Hybridization in Large-Bodied New World Primates

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ABSTRACT

Well-documented cases of natural hybridization among primates are not common. In New World primates, natural hybridization has been reported only for small-bodied species, but no genotypic data have ever been gathered that confirm these reports. Here we present genetic evidence of hybridization of two large-bodied species of neotropical primates that diverged ~3 MYA. We used species-diagnostic mitochondrial and microsatellite loci and the Y chromosome Sry gene to determine the hybrid status of 36 individuals collected from an area of sympatry in Tabasco, Mexico. Thirteen individuals were hybrids. We show that hybridization and subsequent backcrosses are directionally biased and that the only likely cross between parental species produces fertile hybrid females, but fails to produce viable or fertile males. This system can be used as a model to study gene interchange between primate species that have not achieved complete reproductive isolation.

Sequence data for this article have been deposited with the EMBL/GenBank Data Libraries under accession nos. DQ875611–DQ875741.

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Manuscript received April 6, 2007 Accepted for publication May 27, 2007

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DOI: 10.1534/genetics.107.074278

Genetics 176: 2421–2425 (August 2007)
characters), as well as individuals that possessed morphological features of both species. Using a multilocus approach, we present genetic data that show that these howler monkeys are hybridizing in Mexico.

MATERIALS AND METHODS

Blood and/or hair samples were collected from A. palliata and A. pigra individuals from sites in Tabasco, Mexico and other areas throughout Mexico (Figure 1). Genomic DNA was extracted using the DNeasy tissue kit (QIAGEN, Valencia, CA). Primers for eight microsatellite loci (Ap68 (Ellsworth and Hoelzer 1998), Ap74 (Ellsworth and Hoelzer 1998), PEPC8 (Escobar-Páramo 2000), and MapPairs (Invitrogen, Carlsbad, CA) loci D5S111, D6S260, D8S165, D14S51, and D17S804) were used to identify diagnostic alleles in each species and to identify hybrid individuals on the basis of the presence of these alleles. We used primers CB1-5’ and CB2-3’ (Palumbi 1996) to amplify a region of the mitochondrial cytochrome b (cyt b) gene and/or primers LCO-CO2-L and LCO-CO3-H (Cortés-Ortiz et al. 2003) to amplify a fragment of the ATP-synthase 6 and 8 genes (ATPase). A fragment of the Y chromosome Sry gene was amplified using primers SW2 (Whitfield et al. 1993) and SRY (Moreira 2002). To determine whether hybridization and subsequent crosses are directionally biased, we used a chi-square goodness-of-fit test to compare the observed frequencies of genotypes of hybrid individuals to those expected if all possible crosses among hybrids and backcrosses with parental species occur. We also estimated the probabilities of observing the detected genotypes on the basis of equal proportions of alleles/haplotypes in the parental species.

RESULTS AND DISCUSSION

We genotyped 104 individuals of A. palliata and A. pigra hybrid individuals for the eight microsatellite loci. These individuals include 40 A. palliata and 28 A. pigra individuals from outside of the putative hybrid zone and 36 individuals from within this zone (Figure 1). On the basis of the genotypes of A. palliata and A. pigra outside of the zone, three loci contained alleles that were distinct for each species (Ap68, D5S111, and D8S185) (Table 1). Sequences of these alleles confirmed that size differences are due to differences in the number of repeat units. The two species shared alleles at the other loci examined or potentially diagnostic alleles occurred at low frequencies in one or the other species. Several alleles showed clines in allele frequencies through the hybrid zone. We also sequenced a 307-bp region of the mitochondrial cyt b gene and/or an 817-bp fragment of the ATPase locus from the same 104 individuals listed above (GenBank accession nos. DQ875685–DQ875741 and DQ875611–DQ875672, respectively). Sequences from the two parental species have fixed differences at 14 sites for the cyt b fragment and 46 sites for the ATPase fragment, and each locus showed ~5% sequence divergence among species. On the basis of these levels of sequence divergence, A. palliata and A. pigra likely separated ~3 MYA (Cortés-Ortiz et al. 2003).

In total, 23 individuals from the putative hybrid zone wholly possessed alleles of either A. palliata (n = 11) or A. pigra (n = 12) and contained the respective species’ mitochondrial haplotype; this suggests that individuals of both parental species are nearly equally abundant within the hybrid zone. Thirteen other individuals were identified as hybrids on the basis of the mitochondrial and microsatellite data (Table 2). The hybrid individuals included seven adult females, one infant female, and five adult males. Twelve individuals possessed microsatellite alleles, diagnostic of both parental species, although no individuals were F1 hybrids (Table 2). The lack of F1’s may be because these individuals are ephemeral, or the hybrid zone is old, or it could reflect a low incidence of hybridization of pure parental forms (see Goodman et al. 1999). All adult hybrids contained the mitochondrial haplotype of A. pigra. The infant was the only hybrid that possessed A. palliata’s haplotype. The presumed mother of this infant (based on genotypic data and the fact that the female was carrying the infant) was A. palliata based on the genetic markers used here and her appearance and occurred with a hybrid male that was likely the father of this infant based on the genotypic evidence. Hybrid individuals occurred in fragmented habitats where the two species’ distributions overlap and were members of “mixed troops” that contained individuals of both parental species and in some cases individuals with unique or intermediate morphologies (Figure 2).

We attempted amplifications of a region of the Sry gene with genomic DNA of 4 A. palliata males, 2 A. palliata females, 3 A. pigra males, and 2 A. pigra females from outside of the putative hybrid zone and all 13
individuals from within the hybrid zone that were characterized as hybrids. Amplifications were successful only with genomic extractions of males; this and the fact that direct sequencing of amplification products yielded chromatograms without double peaks or other ambiguities strongly imply that the gene amplified occurs on the Y chromosome in these individuals. The sequences obtained from individuals outside of the hybrid zone were ~821 bp in length and showed fixed differences at three sites among species (GenBank accession nos. DQ875673–DQ875684). All male hybrid individuals (n = 5) possessed the Sry gene of A. pigra (Table 2).

If matings of hybrids are random and occur among all possible combinations of hybrids and parental species, we expect to find equal frequencies of the four possible genotypes of males and the two possible genotypes of females at the maternally inherited mitochondrial locus and the paternally inherited nuclear locus located on the Y chromosome of males. Although sample sizes are small, the chi-square goodness-of-fit tests suggest that the observed frequencies of males’ and females’ genotypes differed significantly from these expectations (Table 3). Moreover, probabilities of detecting 12 adult hybrids with the mitochondrial haplotype of A. pigra (P = 2.4 × 10⁻⁴), 12 hybrid individuals with the mitochondrial haplotype of A. pigra plus 1 hybrid individual with the mitochondrial haplotype of A. palliata (P = 1.6 × 10⁻³), and all 5 hybrid males with the Sry gene of A. pigra (P = 3.1 × 10⁻⁴) are low. These patterns imply that the direction of hybridization and subsequent backcrosses is strongly biased. Only crosses between A. pigra females or hybrid females carrying the mitochondrial haplotype of A. pigra and A. palliata males or hybrid males with the Sry gene of A. palliata occur and give rise to female offspring (Figure 3). However, no male

| TABLE 1 | Frequencies of alleles of microsatellite loci of populations of A. palliata and A. pigra from outside and within the putative hybrid zone (including hybrids) |
|---------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Locus   | Allele size | ApaO | ApaHZ | ApaHZ' | ApaO' |
| Ap68    | 187         |      |      | 0.11   | 0.17  |
| 191     |             | 0.64 | 0.41  |        |       |
| 193     | 0.99        | 1.00 | 0.20  |        |       |
| 195     | 0.01        |      |      |        |       |
| 197     |             |      | 0.05  | 0.43   |       |
| 150     |             |      | 0.50  | 0.68   |       |
| 152     | 0.99        | 1.00 | 0.25  |        |       |
| 154     |             |      | 0.14  | 0.07   |       |
| 156     | 0.01        |      |      |        |       |
| D5S111  | 163         | 1.00 | 1.00 | 0.11   |       |
| 167     |             |      | 0.02  | 0.13   |       |
| 169     |             |      | 0.48  | 0.27   |       |
| 174     |             |      |      | 0.02   |       |
| 178     |             |      |      | 0.04   |       |
| 180     |             |      | 0.39  | 0.55   |       |
| D6S260  | 171         |      |      | 0.06   |       |
| 173     |             | 0.25 | 0.02  |        |       |
| 177     |             | 0.75 | 0.07  | 0.02   |       |
| 179     |             |      |      | 0.02   |       |
| 181     |             | 0.18 | 0.27  |        |       |
| 183     |             |      | 0.07  | 0.08   |       |
| 185     |             |      | 0.16  | 0.06   |       |
| 187     |             |      | 0.50  | 0.50   |       |
| D8S165  | 119         | 0.07 | 0.91  | 1.00   |       |
| 143     | 1.00        | 0.93 | 0.09  |        |       |
| D14S51  | 145         | 0.03 | 0.04  | 0.50   | 0.48  |
| 145     |             | 0.03 | 0.05  |        |       |
| 147     |             | 0.96 | 0.45  | 0.52   |       |
| D17S804 | 157         | 1.00 | 0.89  | 0.61   |       |
| 161     |             |      | 0.89  | 0.61   |       |
| 163     |             |      |      | 0.04   |       |
| 165     |             |      | 0.05  | 0.07   |       |
| 167     |             |      | 0.07  | 0.06   |       |
| 169     |             |      |      | 0.07   |       |
| PEPC8   | 239         |      | 0.26  | 0.43   |       |
| 244     |             |      | 0.11  |       |       |
| 246     |             |      | 0.05  | 0.04   |       |
| 248     |             | 1.00 | 0.58  | 0.54   |       |

*Allele sizes are the sizes of the complete sequence of the microsatellite alleles and include both repeat and flanking regions. Diagnostic alleles are shown in italics.  
^ApαO, A. palliata from outside the putative hybrid zone.  
^ApαHZ, A. palliata from within the putative hybrid zone.  
^ApαO', A. palliata from outside the putative hybrid zone.  
^ApαHZ', A. palliata from within the putative hybrid zone.

| TABLE 2 | Hybrid individuals in the area of species overlap that showed mixed A. palliata and A. pigra character states |
|---------|------------------------------------------------------------------------------------------------------|
| ID      | Sex | Phenotype | mtDNA | Microsatellite locus |
| S096    | F   | Apa       | i/a   | Ap68 D5S111 D8S165 Sry |
| S098    | M   | Api       | a/i   |       |
| S154    | M   | Api       | i/i   |       |
| S155    | F   | Api       | i/a   |       |
| S157    | F   | Api       | a/a   |       |
| S161    | F   | Apa       | i/a   |       |
| S162    | F   | Api       | a/a   |       |
| S164    | F   | Api       | i/i   |       |
| S165    | M   | Api       | i/i   |       |
| S166    | M   | Api       | i/a   |       |
| S167    | F   | Api       | i/i   |       |
| S182    | M   | Api       | i/i   |       |
| S183    | F   | Api       | i/i   |       |

*Identification code:  
^All individuals except S157 were adults; S157 was an infant still being carried by its presumed mother.  
^Phenotype based on size and pelage coloration and texture. Apa, A. palliata-like; Api, A. pigra-like individuals.  
^Mitochondrial haplotype. a, A. palliata; i, A. pigra.  
^Identity of alleles for parental species at each diagnostic microsatellite locus. a, A. palliata; i, A. pigra.  
^Identity of the Y chromosome Sry gene. I, A. pigra; NA, primers did not amplify a product and were not expected to on the basis of the sex of this individual.

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hybrids with the Sry gene of *A. palliata* were observed and so, in accordance with Haldane’s rule (Haldane 1922), the data strongly suggest that the aforementioned crosses fail to produce viable males. Furthermore, on the basis of the low probability of detecting only the mtDNA haplotype of *A. palliata* in 12 adult hybrid individuals, *A. palliata* females and *A. pigra* males or hybrid males either mate infrequently or typically fail to produce viable offspring. Nonetheless, the genotypes of the hybrid infant and its suspected parents imply that this infant (S157, Table 2) was produced from a backcross between a male hybrid (S154, Table 2) and a female *A. palliata*. This demonstrates that such matings occur and that female offspring are produced. However, because no adult females were observed with the mitochondrial haplotype of *A. palliata*, we suspect that such crosses are uncommon or this infant is either infertile or will not survive to reproductive age.

We are currently investigating the potential role of morphological, behavioral, genetic, and cytogenetic differences as causes of the bias in direction of hybridization of these species. This work should advance our understanding of the speciation process and origins of reproductive isolation among primates, as well as the role of hybridization in primate evolution (Arnold and Meyer 2006). Moreover, study of the presence of hybrids in fragmented and intact forest tracts will reveal whether human-induced forest fragmentation has instigated hybridization by confining members of both species to small areas and limiting access to conspecific mates.

The Secretaría del Medioambiente y Recursos Naturales, Mexico, and the Autoridad Nacional del Ambiente, Panama, are gratefully acknowledged for providing the collecting, export, and import permits that made our research possible. This research was supported by the PROMEP Ph.D. scholarship no. UVER-98-11-019 and PROMEP 103.5/03/1154EXB-9 to L.C.-O., by the Instituto de Neuroetología, Universidad Veracruzana. Financial support was also provided by the Smithsonian Molecular Systematics program and by the Museum of Zoology, University of Michigan.

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**TABLE 3**

| Genotype* | Frequencies Expected | Observed | Chi-square (d.f.) | P-value |
|-----------|----------------------|----------|---------------------|---------|
| Males (*n* = 5) | | | | |
| aI y | 1.25 | 0 | 15.0 (3) | 1.8 x 10^-3 |
| iI y | 1.25 | 5 | | 
| aA y | 1.25 | 0 | | 
| iA y | 1.25 | 0 | | 
| Females (*n* = 8) | | | | |
| aI y | 4 | 1 | 4.5 (1) | 3.4 x 10^-2 |
| iI y | 4 | 7 | | |

*Lowercase letters refer to the mitochondrial haplotype of *A. palliata* (a) and *A. pigra* (i); uppercase letters denote the genotype of males at the Y chromosome Sry gene for alleles of *A. palliata* (A) and *A. pigra* (I).
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Communicating editor: N. Takahata