MORPHOLOGICAL AND MOLECULAR EVIDENCE REVEALS RECENT HYBRIDIZATION BETWEEN GORILLA TAXA

Rebecca Rogers Ackermann1,2 and Jacqueline M. Bishop3
1Department of Archaeology, Faculty of Science, University of Cape Town, Private Bag, Rondebosch 7701, South Africa
2E-mail: Becky.Ackermann@uct.ac.za
3Department of Zoology, Faculty of Science, University of Cape Town, Private Bag, Rondebosch 7701, South Africa

Received January 16, 2009
Accepted September 12, 2009

Molecular studies have demonstrated a deep lineage split between the two gorilla species, as well as divisions within these taxa; estimates place this divergence in the mid-Pleistocene, with gene flow continuing until approximately 80,000 years ago. Here, we present analyses of skeletal data indicating the presence of substantial recent gene flow among gorillas at all taxonomic levels: between populations, subspecies, and species. Complementary analyses of DNA sequence variation suggest that low-level migration occurred primarily in a westerly-to-easterly direction. In western gorillas, the locations of hybrid phenotypes map closely to expectations based on population refugia and riverine barrier hypotheses, supporting the presence of significant vicariance-driven structuring and occasional admixture within this taxon. In eastern lowland gorillas, the high frequency of hybrid phenotypes is surprising, suggesting that this region represents a zone of introgression between eastern gorillas and migrants from the west, and underscoring the conservation priority of this critically endangered group. These results highlight the complex nature of evolutionary divergence in this genus, indicate that historical gene flow has played a major role in structuring gorilla diversity, and demonstrate that our understanding of the evolutionary processes responsible for shaping biodiversity can benefit immensely from consideration of morphological and molecular data in conjunction.

KEY WORDS: Admixture, developmental anomalies, gene flow, Gorilla beringei, Gorilla gorilla, heterosis, supernumerary teeth, vicariance.

Historical and environmental characteristics of a region influence both the origin and maintenance of its biodiversity at multiple levels (Avise 2000; Vellend and Geber 2005). For example, the repeated glacial cycles of the Pleistocene had a significant impact on the biogeographic patterns of many organisms (Avise et al. 1998; Hewitt 2004a); habitat fragmentation during glacial maxima led to isolation and, in many cases, local extinction as populations retreated and their ranges became subdivided into geographically distinct glacial refugia. During these protracted periods of isolation, populations differentiated to varying degrees; under contrasting selection regimes and together with the increased effects of genetic drift, character divergence under allopatry emerged in many lineages (Endler 1977; Avise 2000). Following climate amelioration and periods of population range expansion, zones of secondary contact are likely to have formed between previously refugial populations, with admixture often resulting in increased variation, the production of character clines, and the emergence of novel phenotypic traits (Endler 1977; Barton and Hewitt 1985; Arnold 1997).

To date, a large number of studies using methods of phylogeographic inference have provided clear evidence across myriad organisms for resumed gene flow among once isolated (Pleistocene) refugial populations (see examples in reviews by Avise et al. 1998; Taberlet et al. 1998; Hewitt 2000, 2004b), and highlight research on hybrid zones as central to our understanding of historic subdivisions and their role in genetic and morphological divergence. A
common finding in these studies is that complete speciation via vicariance is not the predominant outcome of isolation. Indeed, although many do report incipient speciation at hybrid zones that represent several degrees of genetic isolation, a recurring pattern emerging from these studies is that of resumed gene flow via secondary contact (Avise et al. 1998; Hewitt 2000). Even so, because genetic differentiation may substantially predate the onset of resumed gene flow across secondary contact zones, a degree of mismatch between observed contemporary (hybrid) phenotypes and phylogeographic history may exist in studies of contact zones. Unfortunately, although important zoological studies have combined phenotypic and molecular analyses of individuals within hybrid zones (e.g., Patton 1993; Genoways et al. 2008), increasing reliance on molecular analyses alone has meant that our understanding of the level of congruence between molecular and morphological characters, when trying to detect secondary contact hybrids in zoodata, remains limited (Gaubert et al. 2005).

In primates, hybridization at contact zones occurs frequently among a number of closely related taxa, including members of both New and Old World monkeys as well as members of the clade containing apes and humans, and likely played an important role in structuring diversity across the primate order (see overview in Arnold and Meyer 2006). Such contact zones have been particularly well-documented for gibbons (Broockelman and Srijosamatera 1984; Mashall and Sugardjito 1986), baboons (Phillips-Conroy and Jolly 1986; Phillips-Conroy et al. 1991; Jolly et al. 1997; Alberts and Altman 2001), and macaques (Fooden 1964; Bernstein 1966; Supriatna 1991; Froehlich and Supriatna 1996; Bynum et al. 1997; Evans et al. 2001). Additionally, hybridization in primates is known to occur across a variety of taxonomic levels; it is well known that taxa that diverged millions of years ago continue to form natural hybrids in the wild (Jolly 2001). Yet although numerous studies have explored biogeographic explanations for primate diversity (Lehman and Fleagle 2006), our understanding of the role played by the combined effects of evolutionary divergence and subsequent hybridization in shaping this phenotypic/genotypic diversity is poor.

Although gorilla taxonomy has long been debated (Groves 2003), researchers are increasingly recognizing four taxa that represent distinct groups in terms of their geography, morphology, molecules, and behavior: (1) western gorillas, Gorilla gorilla, comprised of two lowland gorilla subspecies (G. g. gorilla and G. g. diehli), and (2) eastern gorillas, Gorilla beringei, comprised of eastern lowland (G. b. graueri) and mountain (G. b. beringei) gorilla subspecies (Sarmiento and Oates 2000; Groves 2001; Stumpf et al. 2003; Taylor and Goldsmith 2003; Clifford et al. 2004; Anthony et al. 2007b). Eastern gorillas are separated from their western counterparts by approximately 1000 km (Fig. 1; Groves 2001; Grubb et al. 2003), and analyses of genetic diversity indicate that divisions between these taxa are substantial, with evidence from both the mitochondrial and nuclear genomes placing the time to most recent common ancestor within the Pleistocene (Ruvolo et al. 1994; Jensen-Seeman et al. 2003; Anthony et al. 2007b; Thalmann et al. 2007). Because gene coalescence predates the timing of an actual population split (Nichols 2001), divergence dates from mitochondrial and nuclear DNA analysis are not indicators of when gene flow ceased between eastern and western gorillas; indeed, simulations of scenarios of population divergence based on nuclear sequences suggest that these taxa may have continued to exchange migrants until as recently as 80,000 years ago (Thalmann et al. 2007). Although it is difficult to estimate divergence dates from molecular data with any certainty, these results do suggest a close relationship between eastern and western gorillas until the late Pleistocene. Within these taxa, genetic structuring correlates with the taxonomic separation of the two eastern gorilla lineages (Anthony et al. 2007b), and their initial divergence (though again, not gene flow cessation) has been estimated to about 400,000 years ago (Ruvolo et al. 1994; Garner and Ryder 1996; Jensen-Seeman and Kidd 2001); divergence times between the two lineages of western gorillas have not been estimated.

Despite extensive research on primate hybrid zones, attempts to quantify the effects of gene flow on primate morphology have been limited. A recent study of baboon crania has, however, provided a means for identifying the presence of gene flow in skeletal morphology by demonstrating that admixture between genetically divergent lineages results in a high incidence of rare nonparental nonmetric traits (Ackermann et al. 2006). In particular, supernumerary teeth and sutural anomalies were shown to be highly sensitive indicators of hybridization, being present at very high frequencies in captive first-generation baboon hybrids, which represent crosses between lineages that diverged over the course of the Pleistocene. Importantly, four museum specimens drawn from proposed wild baboon hybrid zones between other baboon taxa also showed these skeletal signatures of hybridization (Ackermann et al. 2006), whereas studies of a handful of other organisms—i.e., extant and late Pleistocene ground squirrels (Goodwin 1998), monodontids (Heide-Jørgensen and Reeves 1993) and wildebeest (R. R. Ackermann, unpubl. data)—suggest that similar unusual craniodental variation characterizes not just baboon hybridization, but intertaxon mammalian hybridization more broadly. Goodwin (1998) and Ackermann et al. (2006) also noted that the pattern of expression of supernumerary teeth in hybrids (mandibular vs. maxillary, bilateral versus unilateral) differs from what is seen in populations with no recent history of hybridization. For the baboon, which is the only available primate model, mandibular supernumerary teeth and extremely unusual anomalies (both frequently expressed bilaterally) are the best indicators of hybridization. Although none of these studies analyzed large morphological datasets together with locality data, they do suggest that it might be possible to test for the
Figure 1. Summary of morphological and molecular analyses among gorilla taxa with approximate current distributions shown. Although data from museum records (see Supporting Information Google Earth file) indicate the historic presence of gorillas outside of these regions, actual historic ranges are not known. (A) Migration rate estimates (M) and the direction of gene flow (this study) among gorilla taxa are shown by arrows. Gorilla populations are grouped as I–VII and represent regional haplogroups identified in Clifford et al. (2004) and Anthony et al. (2007b) (See Materials and Methods for details). (B and C) Pie charts illustrate the percentages (values indicated within pie slices) of individuals with one or more mandibular supernumerary molars (orange), maxillary supernumerary molars only (purple), and other supernumerary teeth that are not diagnostic (green) for G. g. gorilla and G. g. diehli (II) and G. b. beringei and G. b. graueri (III). These charts also indicate the frequency of malar sutures (yellow), calculated relative to adjusted sample sizes presented in Table 1. Abbreviations for regions are indicated in Table 1. For further details on localities and group assignments, see Supporting Information Google Earth file.

The presence of hybrid zones using such combined approaches. However, although the frequency of such a variation was extremely high in baboon first-generation hybrids (50% of F1 males had supernumerary teeth; Ackermann et al 2006), expectations are necessarily lower when detecting wild hybrid zones for a number of reasons. Most importantly, active contact zones are expected to be complicated—containing a mixture of first-generation hybrids, backcrossed hybrids, and parental populations (Jolly 1993; Barton 2001). Moreover, in zones that are no longer active (representing past hybridization) it is not always known how long lineages remained in contact, how much gene flow occurred, or when gene flow finally ceased among lineages. So although the absence of “hybrid morphologies” cannot be used to rule out gene flow, the presence of such anomalies—especially when they exist at frequencies higher than typically seen in parental (or other model) populations, display different patterns of expression, or are extremely rare or novel—strongly point to hybridization.

In gorillas, habitat fragmentation, isolation, and small local population size across the gorilla range may have led to significant divergence in genetic and morphological traits via drift and selection, as suggested by considerable mitochondrial haplotype substructuring (Anthony et al. 2007b). Refuge theory predicts that admixture should be present at the borders between these refugial populations (Haffer 1969; Hewitt 1996), and indeed evidence from mitochondrial DNA suggests a degree of admixture among the regionally diverse western gorillas (Anthony et al. 2007b). Hybrid morphologies (as evidence of resumed gene flow) are also predicted to occur in zones of contact. Here, we test for the presence of such contact zones across the entire gorilla range by examining the frequencies of unusual nonmetric trait variation in the skull. Importantly, because the morphological analyses sample museum specimens, this approach allows us to take advantage of the much greater sample sizes and broader geographical coverage available in such collections relative to the fairly limited molecular datasets; these museum collections undoubtedly represent diversity and a record of evolutionary process that no longer exist in living populations. This is particularly important in the eastern lowland (G. b. graueri) and mountain (G. b. beringei) gorillas, where genetic sampling has been limited, and where current ranges (and assumedly diversity) are reduced. Morphological data are complemented by molecular estimates of gene flow, generated in this study using published mtDNA sequence data, which
allow us to hypothesize about past population connectivity and inform our expectations regarding the origin of potential parental groups. Finally, because our analyses suggest the presence of a recent hybrid zone in eastern lowland gorillas (*G. b. graueri*), multivariate and univariate analyses of published craniometric data are also performed, to quantify the overall morphological position of—and variation within—these gorillas relative to the other subspecies.

**Materials and Methods**

**CRANIAL SAMPLE FOR NONMETRIC MORPHOLOGICAL ANALYSES**

All adult gorilla skulls in the following museum collections were examined: Musée Royal de l’Afrique Centrale, Tervuren, Belgium (here abbreviated as MRAC); Museum für Naturkunde Berlin, Germany (MFNB); Powell-Cotton Museum, Kent, United Kingdom (PWCT); Royal College of Surgeons, London, United Kingdom (RCS); Natural History Museum, London, United Kingdom (BMNH); Cleveland Museum of Natural History, Cleveland, OH, USA (CMNH); American Museum of Natural History, New York, NY, USA (AMNH); National Museum of Natural History, Smithsonian Institution, Washington, D.C., USA (NMNH). This represents the bulk of gorilla specimens in curated collections. Relatively complete crania (including mandibles) were selected for this study. The exception to this was for the *G. g. diehli* specimens, where specimens without mandibles were included due to the paucity of crania in collections. This makes the results for this taxon less definitive, as discussed below. Specimens were collected primarily in the late 19th to mid-20th centuries. Care was taken to use only well-provenanced individuals, or ones for which provenance could be determined with some certainty. Geographic coordinates were assigned to these localities; some were provided either by collectors or later researchers, whereas other data had to be estimated by locating town or community names on maps or in historical documentation. Care was taken to correct misspellings, but undoubtedly some crept in as names were often recorded phonetically. A Google Earth file with individual localities was compiled, and localities containing hybrid-like individuals were marked. Many localities are known from just a single individual or a few individuals, and therefore locality information was used to assign each individual to one of 11 regions (modeled primarily after Groves 1966; Groves 1970) in order to provide statistical and visual summaries by region. The final sample for nonmetric analyses consisted of 582 specimens (225 females and 357 males; Table 1).

**NONMETRIC CRANIAL VARIATION**

Individual gorilla crania were examined and scored for the presence of a suite of cranial nonmetric traits, with emphasis on dental and sutural anomalies known to be present in hybrid baboons (Ackermann et al. 2006; Ackermann 2007). Although some of these anomalies do exist in humans and other nonhuman primates with no documented history of recent hybridization (as will be discussed further in the Results and Discussion), they occur at a very low frequency (<5%) and often differ in expression from what is seen in other primates. The crania were also examined for evidence of developmental abnormalities or other abnormal trait variation that might suggest a breakdown in coordination of development. Nonmetric traits scored include: supernumerary

### Table 1. Sample sizes (N) and distribution of taxa by region and sex. Overall frequencies of supernumerary teeth and malar sutures are presented for each taxon. Differences in frequencies by subregion can be examined in Figure 1 and in the Supporting Information Google Earth file.

<table>
<thead>
<tr>
<th>Species</th>
<th>Region</th>
<th>N</th>
<th>Females</th>
<th>Males</th>
<th>Supernumerary teeth (% with one or more mandibular supernumerary tooth)</th>
<th>Malar sutures (N&lt;sup&gt;1&lt;/sup&gt;)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>G. b. berengei</em></td>
<td>Virungas (VIR)</td>
<td>25</td>
<td>9</td>
<td>16</td>
<td>0% (0%)</td>
<td>0 (6)</td>
</tr>
<tr>
<td><em>G. b. graueri</em></td>
<td>Mwenga-Fizi (Itombwe) (FIZ)</td>
<td>15</td>
<td>6</td>
<td>9</td>
<td>12% (9%)</td>
<td>45% (60)</td>
</tr>
<tr>
<td></td>
<td>Kahuzi (KAH)</td>
<td>4</td>
<td>2</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tshiaberimu (TSH)</td>
<td>18</td>
<td>12</td>
<td>6</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Utu (UTU)</td>
<td>41</td>
<td>15</td>
<td>26</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>G. g. gorilla</em></td>
<td>Coast (CST)</td>
<td>27</td>
<td>7</td>
<td>20</td>
<td>5% (1%)</td>
<td>15% (293)</td>
</tr>
<tr>
<td></td>
<td>Gabon (GAB)</td>
<td>22</td>
<td>6</td>
<td>16</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Plateau (PLT)</td>
<td>317</td>
<td>134</td>
<td>183</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>River (RIV)</td>
<td>32</td>
<td>5</td>
<td>27</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sangha (SNG)</td>
<td>21</td>
<td>5</td>
<td>16</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>G. g. diehli</em></td>
<td>Cross River (CRV)</td>
<td>60</td>
<td>24</td>
<td>36</td>
<td>12% (0%)</td>
<td>7% (42)</td>
</tr>
</tbody>
</table>

<sup>1</sup>Sample sizes for calculation of malar suture frequencies based on animals with visible suture.
RECENT HYBRIDIZATION BETWEEN GORILLA TAXA

teeth, extra sutures or ossicles in the zygomatic region, rotated teeth, anterior and posterior dental crowding, ossicles at lambda and asterion, bregmatic bones, coronal ossicles, epipetric bones, and parietal notch bones. Of these traits, only supernumerary teeth, extra sutures in the zygomatic region, ossicles in the zygomatic region, rotated teeth, anterior dental crowding, and posterior dental crowding were present in the sample. The expression of supernumerary teeth was also documented, with special attention given to whether they are mandibular, which has been shown to be a good indicator of hybridization in baboons. It is important to note that in mature gorillas facial and neurocranial sutures are typically fused and/or obliterated by muscle markings and associated bony changes, and it is possible that some sutural variation was present but no longer observable in a number of individuals. Therefore, individuals were also scored in terms of the visibility/observability of sutures, and frequencies of sutural anomalies were calculated for the subsample of animals with visible sutures. Differences between the four gorilla taxa were tested for a statistical significance with a chi-square test for each of the analyzed variables.

ESTIMATES OF GENE FLOW

Shaped both by barriers to dispersal in the form of major rivers, and population expansions from Pleistocene forest refugia (Jensen-Seaman and Kidd 2001; Clifford et al. 2004; Anthony et al. 2007b), the evolutionary history of gorillas in central Africa is inherently difficult to unravel. The study of gene flow among extant gorilla taxa is currently limited to estimates between eastern and western gorillas; data from 16 noncoding autosomal loci reveal primarily male-mediated gene flow, with a greater number of migration events into western gorillas (Thalmann et al. 2007). Although data from nuclear gene regions can provide detailed insight into male- and female-mediated gene flow patterns, these simulations were based on only 15 western and three eastern gorilla samples (Thalmann et al. 2007). To date, the most comprehensive spatial sampling of genetic diversity in gorillas is represented by mitochondrial DNA sequences (Clifford et al. 2004; Anthony et al. 2007b). Therefore, to understand past connections between gorilla taxa at a finer scale, we have used results from Thalmann et al. (2007), together with our own analysis of gene flow based on mitochondrial sequences, to inform our expectations and conclusions with regard to patterns in the nonmetric morphological analysis and the possible parentage of hybrid groups. We analyzed available mitochondrial sequence data from previously published studies available on GenBank (Garner and Ryder 1996; Jensen- Seaman and Kidd 2001; Morgan et al. 2003; Clifford et al. 2004; Jensen-Seaman et al. 2004; Thalmann et al. 2005; Anthony et al. 2007b); these sequences (N = 163) represent 233 base pairs of the hypervariable I (HV1) domain of the mtDNA control region. Only published sequences with geographic provenance were included in our analyses. The study of gorilla mtDNA has been plagued by the occurrence of nuclear copies of mitochondrial regions (numts); all data analyzed in this study were previously screened for candidate nuclear copies using methods described in Anthony et al. (2007a). To obtain estimates of gene flow among contemporary gorilla populations, we used a maximum likelihood method based on the coalescent, as implemented in MIGRATE version 2.4.3 (Beerli and Felsenstein 1999; Beerli and Felsenstein 2001). MIGRATE uses an equilibrium model that estimates migration rates averaged across the coalescent history. Coalescent-based estimates of gene flow in gorillas using mitochondrial DNA sequences are unreported in the literature. To circumvent problems with estimating migration rates from small sample sizes (Pfenninger and Posada 2002), we pooled individuals into seven regional populations, representing a priori geographically defined haplogroups (original haplotype group designations and geographic distributions from Clifford et al. (2004) and Anthony et al. [2007b] are reported in parentheses): (I) mountain gorilla n = 12 (A; Rwanda/Uganda); (II) eastern lowland n = 20 (B; Democratic Republic of Congo [DRC]); (III) western lowland n = 21 (D2; Central African Republic [CAR]/Cameroon/Congo); (IV) western lowland n = 11 (C2; Cameroon/Gabon); (V) western lowland n = 52 (D3; Gabon/Congo); (VI) western lowland n = 15 (D1; Equatorial Guinea); (VII) western lowland n = 32 (C1; C2; Nigeria/Cameroon/Gabon) (regional localities I–VII indicated in Fig. 1). Although pooling individuals in this way may restrict the interpretation of estimates of local population size (because of possible within-region structuring), it does provide robust estimates of gene flow (Beerli and Felsenstein 2001). MIGRATE version 2.4.3 was used to estimate the parameter Θ, the effective population size scaled by mutation rate where Θ = N,eμ, together with the effective number of migrants N,m, where N,e is the effective population size, μ is the mutation rate, and m is the rate of migration into a population. Starting values for all parameter estimates were initially obtained using FST (Beerli and Felsenstein 1999); for other settings within the program default values were used. The following search parameters were used for each analysis: 10 short chains with 100,000 recorded genealogies each and three long chains with 50,000 recorded genealogies; each chain was sampled every 100 steps with an initial burn-in of 10,000 steps. Multiple long chains were averaged for estimates and five replicates were performed.

METRIC CRANIAL VARIATION

Analysis of nonmetric cranial data was complemented by metric analyses of 40 cranial metric variables for three of the gorilla taxa (N = 612), drawn from Groves (1970) (Table S1). As will be discussed further in the section “Results,” the nonmetric evidence for hybridization is strongest in the eastern lowland gorillas (G. b. graueri), suggesting that this region may represent a hybrid

EVOLUTION 2009 | 5
zone, whereas the genetic results indicate that the best explanation for such a phenomenon is the movement of western gorillas (G. g. gorilla) into the eastern lowland localities. Making an assumption that parental groups for G. b. graueri might have been drawn from or resemble G. g. gorilla and G. b. beringei, it is therefore useful to consider whether this taxon lies closer to one or the other group for each variable. To assess this, a series of univariate and multivariates analyses were conducted on these three taxa as detailed below. There is considerable missing data in this dataset, and many individuals are missing mandibles in particular. Multivariate analyses were therefore performed with both the full dataset (variable number = 40; N = 356; 256 cases deleted due to missing data), and a reduced one (variable number = 26; N = 423; 189 cases deleted due to missing data; all mandibular variables removed), to maximize variables in the former and number of individuals in the latter.

First, to test for the potentially confounding factor of sexual dimorphism, multivariate analysis of variance (MANOVA) including all traits and the reduced trait set were performed, and indicated statistically significant morphological differences between the sexes (P < 0.0001). Males are larger than females for all but one trait (PERPPROJ). Univariate analysis of variance indicated that all traits except one (PERPPROJ) were different between males and females at the P < 0.001 level of significance. Because of these differences, all traits were adjusted to the female mean for each of the three taxa [adjusted male value = original male value + (female mean – male mean)]. Second, to test whether significant differences exist between the model parental populations (G. g. gorilla and G. b. beringei) and the purported hybrid population (G. b. graueri), univariate and multivariate analyses of variance were performed on this adjusted data. Pairwise t-tests for all variables were also made comparing the means of the two “parental” taxa to each other and to the means of the putative hybrid population. Third, G. b. graueri mean values were then judged relative to the midpoint between G. g. gorilla and G. b. beringei for each examined variable. Previous researchers have taken a similar approach to test for size heterosis in primates (Cheverud et al. 1993; Kohn et al. 2001; Schillaci et al. 2005; Ackermann et al. 2006), where heterosis was measured as the difference between the hybrid and expected values (Turner and Young 1969). However, quantitative genetic expectations differ depending on whether a sample consists of first generation hybrids, backcrossed hybrids, etc (Falconer and Mackay 1997). Because it is not possible to determine such hybrid-zone composition from field-collected museum specimens, here we compared the mean values to a midparental value (PO) of the means of the pooled-sex mountain (G. b. beringei) and western lowland (G. g. gorilla) gorillas, and also considered these values relative to the mean values for each of these two taxa, simply to assess the relative position of these trait values (i.e., whether G. b. graueri is more G. g. gorilla-like or more G. b. beringei-like), and to make qualitative comparisons with these previous primate studies. For each variable, the statistical significance of deviation from a midpoint value is evaluated using a t-test, where the standard error of the expected value is half the square root of the sum of the sampling variances obtained from the G. g. gorilla and G. b. beringei samples. Finally, a principal components analysis (PCA) was performed on a total correlation matrix of log-transformed variables (without above size-correction), to quantify and visualize within- and between-group size and shape variation across the taxa, and especially the position of G. b. graueri relative to both G. g. gorilla and G. b. beringei. This PCA was based on the reduced dataset only to maximize sample size.

Results

NONMETRIC CRANIAL VARIATION

Of the scored dental and sutureal nonmetric trait variation, statistically significant differences exist among the four gorilla taxa only for the presence of supernumerary teeth ($\chi^2 = 7.75; P = 0.05$) and the presence of one or more additional sutures in the zygomatic region (henceforth referred to as a malar suture; $\chi^2 = 28.81; P < 0.001$; Table 1). The observed malar suture runs from the inferior border of the orbit to the maxillary foramen, and its incidence is higher than expected in the eastern lowland gorillas, relative to the other taxa (Table 1). The incidence of supernumerary teeth is higher than expected in both the G. b. graueri and G. g. diehli samples, and lower than expected in G. g. gorilla and G. b. beringei samples (Table 1). Statistically significant differences also exist among the taxa for frequency of one or more mandibular supernumerary teeth ($\chi^2 = 24.53; P < 0.001$)—an expression most consistent with hybridization (see below and Discussion); again, the incidence is higher than expected in eastern lowland gorillas relative to the other taxa. Although the supernumerary teeth vary in expression across individuals, the majority are permanent distomolars; other supernumerary teeth include incisors (n = 4) and one individual with a supernumerary permanent maxillary P4, lingual to the existing P4, and rotated 180 degrees (Appendix 1). There are also two individuals with small pieces of supernumerary root or enamel lodged in bone near the tooth row. The bulk of individuals with supernumerary teeth are male; this is consistent with what has been demonstrated in first-generation baboon hybrids (Ackermann et al. 2006), and suggests that the frequency of supernumerary teeth may differ by sex.

Regional differences in the frequencies of nonmetric trait variation are shown in Figure 1B,C. Pie charts indicate the frequencies of individuals with supernumerary teeth, with the information presented in Table 1 further broken down to indicate regional differences in the pattern of expression as follows:
individuals with one or more mandibular supernumerary molars, maxillary supernumerary molars only, and other supernumerary teeth. These charts also indicate the frequency of malar sutures, although it is important to note: (1) that the presence of sutures and supernumerary teeth are not mutually exclusive, and (2) frequencies were calculated relative to different sample sizes, as individuals without any visible sutures were not included for the suture calculations. Frequencies are calculated based on pooled samples of geographically proximate localities (see Table 1), with the exception of the Gabon (GAB) sample, which pools data from the western region of Gabon with samples further down the coast, in the Central African Republic and the southwestern-most regions of the Congo, due to small sample sizes. For further details on which localities were assigned to these groups, see Google Earth file. Because differences in individual expression (as well as higher population frequency) relative to “normal” primate trait variation are important for diagnosing hybridization, we also provide detailed descriptions of individual abnormalities and rank these individuals in terms of the likelihood that their pattern of expression indicates that this is a hybrid (Appendix 1). Ranking proceeds as follows: most unusual and consistent with hybrid morphology (hybrid status marked as∗∗∗, indicating bilateral mandibular and maxillary distomolars), somewhat less rare although still consistent (marked as∗∗, indicating the presence of one or two mandibular distomolars, but not all four distomolars), and more typical of “normal” nonhybrid primate variation (marked as∗). Other dental anomalies are also presented and discussed in detail (no asterisk).

To summarize these results for the supernumerary teeth, mountain gorillas (G. b. beringei) have no supernumerary teeth, whereas a high percentage (12%) of eastern lowland gorillas (G. b. graueri) have supernumerary teeth, the bulk of which are mandibular distomolars. One-third of these individuals display both bilateral mandibular and maxillary distomolars. All eastern lowland individuals with distomolars have morphological patterns consistent with hybridization. In contrast, the vast majority of supernumerary teeth in western gorillas are maxillary. The G. g. gorilla sample has a low frequency of supernumerary teeth (5%), most of which are maxillary (1% of individuals have one or more mandibular distomolars). In the G. g. diehli gorilla sample, although the frequency of supernumerary teeth is high (12%), these are all maxillary teeth; because this expression has been observed in a nonhybrid primate population (Ackermann et al. 2006), it is not considered a strong indicator of hybridization. However, it is important to note that 63% of the total sample, including three of the seven specimens with supernumerary teeth, had no mandibles, making it impossible to reject the hypothesis that we are sampling a contact zone (presumably, because this taxon lies at the most northwest limits of the gorilla range, this would reflect gene flow in from regions further south).

Two eastern lowland (G. b. graueri) specimens also exhibit an extremely rare sutural variant, os parietale divisum, i.e., division of the parietal bone: (1) MRCA 21,537, from the Tshiaberimu region, bilaterally (this individual also displays bilateral maxillary and mandibular fourth molars), (2) MRCA 34,476, from the Kahuzi region, on the right side only. These divisions of the parietal bone are complete, with the suture running anterior–posterior dividing the parietal fairly evenly, nearly parallel with the sagittal suture (Fig. 2).

**ESTIMATES OF GENE FLOW**

Maximum likelihood estimates of gene flow were used to complement the analyses of nonmetric variation, by providing relative estimates of migration. These estimates revealed highly asymmetrical patterns of past gene flow among gorilla taxa (Fig. 1). Estimates of migration rates are summarized as $M = ml/\mu$, where $m$ is the number of migrants exchanged per generation, scaled by $\mu$, the mutation rate; estimates are reported with approximate 90% likelihood credibility intervals, as calculated for $M$.

In western gorillas, MIGRATE recovered a range of levels of immigration among adjacent regions known to show distinct haplotype substructuring (Anthony et al. 2007b). Although overall values were all low, the highest values of $M$ were observed from populations in group V (Gabon/Congo) into populations in Equatorial Guinea (group VI; $M = 1.32, 0.27–2.9$) and group VII (Nigeria/Cameroon/Gabon; $M = 1.208, 0.37–2.17$). Evidence for lower levels of gene flow was observed from group VII into III (CAR/Cameroon/Congo; $M = 0.365, 0.12–1.72$) and from III into group IV (Cameroon/Gabon; $M = 0.402, 0.09–0.63$). Low levels of directional gene flow are also indicated between western and eastern gorilla species ($M = 0.137, 0.01–1.28$) and between eastern lowland and mountain gorillas ($M = 0.249, 0.03–1.81$). A striking aspect of these results is the generally unidirectional pattern of mitochondrial gene flow among western gorilla populations, and between western and eastern gorillas where gene flow occurs from the west to the east. Throughout the range of gorillas these patterns, together with relatively low levels of mitochondrial gene flow, likely reflect the reduced levels and shorter overall distances covered by dispersing female gorillas (Douadi et al 2007). Gene flow from western into eastern gorillas is consistent with the hybrid morphologies we report, although the observed discordance between molecular evidence of gene flow into the mountain gorillas and the lack of hybrid morphologies in this sample (as reported above) could result from a number of factors: (1) it may be an artifact of small sample sizes for these two gorilla taxa, (2) the two hybridizing parental lineages may not be sufficiently distinct genetically to present classic hybrid characters in offspring, or (3) the molecular signal of gene flow might be the result of ancestral polymorphisms retained within both lineages from a time before they diverged. MIGRATE does not provide
Figure 2. Two eastern lowland gorillas with os parietale divisum: MRCA 21,537 (Tshiaberimu region, Kivu N.E. by Lubero) viewed (A) laterally and (B) superiorly, and MRCA 34,476 (Kahuzi region, Kabare territory) viewed (C) laterally and (D) superiorly. Extra parietal sutures are indicated with arrows.

a temporal framework across which gene flow has occurred and cannot be used to distinguish between short divergence times with (very) low levels of gene flow (incomplete lineage sorting) and longer divergence times with moderate gene flow. Yet, given the time frame of the marker and together with the estimates from (Anthony et al. 2007b; Thalmann et al. 2007), the results do suggest that the pattern is more likely to represent past gene flow than retention of ancestral polymorphisms alone.

METRIC CRANIAL VARIATION

Because the nonmetric evidence for hybridization is strongest in the eastern lowland gorillas (G. b. graueri), we suggest that this taxon—or some portion of it—represents a hybrid zone. The genetic results indicate that the best explanation for such a phenomenon is the movement of western gorillas into the eastern lowland localities. Assuming that the parental populations of eastern lowland gorillas are comprised of (or closely resembled) mountain gorillas (G. b. beringei) and western lowland gorillas (G. g. gorilla; see Materials and Methods), it is interesting to consider the variation in eastern lowland gorillas relative to these taxa.

Significant differences exist between the model parental populations (G. g. gorilla and G. b. beringei) and the purported hybrid population (G. b. graueri) (MANOVA on both full and reduced datasets; P < 0.0001). Univariate analysis of variance also indicated significant differences (P < 0.05) between the three taxa for 78% of the traits, whereas we expect only 5% to be significant by chance. Pairwise t-tests were also made by comparing the means of the two-model parental taxa to each other and to the means of the putative hybrid population, and indicated that the mountain (G. b. beringei) and western lowland (G. g. gorilla) gorillas were significantly different from each other for 60% of the traits, whereas the eastern lowland (G. b. graueri) gorillas differ significantly from the mountain and western lowland gorillas for 43% and 73% of the traits, respectively (Table S2). Finally, univariate comparisons among traits indicate eastern lowland gorillas have 25 traits (63%) that deviate significantly from a midpoint value, with 17 traits (43%) that are above the midpoint value, with the bulk of these traits (11 traits) exceeding the mean values of both parental populations (Table S2). All eight traits (20%) that are below the midpoint value are lower than the mean values of both
RECENT HYBRIDIZATION BETWEEN GORILLA TAXA

Figure 3. Principal Components Analysis plots of the first five PCs for three gorilla taxa: G. g. gorilla (red circles); G. b. beringei (light green x’s); G. b. graueri (dark blue triangles – females are filled). 80% confidence ellipses are shown.

The results presented here provide evidence for past gene flow among evolutionarily distinct populations of gorillas. The strongest evidence for admixture comes from the high frequencies of rare craniodental variants (i.e., supernumerary teeth and sutural anomalies). These traits are comparable to that seen in first-generation baboon hybrids (Ackermann et al. 2006).
consistent with trait variation seen in other hybrid mammals (Heide-Jørgensen and Reeves, 1993; Goodwin, 1998; R. R. Ackermann and Brink, unpubl. data), and may indicate a breakdown in the coordination of early development associated with the mixing of evolutionarily divergent genotypes/phenotypes (Ackermann et al. 2006; Ackermann 2007). This is especially true as abnormal traits that appear in hybrid offspring but not in parental animals are generally considered to be the result of mixing two separately co-adapted gene complexes (Falconer and Mackay 1997; Vrana et al. 2000), with hybridization resulting in the breakdown of these complexes (Dobzhansky and Pavlovsky 1958; Templeton 1987).

The distribution of these gorilla hybrid craniofacial anomalies—both in terms of overall trait frequencies (Fig. 1) and the precise localities of individuals (Appendix 1; Supporting Information Google Earth file)—shows clear regional differences. Moreover, the pattern of expression of these anomalies, especially supernumerary teeth, differs among the four gorilla taxa. This is particularly important, because although supernumerary teeth do exist in humans and in other nonhuman primates with no documented history of recent hybridization, they occur at a very low frequency (<5%), and are overwhelmingly unilateral, anterior (usually incisors) and maxillary (Lavelle and Moore 1973; Rajab and Hamdan 2002; Hallgrímsson et al. 2005). Indeed, in humans a direct relationship has been shown between increasing rarity of nonmetric traits and frequency of unilateral expression (Ossenberg 1981; Hallgrímsson et al. 2005). In contrast, recent studies of baboon dental variation have shown that supernumerary teeth in hybrids are atypical relative to this primate pattern, occurring at a very high frequency (as much as 50% in F1 baboon males) and being remarkably different in their pattern of expression (i.e., most frequently bilateral, posterior [distomolars], and mandibular [Ackermann et al. 2006; Ackermann 2007]). In the east, mountain gorillas (G. b. berengei) have no supernumerary teeth, whereas an unusually high percentage of eastern lowland gorillas (G. b. graueri) have supernumerary teeth. Most importantly, every eastern lowland individual with distomolars has morphological patterning consistent with hybridization. This is in contrast to the pattern seen in the west. For the subspecies G. g. gorilla, the frequency of supernumerary teeth is low, providing no evidence that this taxon as a whole represents a hybrid zone. However, keeping in mind that unusual patterning (even arguably on a single individual) can be as important as frequency, it is interesting to note that the three individuals that do have hybrid-like supernumerary teeth (Appendix 1; Supporting Information Google Earth file) are present at locations predicted by Pleistocene refugia and riverine barrier hypotheses (Anthony et al. 2007b): (1) near major waterways that separate known haplogroups, (2) in a region of southern Cameroon with signs of haplotype admixture, and (3) at the boundaries of a geographically widespread haplotype

group (D3 in Anthony et al. 2007b) shown to exhibit signatures of a population bottleneck and subsequent expansion. In the G. g. diehli sample, the expression of dental anomalies is like that seen in primates that have not undergone recent hybridization, and therefore is not consistent with a scenario of admixture, although the lack of mandibles for many specimens necessarily renders this conclusion tentative.

The incidence of unusual zygomaxillary (malar) sutures has also been shown to occur at an increased frequency in hybrid baboons relative to parental populations (Ackermann et al. 2006), and the high frequency of them here (albeit a different suture) also points to a high level of hybridization in eastern lowland gorillas (G. b. graueri; Table 1; Fig. 1). Moreover, the presence of an extremely rare sutural variant, os parietale divisum, in two eastern lowland gorillas—one of which expresses the trait bilaterally—also supports this scenario. Os parietale divisum is present in humans and other primates at an incidence well below 1%; few cases (overwhelmingly unilateral) have been described in the anatomical literature (Turner 1890; Shapiro 1972; Hrdlicka 1903; Berry 1910; Fenton et al. 2000; Bessell-Browne and Thonell 2004; Becker et al. 2005). Complete divisions in apes have been reported once in the literature, in two orangutans and a chimpanzee—these were both unilateral (Hrdlicka 1903).

Although this condition is considered to have no functional consequences (Hauser and De Stefano 1989), it has recently been shown that it can be associated with other congenital anomalies (Becker et al. 2005). As such, it may result from, or at least be correlated with, wider developmental abnormalities in these two specimens.

The molecular evidence provides an interesting complement to the nonmetric data, suggesting that low levels of gene flow have occurred primarily in a west-to-east direction, supporting both a scenario of admixture along the borders between refugial populations in the west, as well as past dispersal across more fragmented habitats. Although both sexes in gorillas emigrate from their natal groups, males are known to disperse longer distances than females (Douadi et al. 2007); estimates of gene flow based on mitochondrial sequences alone are therefore likely to underestimate the degree to which gorilla populations are linked via the past immigration of individuals. Unfortunately, microsatellite, autosomal non-coding, or Y chromosome data are not currently available from as broad a range of gorilla samples as mitochondrial sequence data. Notwithstanding the limitations of using mitochondrial markers, the analyses do indicate that low levels of mitochondrial gene flow have occurred between western and eastern gorillas, suggesting that these species must have been connected via dispersal events in the past. Our findings are complementary to those of Thalmann et al. (2007) in which nuclear loci support a scenario of gene flow between eastern and western lowland gorillas. Within eastern lowland gorillas, the substantial morphological signatures of
hybridization in this taxon (discussed above; Table 1 and Fig. 1), combined with molecular evidence for migration of individuals from further west (Fig. 1), strongly suggest that this region in particular—or some portion of it—represents a zone of introgression. The results of the analyses of metric cranial variables provide further support for this scenario, with aspects of G. b. graueri morphology shared with both G. b. beringei and G. g. gorilla, while some G. b. graueri individuals display morphological attributes outside the range of either of these taxa. Because the specimens were collected during the past two centuries, it is possible that the hybrid morphologies expressed in these individuals represent recent—e.g., historical—signatures of hybridization. Under such a scenario, substantial genetic exchange would have occurred between the western and eastern gorillas until about 80,000 years ago, the species then became allopatric, and subsequently gene flow resumed in the recent past. This scenario is in stark contrast with previous molecular studies that estimate termination of gene flow between the two gorilla species in the distant past, circa 80,000 years ago (Thalmann et al. 2007). However, there is a temporal conundrum here; although cranial material with evidence for hybridization was collected as recently as 50–100 years ago, it is not possible to tell from this evidence precisely when hybridization resumed, or indeed whether gene flow ever terminated completely between the species. Moreover, it is not known how long hybrid traits persist into future generations in the absence of hybridization, although recent work on sunflowers suggests that recombination may continue for hundreds of generations before the hybrid genome becomes stabilized (Buerckle and Rieseberg 2008). Given that the separation of these taxa was likely to have been marked by low levels of gene exchange across a considerable time period (especially given the long generation time of gorillas), it is highly likely that we are sampling multiple-generation hybrids and recombinants. Regardless of which of these (untestable) scenarios is true, the nature of the cranial metric variation in eastern lowland gorillas, together with the high frequency of unusual nonmetric trait variation in this taxon, is consistent with a scenario of hybridization between adaptively divergent parental populations similar to (if not exactly like) existing mountain and western lowland gorillas. This further supports the conclusion that these hybrids likely result from crosses between local eastern (G. b. beringei-like) gorillas and western (G. g. gorilla-like) gorilla immigrants. Of course, it is recognized that the parental groups might be represented by populations that no longer exist, having succumbed to recent (anthropogenic) extinction, and would have occupied regions geographically more proximate (both to the east and west) to the eastern lowland localities represented here. In this context, it is interesting to note that museum specimens from Bondo, a locality intermediate between the current taxa and that has often been used as evidence for a historic corridor between these taxa (e.g., Sarmiento 2003), have been shown to be genetically indistinguishable from western gorillas (Hofreiter et al. 2003).

Interestingly, the strong morphological evidence for hybridization in eastern lowland localities provides some explanation for the longstanding discordance of taxonomic interpretations based on molecular versus morphological data. In contrast to molecular evidence, traditional morphology-based interpretations of gorilla taxonomy place all gorillas in a single species, comprised of two (i.e., G. g. gorilla, G. g. beringei) (Coolidge 1929) or more recently three (i.e., G. g. gorilla, G. g. beringei, G. g. graueri) subspecies. Support for this has come in part from studies showing that aspects of eastern lowland morphology are “intermediate” between mountain gorillas and western lowland populations, including ones focusing on cranial discrete trait analysis (Leigh et al. 2003), craniometrics (Groves 1970), and jaw morphology (Taylor 2003). Specific populations have also proven enigmatic. For example, Groves and Stott (1979) showed that eastern lowland gorillas from Tshiaberimu have some skull traits that are similar to mountain gorillas, whereas hair and other aspects of external morphology are more lowland-like. Kahuzi populations, on the other hand, share similarities with mountain gorillas in terms of their postcranial skeleton (Groves and Stott 1979). “Among eastern gorillas, the Virunga skulls stand out from the other populations more than any of the western groups differ from one another. The other three eastern groups do not differ very much from each other, but show varying degrees of resemblance to the western or the Virunga gorillas” (Groves 1967; italics ours).

Again, this increased variation—where individuals can resemble one parental population or the other, or can display morphological attributes unlike that seen in either group—is precisely what we expect in primate hybrid populations (Ackermann et al. 2006).

Although this study set out specifically to test for the presence of traits known to be associated with primate hybrids, it is important to consider the possibility that the observed craniofacial anomalies arise from other factors, especially inbreeding. This is plausible because eastern lowland gorillas occupy fragmented habitats and are represented by a fairly low population size of approximately 17,000 individuals (Plumptre et al. 2003). Studies of captive-bred primates have attributed a wide range of skeletal malformations to inbreeding, including anencephaly (Rawlins and Kessler 1983), polydactyly and syndactyly (Chalifoux and Elliot 1986), and limb malformations (Nakamichi et al. 1997). However, most of these studies have not quantified inbreeding depression. Studies of wild and captive primates that have quantified inbreeding depression rely primarily on common measures of fitness (e.g., survival, birth weight, reproductive success) rather than morphology (see Charpentier et al. 2007 and references therein). In all of this work, there is no evidence that inbred matings result in the craniofacial anomalies discussed herein. Moreover, mountain gorillas (G. b. beringei)—which have been studied extensively
for many decades (Schaller 1963; Fossey 1983), occupy an extremely limited habitat, and are represented by very few individuals (~650–700; Plumptre et al. 2003)—are the gorilla subspecies with the best evidence for inbreeding (Fossey 1983), containing individuals with misaligned eyes (strabismus) and syndactyly, and yet as shown here they have no dental or suture anomalies.

From a broader perspective, two aspects of this study are worth emphasizing. First, it highlights the complex nature of evolutionary divergence—and especially the important role of hybridization in structuring biodiversity—in equatorial Africa over the Pliocene. Pleistocene range retraction followed by climate amelioration has undoubtedly resulted in secondary contact among many plant and animal lineages, and the study of genetic and morphological divergence and/or introgression across these zones of contact is central to our understanding of speciation and the evolution of biodiversity. Increased aridity associated with Plio-Pleistocene cooling phases in Africa has been implicated in the evolution of a number of animals, including bovids (Vrba 1995) and hominins (deMenocal and Bloemendal 1995), and is also consistent with explanations for the distributions of other African equatorial primates that have diverged fairly recently (Hamilton 1988; Oates 1988; Colyn et al. 1991; Telfer et al. 2003; Eriksson et al. 2004), yet the role of ongoing or subsequent gene flow in all of these taxa has been underexplored.

For primates in particular, hybridization is common among many extant lineages, and yet the link between this evolutionary process and biodiversity remains poorly understood. This is complicated by the fact that many extant primate populations have been drastically reduced by habitat destruction and the bush meat trade, and current distributions are often relict relative to even the fairly recent past (Cowlishaw and Dunbar 2000). Clearly, more studies aimed at understanding the complex nature of divergence in primates are needed, and our interpretation of extant morphological and genetic similarity needs to be informed by such knowledge. Second, this study demonstrates that coordinated, multi-faceted analyses of morphological and molecular data, interpreted in a geographic context, can provide greater insight into the evolutionary outcomes of secondary contact via hybridization than either alone. Yet despite strong argument for an integrated approach in evolutionary biology, studies that combine morphological and molecular data, interpreted in a phylogenetic context, are still rare. It is not always possible to analyze both types of data from the same specimens, nor is it necessarily beneficial; as shown here, museum collections can provide large samples of historical data drawn from regions no longer occupied—data that are simply not available in the modern context. Such integrated studies would be extremely valuable for understanding the evolutionary underpinnings of contemporary biodiversity—and for allowing a more nuanced interpretation of fossil diversity—across myriad organisms, including our hominin ancestors.

Finally, while the evidence presented here points to complex histories of genetic exchange across all gorillas, from a conservation perspective it highlights the exceptional—and exceptionally complicated—evolutionary history of the eastern lowland gorillas. Unfortunately, given sample size constraints, it is not possible to determine here whether significant molecular and morphological differences exist among the eastern lowland populations themselves; this is an ideal question for finer scale biogeographic studies. It would also be extremely interesting to know the genetic make up of the museum specimens that show hybrid morphologies. These and other similar studies could further help us to understand the composition and evolutionary history of this group, and especially whether eastern lowland gorillas as a whole originated as a hybrid taxon. Regardless, the results of this study provide one more piece of evidence in support of the conservation of the unique, increasingly endangered gorillas of the eastern Congo.

ACKNOWLEDGMENTS

Many thanks to J. Cheverud, C. Groves, and A. Taylor for providing helpful comments on earlier drafts of this manuscript; to N. Anthony for generously assisting us with the sequence dataset; and to C. Groves and R. Stumpf for providing the cranial metric data and descriptions. We also thank the three reviewers and Associate Editor, G. Marroig, whose comments were extremely helpful for revising this manuscript. Finally, special thanks to the museum curators and staff who provided access to skeletal materials in their care and helped in numerous other ways: E. Westwig (AMNH), P. Jenkins (BMNH), J. Haile-Selassie and L. Jellema (CMNH), H. Turni (MFNB), W. van Neer and E. Gilissen (MRAC), R. Thorington and L. Gordon (NMNH), M. Harman (PWCT), and S. Chaplin (RCS).

LITERATURE CITED


R. R. ACKERMANN AND J. M. BISHOP


Associate Editor: G. Marroig
**Appendix 1.** Details of individual gorillas with supernumerary teeth, including museum catalogue and locality information. Presence of right and left maxillary (R MAX; L MAX), right and left mandibular (R MAND; L MAND), and other supernumerary teeth are indicated with an X. Hybrid status refers to patterning strongly showing hybridization (****), consistent with hybridization (**), consistent with “normal” maxillary (*) and other nondiagnostic (no asterisk) trait variation. Museum abbreviations are as indicated in Materials and Methods. Country abbreviations: CAM, Cameroon; GAB, Gabon; NIG, Nigeria; CNG, Republic of Congo; CAR, Central African Republic; DRC, Democratic Republic of Congo. For Region abbreviations see Table 1.

<table>
<thead>
<tr>
<th>Museum ID</th>
<th>Hybrid status</th>
<th>Subspecies</th>
<th>Country</th>
<th>Region</th>
<th>Locality</th>
<th>Mandible present?</th>
<th>R MAX</th>
<th>L MAX</th>
<th>R MAND</th>
<th>L MAND</th>
<th>OTHER</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>MFNB 83,539</td>
<td>DIEHLI</td>
<td>CAM</td>
<td>CRV</td>
<td>Ossidinge</td>
<td>N</td>
<td>X</td>
<td>Right maxillary incisor.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MFNB 83,541</td>
<td>DIEHLI</td>
<td>CAM</td>
<td>CRV</td>
<td>Ossidinge</td>
<td>Y</td>
<td>X</td>
<td>Empty socket suggests tooth is very small, probably peg-like.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MFNB 83,553</td>
<td>DIEHLI</td>
<td>CAM</td>
<td>CRV</td>
<td>Basho</td>
<td>Y</td>
<td>X</td>
<td>Teeth missing, but with normal sized sockets.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MFNB 12,790</td>
<td>DIEHLI</td>
<td>CAM</td>
<td>CRV</td>
<td>Forest near Obonyi</td>
<td>BROKEN</td>
<td>X</td>
<td>Normal-sized. Do not know if there was a right one (maxilla broken).</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MFNB 83,522</td>
<td>DIEHLI</td>
<td>CAM</td>
<td>CRV</td>
<td>Ossidinge</td>
<td>N</td>
<td>X</td>
<td>Small, peg-like.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MFNB 83,544</td>
<td>DIEHLI</td>
<td>CAM</td>
<td>CRV</td>
<td>Ossidinge</td>
<td>N</td>
<td>X</td>
<td>Both with some occlusal morphology.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMNH 35.3.19.1</td>
<td>DIEHLI</td>
<td>NIG</td>
<td>CRV</td>
<td>Obudu, Ogoja Provence</td>
<td>BROKEN</td>
<td>X</td>
<td>Teeth missing. Based on sockets, the left is probably normal-sized, while the right is smaller.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMNH 1939.938</td>
<td>GORILLA</td>
<td>GAB</td>
<td>GAB</td>
<td>Ogowe, headwaters of Nguni River</td>
<td>Y</td>
<td>X</td>
<td>Right maxillary first incisor. May be a remaining deciduous tooth.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NMNH 174,712</td>
<td>GORILLA</td>
<td>GAB</td>
<td>GAB</td>
<td>Lake Feman Vaz</td>
<td>Y</td>
<td>X</td>
<td>Left tooth is erupting, right is missing (socket only).</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CMNH HTB 1907</td>
<td>GORILLA</td>
<td>CAM</td>
<td>PLT</td>
<td>Abong Mbang</td>
<td>Y</td>
<td>X</td>
<td>All four teeth completely in crypt, so evaluating size is difficult. Appear to have at least some occlusal morphology.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CMNH HTB 1992</td>
<td>GORILLA</td>
<td>CAM</td>
<td>PLT</td>
<td>Abong Mbang</td>
<td>Y</td>
<td>X</td>
<td>Left central maxillary incisor. May be a remaining deciduous tooth.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CMNH HTB 3426</td>
<td>GORILLA</td>
<td>CAM</td>
<td>PLT</td>
<td>Djaposten, Abong Mbang</td>
<td>Y</td>
<td>X</td>
<td>Full-sized.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Continued.*
### Appendix 1. Continued.

<table>
<thead>
<tr>
<th>Museum ID</th>
<th>Hybrid status</th>
<th>Subspecies</th>
<th>Country</th>
<th>Region</th>
<th>Locality</th>
<th>Mandible present?</th>
<th>R MAX</th>
<th>L MAX</th>
<th>R MAND</th>
<th>L MAND</th>
<th>OTHER</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>PWCT M798</td>
<td>*</td>
<td>GORILLA</td>
<td>CAM</td>
<td>PLT</td>
<td>Batouri district</td>
<td>Y</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td>Difficult to assess. The 3rd right maxillary molar is slightly small, and there is a big (3rd molar-looking) tooth coming up underneath it.</td>
<td></td>
</tr>
<tr>
<td>PWCT M877</td>
<td>*</td>
<td>GORILLA</td>
<td>CAM</td>
<td>PLT</td>
<td>Batouri district</td>
<td>Y</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td>Some occlusal morphology, but teeth are quite small (still in crypt) and impacted on M3 roots (e.g., would never have erupted).</td>
<td></td>
</tr>
<tr>
<td>PWCT M490</td>
<td>*</td>
<td>GORILLA</td>
<td>CAM</td>
<td>PLT</td>
<td>Obala, Batouri district</td>
<td>Y</td>
<td></td>
<td>X</td>
<td></td>
<td></td>
<td>Tiny nub of a tooth not in the tooth row, buccal and slightly posterior to M3.</td>
<td></td>
</tr>
<tr>
<td>MRCA 73018M10</td>
<td>*</td>
<td>GORILLA</td>
<td>CAM</td>
<td>PLT</td>
<td>Messea region</td>
<td>Y</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td>Normally shaped but slightly small.</td>
<td></td>
</tr>
<tr>
<td>MFNB 83,530</td>
<td>*</td>
<td>GORILLA</td>
<td>CAM</td>
<td>PLT</td>
<td>Dume</td>
<td>BROKEN</td>
<td></td>
<td>X</td>
<td></td>
<td></td>
<td>A supernumerary root, on the mandible near the right 3rd premolar. Appears to be a touch of enamel at the top of the root.</td>
<td></td>
</tr>
<tr>
<td>MFNB 83,543</td>
<td>*</td>
<td>GORILLA</td>
<td>CAM</td>
<td>PLT</td>
<td>Lomie or Dume-Mundung</td>
<td>Y</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td>Teeth missing, but double root sockets indicate they are likely full sized.</td>
<td></td>
</tr>
<tr>
<td>CMNH HTB1919</td>
<td>*</td>
<td>GORILLA</td>
<td>CAM</td>
<td>PLT</td>
<td>Abong Mbang</td>
<td>BROKEN</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td>Full-sized.</td>
<td></td>
</tr>
<tr>
<td>CMNH HTB1947</td>
<td>*</td>
<td>GORILLA</td>
<td>CAM</td>
<td>PLT</td>
<td>Abong Mbang</td>
<td>Y</td>
<td></td>
<td>X</td>
<td></td>
<td></td>
<td>Tooth missing, but small socket suggests peg-like morphology.</td>
<td></td>
</tr>
<tr>
<td>CMNH HTB1966</td>
<td>**</td>
<td>GORILLA</td>
<td>CAM</td>
<td>PLT</td>
<td>Abong Mbang</td>
<td>BROKEN</td>
<td></td>
<td>X</td>
<td></td>
<td></td>
<td>In crypt. Full-sized.</td>
<td></td>
</tr>
<tr>
<td>CMNH HTB1979</td>
<td>*</td>
<td>GORILLA</td>
<td>CAM</td>
<td>PLT</td>
<td>Abong Mbang</td>
<td>BROKEN</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td>Small, but with some occlusal morphology.</td>
<td></td>
</tr>
<tr>
<td>CMNH HTB2826</td>
<td>*</td>
<td>GORILLA</td>
<td>CAM</td>
<td>PLT</td>
<td>Abong Mbang</td>
<td>Y</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td>Less than full-sized but with occlusal morphology.</td>
<td></td>
</tr>
<tr>
<td>CMNH HTB1746</td>
<td>*</td>
<td>GORILLA</td>
<td>CAM</td>
<td>PLT</td>
<td>Ebolowa</td>
<td>Y</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td>Tooth missing, normal-sized socket.</td>
<td></td>
</tr>
<tr>
<td>PWCT M766</td>
<td>*</td>
<td>GORILLA</td>
<td>CAM</td>
<td>PLT</td>
<td>Batouri district</td>
<td>Y</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td>Tiny nubbins.</td>
<td></td>
</tr>
<tr>
<td>PWCT M854</td>
<td>**</td>
<td>GORILLA</td>
<td>CAM</td>
<td>PLT</td>
<td>Batouri district</td>
<td>Y</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
<td>Maxillary teeth are full-sized, but mandibular tooth is a small nubbin.</td>
<td></td>
</tr>
<tr>
<td>Museum ID</td>
<td>Hybrid status</td>
<td>Subspecies</td>
<td>Country</td>
<td>Region</td>
<td>Locality</td>
<td>Mandible present?</td>
<td>R MAX</td>
<td>L MAX</td>
<td>R MAND</td>
<td>L MAND</td>
<td>OTHER</td>
<td>Description</td>
</tr>
<tr>
<td>-----------</td>
<td>---------------</td>
<td>------------</td>
<td>---------</td>
<td>--------</td>
<td>----------</td>
<td>------------------</td>
<td>------</td>
<td>------</td>
<td>--------</td>
<td>--------</td>
<td>-------</td>
<td>-------------</td>
</tr>
<tr>
<td>AMNH 214,111</td>
<td>GORILLA</td>
<td>CNG</td>
<td>RIV</td>
<td>Oka, west of Okio</td>
<td>Y</td>
<td>X</td>
<td>Maxillary: normal shaped, slightly smaller than M3s. Mandibular: right 3/4-sized; left residual but with some occlusal morphology.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMNH 49,664</td>
<td>GORILLA</td>
<td>CAR</td>
<td>SNG</td>
<td>Ziendi, Oubangui</td>
<td>Y</td>
<td>X</td>
<td>Small, peg-like nubbin.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AMNH CA502</td>
<td>GORILLA</td>
<td>CNG</td>
<td>SNG</td>
<td>Ouesso region</td>
<td>Y</td>
<td>X</td>
<td>All full-sized 4th molars. Max normal shaped, slightly smaller than m3. Mand full on left, right tooth lost – socket only. Mandible very crowded.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MRCA RG999</td>
<td>GRAUERI DRC</td>
<td>FIZ</td>
<td>Baraka, Forest Sibatwa</td>
<td>Y</td>
<td>X</td>
<td>All normal shaped, but slightly smaller than M3s.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MRCA 22,924</td>
<td>GRAUERI DRC</td>
<td>FIZ</td>
<td>Mavabi, terr Mwenga, Kivu</td>
<td>Y</td>
<td>X</td>
<td>Left maxillary is missing, but rest smaller than M3s, and retain occlusal morphology. Right maxillary tooth turned medially.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MRCA 14,769</td>
<td>GRAUERI DRC</td>
<td>UTU</td>
<td>Shabunda region</td>
<td>Y</td>
<td></td>
<td>Very unusual and difficult to diagnose. There are at least two pieces of enamel and some separate root in the palate. Occlusal fragment of enamel and the root are medial to P4, and the other enamel fragment is medial to P3/Canine.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MRCA 18,191</td>
<td>GRAUERI DRC</td>
<td>UTU</td>
<td>Chefferie Wazimu, terr Shabunda, Kivu</td>
<td>Y</td>
<td>X</td>
<td>Teeth missing, but small sockets indicate that both are residual.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Continued.
### Appendix 1. Continued.

<table>
<thead>
<tr>
<th>Museum ID</th>
<th>Hybrid status</th>
<th>Subspecies</th>
<th>Country</th>
<th>Region</th>
<th>Locality</th>
<th>Mandible present?</th>
<th>R MAX</th>
<th>L MAX</th>
<th>R MAND</th>
<th>L MAND</th>
<th>OTHER</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>MRCA 86044M15</td>
<td>***</td>
<td>GRAUERI</td>
<td>DRC</td>
<td>UTU</td>
<td>Kasese</td>
<td>Y</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
<td>All teeth in crypt. Left maxillary M4 is not clearly visible. Rest look full-sized, but it is difficult to be certain.</td>
</tr>
<tr>
<td>MRCA 86044M16</td>
<td>**</td>
<td>GRAUERI</td>
<td>DRC</td>
<td>UTU</td>
<td>Kasese</td>
<td>Y</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td>Normal-sized.</td>
</tr>
<tr>
<td>MRCA 15,352</td>
<td></td>
<td>GRAUERI</td>
<td>TSH</td>
<td>Ibatsero</td>
<td></td>
<td></td>
<td>Y</td>
<td></td>
<td>X</td>
<td></td>
<td></td>
<td>Right lateral mandibular incisor.</td>
</tr>
<tr>
<td>MRCA 21,537</td>
<td>***</td>
<td>GRAUERI</td>
<td>TSH</td>
<td>Kivu N.E. by Lubero</td>
<td>Y</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>In crypt. Small, but with occlusal morphology.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MRCA 17,161</td>
<td>**</td>
<td>GRAUERI</td>
<td>TSH</td>
<td>Volcans</td>
<td></td>
<td>Y</td>
<td></td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td>Tooth missing, but single-root crypt loos residual.</td>
</tr>
</tbody>
</table>

1Museum records/labels allocate these to the taxon G. g. rex-pygmaeorum, currently recognized as a synonym of G. b. graueri, as it has been shown to be a regional variant from Tshiaberimu (Groves, 1979).

2Although “Volcans” is an unspecific locality, this specimen was placed in Tshiaberimu based on its identification as G. g. rex-pygmaeorum (see above footnote). It is, however, possible that this specimen is misidentified, and actually belongs with G. b. beringei.
Supporting Information

The following supporting information is available for this article:

**Table S1.** Descriptions of Gorilla skull measurements.

**Table S2.** Sample sizes (N), Means*, Standard deviations (SD), and Univariate probabilities of intergroup differences in cranial measurements among all three groups (P) and among pairs of taxa.

**Table S3.** Component loadings for PCA of logged data for three gorilla subspecies (G. g. gorilla, G. b. berengei, G. b. graueri).

**Google Earth file.** Specimen localities, with coordinate data reconstructed as best possible from museum records. Each point represents a locality, and colors indicate regionally pooled samples (per Table 1). Abbreviations for regions indicated in Table 1. Note that a single locality may have more than one individual—in fact, this is typically the case. Localities that contain one or more individuals with supernumerary teeth are marked with an X. This file must be opened with the program Google Earth (available for free download at http://earth.google.com.

Supporting Information may be found in the online version of this article.
(This link will take you to the article abstract).

Please note: Wiley-Blackwell is not responsible for the content or functionality of any supporting information supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.