resumen: *El gibón plateado (*Hylobates moloch*), endémico a la isla de Java, depende de un dosel cerrado del bosque compacto siempre-verde para su supervivencia. Debido a que Java a perdido cerca de un 91% de su bosque original, los gibones plateados actualmente ocupan fragmentos de bosque pequeños y aislados y están amenazados de extinción. Para contribuir con una estrategia de conservación comprensiva de esta especie, analizamos la región de control del ADNm de 31 gibones representantes de la mayoría de las poblaciones remanentes. Nuestros resultados sugieren la presencia de al menos dos linajes genéticamente diferenciados: el linaje "occidental" representado por la población natural remanente más grande en el parque nacional Gunung Halimun y un linaje "central" consistente de poblaciones pequeñas más aisladas en y alrededor del complejo Gunung Masigit/Simpang/Tilu, Gunung Gede/Pangrango y Gunung Slamet. Estos dos linajes por lo menos representan unidades de manejo diferentes que deberían ser manejadas por separado, excepto bajo las más desastrosas circunstancias.*

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Introduction

In Indonesia the genus *Hylobates* is represented by five species, two of which—the silvery gibbon of Java (*H. moloch*) and Kloss's gibbon of the Mentawai islands (*H. klossi*)—are endemic (Corbet & Hill 1992). All five species are currently under extreme pressure because primary rainforests in Sumatra, Borneo, and Java are disappearing rapidly. The most populous island in Indonesia, Java has lost over 91% of its forest cover (Collins et al. 1991; BAPPENAS 1993); thus, the threat of extinction to the silvery gibbon is extremely serious (Eudey 1997).

Like other gibbon species, the silvery gibbon is a highly specialized forest dweller that relies on closed-canopy lowland (0–1600 m) evergreen forests (Gittins 1982; Chivers 1984; Kappeler 1984*b*). Its narrowly defined niche makes it highly vulnerable to environmental change (MacKinnon & MacKinnon 1984). This species is also hunted extensively and kept as pets, practices that pose additional threats. Consequently, silvery gibbons occur only in small, isolated forest fragments on the western half of Java.

Of 37 forest patches previously inhabited by this species (Kappeler 1984*a*), many have been severely degraded and no longer support gibbon populations (Asquith et al. 1995). An estimated total of 386 to 1957 silvery gibbons survive today in approximately 23 forest patches (Fig. 1; Supriatna et al. 1994). Although fairly large populations can still be found in national parks (e.g., Gunung Halimun, Gunung Gede-Pangrango), many remnant forest patches support fewer than 10 individuals (Supriatna et al. 1994). This is well below the demographic and genetic threshold for a population to persist over time (Lande 1988, 1993). Hence, most populations are clearly at high risk of extirpation, and intensive conservation efforts are necessary to secure their survival.

We analyzed the mtDNA control region to assess the extent and pattern of genetic variability among remaining silvery gibbon populations. The ability to detect local differentiation is enhanced by the rapid pace of mtDNA evolution, which is generally considered to be 5–10 times higher than that of nuclear DNA in most species of mammals (W. M. Brown et al. 1982; Avise 1995; Douzery & Randi 1997). The mtDNA control region contains blocks that evolve even faster than the rest of the molecule, making this part of the genome particularly well suited to the study of conspecific populations (Horai & Hayasaka 1990; J. R. Brown et al. 1993). This study constitutes the first mtDNA sequence-based population genetic analysis of any gibbon species and provides data crucial to the development of an informed conservation strategy for the long-term sustainability of the silvery gibbon.

Methods

We included 31 silvery gibbons and 5 agile gibbons (*Hylobates agilis*) in our study. Samples from pets in villages close to gibbon habitat were included if, after extensive interviews with the owners, they were determined to be of local origin. Samples from zoos were considered of unknown origin. Blood and fecal samples collected in the field were preserved in lysis buffer (100 mM EDTA, 100 mM Tris pH 8.0, 2% SDS). We extracted total genomic DNA from these samples with a commercially available kit (QIAamp Tissue Kit from QiaGen, Valencia, California) and through standard phenol/chloroform procedures (Sambrook et al. 1989).

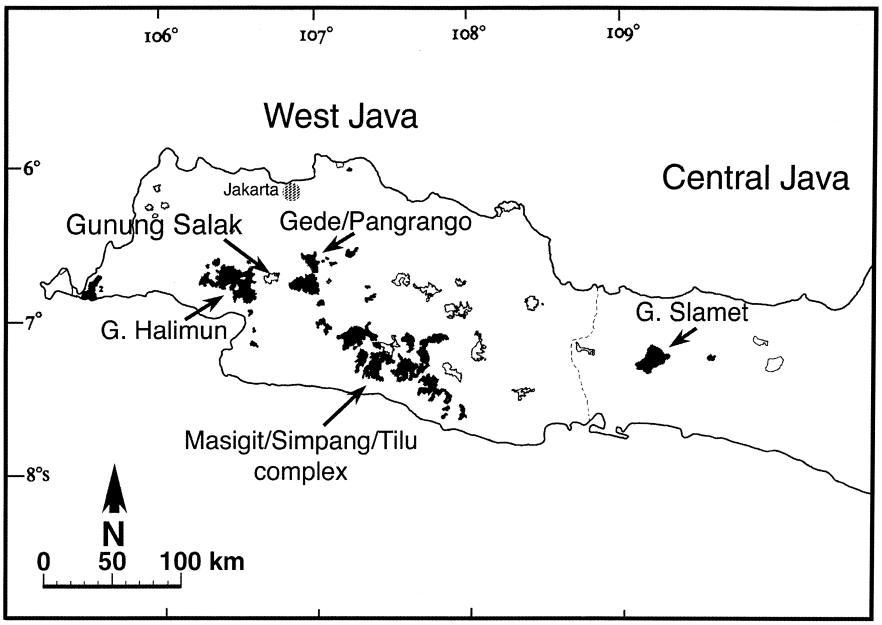

*Figure 1. Distribution of the silvery gibbon (*Hylobates moloch*) in the western portion of the island of Java, Indonesia. Darkened areas represent forest patches where gibbons are still present. Outlined light areas represent potentially suitable habitat, but where no gibbons were detected in recent surveys (modified from Kappeler 1984a; Supriatna et al. 1994). The broken line indicates the political boundary between West Java and Central Java.*

We PCR-amplified the mtDNA fragment between the cytochrome b and 12S RNA genes, which encompasses the entire control region, using primers GIBDLF2 (5′GTA CTA TTC TCA CCC GAC CTC CTA GGC GA3′) and H815 (5′GTT TCC CGT GGG GGT GTG GCT AGG3′). The 3′ end of the first primer corresponds to position 14921, and the second primer to position 215 of the published sequence of *Hylobates lar* (Genbank ID 5835820; Arnason et al. 1996). Double-stranded PCR amplifications were performed in 100 μL reaction volumes, containing 1.5 mM $MgCl_2$, 60 mM Tris, 15 mM $(NH_4)SO_4$, 0.5 mM of each dNTP, 0.1 μM of each primer, 1.0 U/reaction Taq polymerase (Perkin Elmer, Foster City, California), and approximately 1.0 ng of template DNA. Successful amplifications were obtained by the following protocol: 40 cycles of denaturing at 94° C for 1 minute, annealing at 53° C at 1 minute, and extension at 72° C for 3 minutes, ended by a final polymerization step at 72° C for 6 minutes.

We used eight internal primers to sequence the amplified products in both directions: GIBDLF3 (5′CTT CAC CCT CAG CAC CCA AAG C3′); GIBDLF4 (5′GCC CAG CAT CCT CCG TGA AAT CA3′); GIBDLF5 (5′GGA GCA CCT TAT GTC GCA GTA TCT3′); GIBDLR1 (5′GGT GAT AAA TGG GTA GCT CA3′); GIBDLR2 (5′AAG AAC AGG TTA RTA CTG G3′); GIBDLR3 (5′GCT GGT TGG TTT GTA TGT CGT3′); GIBDLR4 (5′GGG TGA TAG GCC TGT GAT C3′); GIBDLR5 (5′GGG TGG CTG TTA AAG GCG TGT A3′). Cycle sequencing was carried out with the BigDye–DNA sequencing kit (Applied Biosystems, Foster City, California) following manufacturer specifications. Sequences were aligned manually against the homologous sequence in *H. lar* (Arnason et al. 1996) and subsequently submitted to Genbank (accession # AF338873 - AF338908).

We examined phylogenetic relationships among aligned sequences through maximum parsimony. We performed two separate analyses by including (1) only samples of presumed known geographic origin and (2) all samples in the data set. Heuristic searches for the most parsimonious trees were conducted with PAUP version 4.0b2 (Swofford 1998). An initial heuristic search was performed on individuals from presumed known origin based on the entire control region with gapped characters excluded. Two outgroups (*H. lar* and *H. agilis*) were used for rooting the phylogenetic trees. Ten random additions were executed for each search, and all minimum trees were stored. We estimated pairwise comparisons of nucleotide divergence among all sequences using the uncorrected *p* distance, which is the total number of nucleotide differences divided by the total number of sites (Swofford et al. 1996).

Results

A total of 992 bp in the mtDNA control region was included in the analysis. For specimens of known origin, 256 sites were variable, of which 93 were phylogenetically informative. Nucleotide substitutions in the control region showed a transition-to-transversion ratio of 2:1, a ratio much lower than substitution biases reported for the control region of other species (Vigilant et al. 1989; W. M. Brown et al. 1982; Douzery & Randi 1997; Eizirik et al. 1998), but regression of transitions on transversions showed no evidence of transition saturation (analysis not shown).

Parsimony analysis with samples of known origin initially resulted in eight shortest trees. The silvery gibbon was divided into two clades, one clade including individuals from Gunung Halimun and a second clade consisting of samples from the Gunung Masigit/Simpang/Tilu complex, Gunung Gede/Pangrango, and Gunung Slamet (Fig. 2). The parsimony analysis of all 31 individuals maintained the two previously established clades, with individuals of unknown origin falling into one clade or

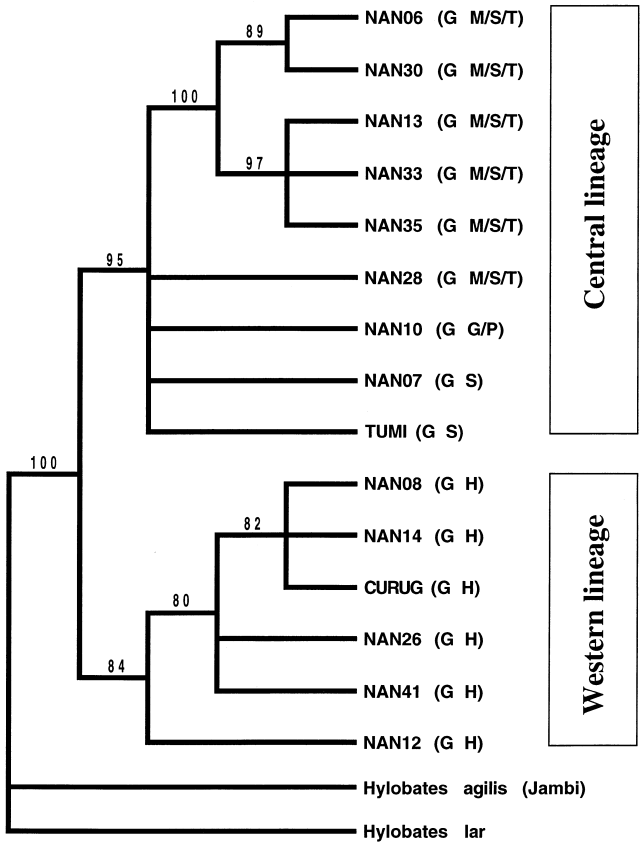

Figure 2. Consensus bootstrap tree (with bootstrap support values indicated on the branches) for the haplotypes of silvery gibbons of assumed known provenance used in this study. The areas of provenance are G M/S/T, Gunung Masigit/Simpang/Tilu; G G/P, Gunung Gede/Pangrango; G S, Gunung Slamet; and G H, Gunung Halimun. The haplotype of H. agilis *is from a sample collected in the Jambi province of Sumatra. The haplotype of* H. lar *was obtained from Genbank.*

the other (not shown). Bootstrap analysis strongly supported the division of silvery gibbons into two major clades, both when only samples of known origin were included and when all samples were included. Neighbor-joining and maximum-likelihood analyses produced virtually identical results (not shown).

Nucleotide sequence divergence between samples of known origin is shown in Table 1. Within the western lineage, the average divergence among haplotypes was 1.3%, whereas in the more widespread central lineage the average divergence was 3.1%. Average divergence between the western and central haplotypes was 3.5%.

Discussion

In their population and habitat viability analysis (PHVA) for the silvery gibbon, Supriatna et al. (1994) made several recommendations, including the translocation of gibbons from 11 unprotected forest patches that supported populations of fewer than 10 individuals. Given the human population growth on Java, protecting these patches is not feasible; therefore, translocating silvery gibbons into larger, more suitable areas is a reasonable conservation strategy. Translocation might be augmented by an intense metapopulation management program involving periodic individual translocations among the larger forest patches, thus increasing the overall effective population size of the silvery gibbon.

Our results, obtained from mtDNA control-region sequence data, suggest the presence of two genetically differentiated lineages of silvery gibbon. The western lineage, represented by the largest natural population of silvery gibbons in Gunung Halimun National Park, and the central lineage, represented by a series of smaller, more isolated populations in and around the Gunung Masigit/Simpang/Tilu complex, Gunung Gede/Pangrango, and Gunung Slamet (Figs. 1 & 2).

Within the central lineage, no obvious phylogeographic structuring is present, suggesting that no significant barrier existed to gene flow across the vast area it occupied prior to the recent fragmentation of gibbon habitat. In contrast, a clear phylogenetic division between the western and central lineages indicates that the Gunung Halimun population has been isolated from its conspecifics to the east for a considerable period of time. Indeed, the mean pairwise control-region nucleotide divergence (3.5%) between these two lineages is comparable to nucleotide divergence among long-tailed macaque (*Macaca fascicularis*) populations on different islands (Evans et al. 1999).

A plausible explanation for this division is not readily apparent. One possibility is that sequence divergence does not represent historical barriers to gene flow but rather differential lineage sorting from a polymorphic ancestor whose haplotypes diverged from one another long before populations became separated (Avise 1994). If this is the case, it is hard to imagine why the populations to the east of Gunung Halimun all sorted to haplotypes that share the same common ancestral haplotype, whereas those within Gunung Halimun sorted to haplotypes that share a different common ancestral haplotype. A more parsimonious explanation would be that population divergence predated the current habitat fragmentation and thus the molecular split reflects a more ancient biogeographic separation of the two lineages.

The ecological basis for this separation may be found in the montane forests of Gunung Salak (1700 to 2211 m above sea level), which lie between Gunung Halimun and the forests of Gunung Gede/Pangrango (Fig. 1) and may represent an effective barrier to gene flow. Kappeler (1984*a*) surveyed this area and found no gibbons. He

Table 1. Nucleotide divergence p distances between the haplotypes of silvery gibbon used in this study.*

	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*	*16*
1 NAN06																
2 NAN30	0.0151															
3 NAN13	0.0261	0.0240														
4 NAN33	0.0332	0.0200	0.0261													
5 NAN35	0.0291	0.0160	0.0240	0.0170												
6 NAN28	0.0442	0.0320	0.0521	0.0471	0.0440											
7 NAN10	0.0361	0.0280	0.0390	0.0401	0.0360	0.0280										
8 NAN07	0.0301	0.0260	0.0360	0.0381	0.0340	0.0340	0.0300									
9 TUMI	0.0372	0.0240	0.0401	0.0361	0.0320	0.0241	0.0221	0.0250								
10 NAN08	0.0412	0.0280	0.0440	0.0381	0.0360	0.0340	0.0280	0.0300	0.0190							
11 NAN14	0.0412	0.0280	0.0440	0.0381	0.0360	0.0340	0.0280	0.0300	0.0190	0.0040						
12 CURUG	0.0432	0.0310	0.0471	0.0401	0.0350	0.0371	0.0311	0.0320	0.0221	0.0030	0.0070					
13 NAN26	0.0482	0.0350	0.0511	0.0431	0.0430	0.0411	0.0370	0.0410	0.0291	0.0150	0.0170	0.0181				
14 NAN41	0.0433	0.0300	0.0461	0.0381	0.0320	0.0381	0.0321	0.0341	0.0221	0.0100	0.0120	0.0110	0.0171			
15 NAN12	0.0391	0.0300	0.0451	0.0401	0.0380	0.0361	0.0280	0.0340	0.0241	0.0120	0.0140	0.0150	0.0210	0.0160		
16 NAN39	0.1414	0.1299	0.1401	0.1351	0.1309	0.1321	0.1320	0.1359	0.1282	0.1239	0.1279	0.1251	0.1330	0.1242	0.1270	
17 Hylobat	0.1114	0.1079	0.1152	0.1182	0.1139	0.1080	0.1080	0.1079	0.1061	0.1029	0.1059	0.1061	0.1120	0.1072	0.1050	0.1439

Central lineage

Western lineage

**The polygons surrounding the* p *distances delimit those within the central Java clade (left) and within the western Java clade (right), respectively.*

suggested that their absence was due to an unsuitable habitat of low canopy and limited food. Detailed surveys in all the areas immediately adjacent to Gunung Halimun are required to test this ecological hypothesis.

Given this biogeographical explanation, our results have several implications for the conservation management of the silvery gibbon.

First, silvery gibbons consist of at least two genetically distinct, monophyletic mtDNA lineages. Although these clades satisfy Moritz's (1994) recognition criteria for the designation of two separate evolutionarily significant units, limited sample sizes and recent debates about these criteria (Paetku 1999; Crandall et al. 2000) support a less emphatic conclusion. Conservatively, then, we argue that gibbons in Gunung Halimun should be managed as a separate western management unit and should not be used to reinforce populations in the central management unit unless the status of the latter becomes dire.

Second, the lack of genetic structure among the Gunung Masigit/Simpang/Tilu complex, Gunung Gede/Pangrango, and Gunung Slamet populations presents managers with the option of translocating individuals (Griffith et al. 1989) among different localities within the central unit as a means of genetic and demographic management (Hanski 1999). The alternative strategy of establishing corridors among different isolates would be nearly impossible across the entire range of the central unit. Thus, in the face of extreme fragmentation, we recommend immediate experimentation with translocating silvery gibbons as a prelude to direct metapopulation management of the central unit.

Third, although the gibbons in Gunung Slamet in central Java are not phylogenetically distinct from other central silvery gibbons, this population merits special attention because it occupies the easternmost part of the species' distribution and may be ecologically distinct.

Finally, although our genetic analysis shows that silvery gibbons are monophyletic with respect to all other gibbon species (also supported by Takacs et al., unpublished data), morphological variation within and among *Hylobates* may in some instances make species identification difficult. Therefore, releasing confiscated gibbons, which may come from a number of Indonesian islands, into the wild population on Java should be avoided except under the most stringent conditions of taxonomic identification. Similarly, proper identification should also be made in zoos or other captive facilities because regular hybridization among species of gibbons renders the offspring of little conservation value and greatly reduces their importance for scientific study and public education.

Conclusion

Behavioral, ecological, demographic, and genetic information is sufficient to permit an intensive effort to conserve the highly endangered silvery gibbon. This effort should have two main goals: (1) to muster significant support for the protection of the remaining closed-canopy forests of Java and (2) to devise the means to separately manage the western and central silvery gibbons to forestall loss of genetic diversity and demographic viability (Lande 1993). To accomplish the latter goal, we propose immediate efforts to determine how best to translocate gibbons among Javan forest patches and additional efforts to devise metapopulation models (Hanski 1999) appropriate to the demographic and genetic management of the silvery gibbon.

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