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RESEARCH ARTICLE

Genetic Variation in Gorillas

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This review summarizes what is currently known concerning genetic variation in gorillas, on both inter- and intraspecific levels. Compared to the human species, gorillas, along with the other great apes, possess greater genetic varation as a consequence of a demographic history of rather constant population size. Data and hence conclusions from analysis of mitochondrial DNA (mtDNA), the usual means of describing intraspecific patterns of genetic diversity, are limited at this time. An important task for future studies is to determine the degree of confidence with which gorilla mtDNA can be analyzed, in view of the risk that one will inadvertently analyze artifactual rather than genuine sequences. The limited information available from sequences of nuclear genomic segments does not distinguish western from eastern gorillas, and, in comparison with results from the two chimpanzee species, suggests a relatively recent common ancestry for all gorillas. In the near future, the greatest insights are likely to come from studies aimed at genetic characterization of all individual members of social groups. Such studies, addressing topics such as behavior of individuals with kin and non-kin, and the actual success of male reproductive strategies, will provide a link between behavioral and genetic studies of gorillas. Am. J. Primatol. 64:161–172, 2004.

Key words: phylogeography; mtDNA; noninvasive samples; numt; genotype

INTRODUCTION

Genetic Variation in Wild Animal Populations

Studies of genetic variation within a wild animal taxon commonly address two topics: an estimation of the amount of variation present in both individuals and populations, and a description of how that variation is geographically distributed [Avise, 2000]. Such analyses are necessary for deciphering long-term

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patterns in the interactions of populations of a species, which is an essential element in gaining a comprehensive understanding of the biology of a species. It is generally accepted that the level of genetic variation in wild animal populations is related to population size; however, supporting empirical evidence is surprisingly scarce [Frankham, 1996]. Conservation management recommendations currently emphasize the importance of maintaining genetic variation within populations [Frankham, 1999]. Species that have experienced profound and/or prolonged constrictions in population size are expected to exhibit reduced genetic variation. However, a species may possess relatively low variation despite a lack of evidence of population decline [Amos & Balmford, 2001]. Thus, rather than the amount of genetic variation in a population, a more relevant factor may be the relative change in variation and hence presumed adaptive potential over time in that population.

The contemporary geographical distribution of genealogical lineages, along with the inference of the historical processes that have led to such distributions, is the focus of the discipline termed phylogeography [Avise, 2000]. A comparison of results from scores of studies conducted on both vertebrate and invertebrate animals suggests that the structure of gene genealogies can be interpreted with reference to the behavior and ecology of the species under consideration. For example, small nondispersing animals typically exhibit greater levels of intraspecific genealogical structure than do large, highly mobile animals. Furthermore, instances in which gene genealogies do not correspond to distinctions made on the basis of morphology or behavior are particularly interesting. Examples include situations in which parallel changes in morphology apparently occurred in different genetic lineages in response to local ecological conditions [e.g., Janzen et al., 2002; Richmond & Reeder, 2002]. Interestingly, cases can also be found in which genetic divergence is not accompanied by recognizable morphological differentiation, which suggests the existence of cryptic species. However, some of the most well-studied cases of cryptic species in vertebrates include instances in which the taxonomic divisions inferred from genetic studies were consistent with vocalization differences (e.g., in bats [Barratt et al., 1997; Kingston et al., 2001] and frogs [Dawood et al., 2002]). A standardized scheme for integrating phylogenetic and taxonomic information has not yet been adopted [Avise & Johns, 1999], although increasing attention has been drawn to the potential use of DNA sequence variation as the foundation for an integrated taxonomic reference system [Hebert et al., 2003; Tautz et al., 2003].

Gorillas are a particularly interesting taxon for consideration of the factors that affect the level and pattern of genetic variation in endangered wild animals. The range of gorillas in equatorial Africa is much more limited compared to that of chimpanzees [Yamagiwa, 1999]. Furthermore, the distribution of gorillas exhibits a striking discontinuity between the much more numerous western gorilla (*Gorilla gorilla*) and the perhaps 12,000 remaining eastern gorillas (*G. beringei*) (see Fig. 1 in Doran and McNeilage [1998]). On the basis of habitat and morphology, a further distinction is typically made between eastern lowland gorillas (*G. b. graueri*) and eastern mountain gorillas (*G. b. beringei*). Although much information concerning gorilla behavior and social systems is known from direct observation [Robbins et al., 2001; Taylor & Goldsmith, 2002] (papers in this issue), genetic analysis is essential for determining genetic relationships among individuals so that questions such as the relative success of different male reproductive strategies may be addressed.

In comparison with studies of humans and chimpanzees, there have been relatively few attempts to investigate genetic variation in wild populations of

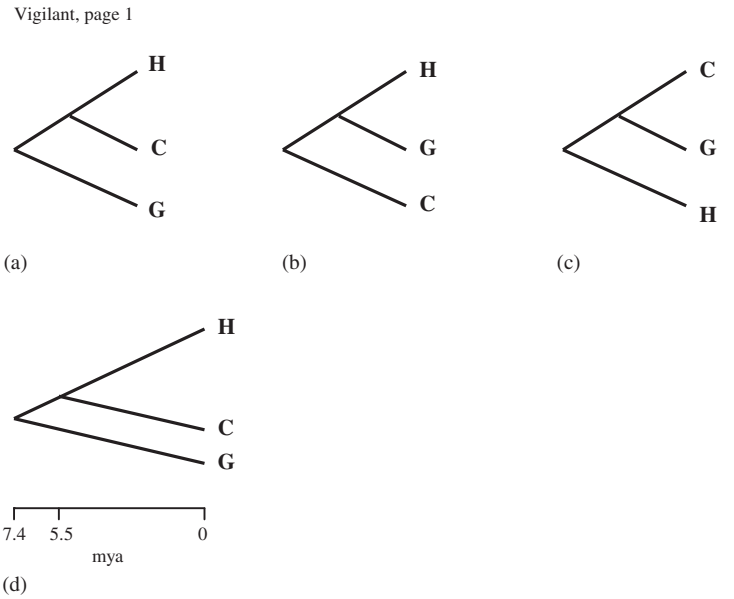

Fig. 1. Schematic tree diagrams illustrating the relationships among humans (H), chimpanzees (C), and gorillas (G). The majority of the DNA segments analyzed support the topology in **a**, but the topologies in **b** and **c** are consistent with results from other segments. The diagram in **d** shows the short time interval between the divergence of the lineage leading to gorillas at about 7.4 million years ago (mya), and the divergence of the lineages leading to chimpanzees and humans around 5.5 mya.

gorillas. Nonetheless, comparisons among these three closely related taxa can potentially illuminate the demographic, social, or ecological factors that contribute to the differences in the characteristics of genetic variation observed. A prerequisite is a description of the molecular phylogeny of these three African apes.

Gene Trees Are Not Species Trees

One of the first questions concerning the evolution of the great apes that was addressed by molecular means involved the evolutionary relationships among humans, chimpanzees, and gorillas [Goodman et al., 1990; Sibley et al., 1990]. After years of debate this question appears to be resolved: multiple independent studies have strongly supported a *Homo-Pan* clade, with an earlier divergence of *Gorilla* [Chen & Li, 2001; Ruvolo, 1997; Satta et al., 2000]. Interestingly, not all DNA segments examined produced gene trees consistent with this species tree, due to the fact that these three species apparently diverged over a short period of time from a large common ancestral population. In an analysis of more than 24,000 base pairs (bp) of sequence from 53 autosomal, intergenic, nonrepetitive DNA segments from humans, chimpanzees, and gorillas, only 31 segments were found to individually support the *Homo-Pan* clade [Chen & Li, 2001]. A further 10 segments supported the *Homo-Gorilla* clade, while 12 supported the *Pan-Gorilla* clade (Fig. 1). Incongruities such as these between gene trees and species trees are most likely to arise when the time span between two speciation events is short, and the effective size of the population in that time span is large [Nei, 1987]. In other words, the time of divergence of polymorphic genes present in the ancestral population necessarily precedes that of the species divergence, potentially giving

rise to discordances between gene and species trees. This phenomenon (also termed ''differential lineage sorting'') is likely also responsible for the current difficulties researchers are encountering in establishing a consensus molecular phylogeny of New World monkey families [Disotell et al., 2003] and colobine monkey genera [Sterner et al., 2003].

The next question of interest concerning the relationships among gorillas, chimpanzees, and humans is the divergence times of the ancestral populations. After they demonstrated that the assumption of constant rates of evolution (the ''molecular clock'') was valid in hominoids, Chen and Li [2001] calculated the times of divergence of gorillas and *Homo-Pan*. Assuming a speciation time for orangutans of 12–16 million years ago [Goodman et al., 1998], the time of divergence of the lineage leading to gorillas would be 6.3–8.5 million years ago, and the human–chimpanzee divergence would be 4.8–6.4 million years ago. Thus, only about 1.5–2.1 million years separate the gorilla speciation and the human–chimpanzee common ancestor [Chen & Li, 2001] (Fig. 1).

Genetic Variation in Gorillas: Nuclear DNA

While the work mentioned above focused on interspecific phylogenetic relationships, few investigations have examined the relative amount of genetic variation found *within* the nuclear genomes of gorilla and other nonhuman great ape species. One practical obstacle is that many analytical approaches require the use of DNA of a quality that can only be obtained from blood or tissue samples, which limits the study to samples from captive animals. In one such study, Kaessmann et al. [2001] examined variation in great apes at an approximately 10,000 bp noncoding locus on the X chromosome, and found reduced variation in humans relative to apes. When calculated in a way that takes into account varied sample sizes, the diversity found in gorillas is about twice that in humans, while chimpanzees and orangutans each possess more than three times the variation in humans. Phylogenetic analysis does not sort the sequences from gorillas, chimpanzees, or orangutans by subspecies. This lack of sorting suggests that it is not population structure in the ape species that produces the higher diversity values, but rather that humans are less diverse because the population history of humans is fundamentally different from that of the African apes. In fact, the data from gorillas and other great apes are consistent with a constant long-term population size, while only the human species shows a strong signal of expansion in population size. A similar finding of relatively low diversity in humans relative to chimpanzees and gorillas was obtained in a study nearly 4,000 bp of variation at five autosomal loci were examined [Jensen-Seaman et al., 2001b]. This study also inferred a lower long-term effective size for gorillas as compared to chimpanzees, but it is unclear to what extent the use of ape samples from individuals of diverse, unknown geographic origins may have biased these results. For one of the loci (DRD4), the authors obtained sufficient data to examine differentiation within *Pan* (chimpanzees and bonobos) and *Gorilla*. While the chimpanzees and bonobos were reciprocally monophyletic and did not share any sequences, the eastern and western gorillas could not be sorted and the variation seen in eastern gorillas was encompassed within that of western gorillas [Jensen-Seaman et al., 2003]. Interestingly, this contrast between patterns of variation in *Pan* and *Gorilla* has not been found in studies of maternally inherited mtDNA (see below), and hints that *Pan* and *Gorilla* differ in population structure as a result of differences in sex-specific gene flow.

Hence, an outstanding question of particular interest concerns the pattern of variation on the paternally transmitted Y-chromosome relative to that found in other species. A comparison of the levels of variation seen in gorillas to those in chimpanzees should prove particularly interesting, since social groups containing one or few adult males are common in gorillas, whereas multi-male groups are typical for chimpanzees. This leads to an expectation of greater differences in reproductive success (i.e., higher reproductive skew) among gorilla males as compared to chimpanzee males. Results of genetic analysis are consistent with this prediction. Preliminary reports indicate that while individual gorillas can monopolize paternity of group offspring over a period of years [Bradley et al., 2001], dominant chimpanzee males are less successful in monopolizing paternity within the group [Constable et al., 2001] (Boesch and Vigilant, unpublished results). This in turn leads to an expectation of low variability in Y-chromosome sequences in gorillas as compared to chimpanzees and humans, an inference supported by some preliminary work [Jensen-Seaman et al., 2001a].

Genetic Variation in Gorillas: Mitochondrial DNA

To date, all results describing how genetic variation is distributed among gorilla populations in the wild have come from studies of a single genetic locus, the mitochondrial DNA (mtDNA) molecule. In terms of applicability for intraspecific analyses, the advantages of this molecule as compared to nuclear DNA include a higher rate of evolution, greater abundance in cells, and apparent maternal inheritance and lack of recombination [Stoneking, 1993]. The higher rate of evolution means that variation accumulates more quickly than in the typical segment of genomic DNA, and thus more recent evolutionary history can be studied. The fact that mitochondria and mtDNA are present in multiple copies per cell makes it easier to analyze mtDNA (compared to nuclear DNA) in noninvasive samples, which typically yield low amounts of DNA. The final two factors allow for the construction of genealogical trees depicting the phylogenetic relationships among the mtDNA molecules characterized. One disadvantage associated with mtDNA is that it constitutes a single genetic locus that represents a miniscule percentage of the overall genome. A potentially serious difficulty of working with mtDNA arises from the fact that the mitochondrial and nuclear genomes of a cell do not evolve in isolation, and thus pieces of genetic information can and do transfer between the genomes [Zullo et al., 1991]. A piece of mtDNA that has transferred to the nuclear genome will subsequently evolve at the rate of the surrounding nuclear DNA, rather than that of its mitochondrial source. Such nuclear insertions of mtDNA (numts) may be inadvertently analyzed in place of genuine mtDNA, and hence seriously confound results [Bensasson et al., 2001]. These artifactual copies of mtDNA (also termed mitochondrial pseudogenes) have been found in a wide variety of animal species [Bensasson et al., 2001]. Analysis of the complete human genome has revealed that segments encompassing all parts of the mitochondrial genome have been transposed to the nucleus, and that this transfer is a continual process [Mourier et al., 2001; Tourmen et al., 2002; Woischnik and Moraes, 2002]. It is therefore essential that in studies of mtDNA, one must consider and explicitly exclude the possibility that numt sequences have been inadvertently included.

An intraspecific study of mtDNA control region sequences from gorillas reported a total of 26 unique sequences derived from 63 individuals [Garner & Ryder, 1996]. The wild individuals sampled included representatives of eastern gorilla populations (mountain and lowland) and a few western gorilla populations,

with additional samples from captive western gorillas. The phylogenetic tree relating the sequences was characterized by a primary split between lineages leading to western and eastern gorillas, and multiple deep branches within the grouping of western gorillas. While deep splits within a clade representing a single taxa are often indicative of population substructuring, interpretation of the pattern obtained for western gorillas was hampered by a lack of information concerning the geographic origin of the captive individuals examined. More problematically, reanalysis of DNA from some of the same gorillas has revealed that the deepest branches in the western gorilla clade (leading to WL495, WL287, and WL759) are in fact spurious, as those three sequences were apparently derived from inadvertent amplification of numts [Jensen-Seaman, 2000] (Bradley and Vigilant, unpublished observations).

If the other gorilla sequences reported in publications to date [Garner & Ryder, 1996; Jensen-Seaman & Kidd, 2001; Xu & Arnason, 1996] are taken to be valid mtDNA sequences, one can estimate amounts of variation in mtDNA for defined groups of gorillas by calculating the nucleotide diversity, a measure of the average number of nucleotide differences between any two randomly chosen sequences [Nei, 1987]. Jensen-Seaman and Kidd [2001] found that nucleotide diversity was an order of magnitude higher in western gorillas as compared to either eastern mountain or eastern lowland gorillas (Table I). However, since the eastern mountain and eastern lowland gorillas analyzed almost certainly came from much more limited geographic regions compared to the mostly captive western gorillas, it is appropriate to recalculate the values and compare nucleotide diversity for what are now considered two species: western and eastern gorillas. Very similar estimates of nucleotide diversity for western and eastern gorillas can then be obtained (Table I), suggesting that despite very different total current-day population sizes and distributions, the evolutionary histories of western and eastern gorillas may have been broadly similar.

The date of divergence of the lineages leading to western and eastern gorillas has been estimated with the use of information from control region sequences, as well as from other segments of the mtDNA molecule. The depth of the divergence between western and eastern gorillas appears to approximate the depth of the split between chimpanzees and bonobos, and to date back some 2 million years [Jensen-Seaman et al., 2001b; Ruvolo, 1996]. The split between the lineages leading to eastern lowland and eastern mountain gorillas is more recent: approximately 400,000 years ago. It is important to note that, as mentioned above, due to polymorphism in the ancestral populations these dates represent estimates of divergences of gene lineages, not of actual populations or species. The time that populations split is expected to be more recent than these estimates. Interestingly, calculations from the limited data available from sequences of noncoding nuclear segments do not produce an equivalent time estimate of the split within *Pan* and *Gorilla*; rather, they indicate that the chimpanzee–bonobo

Table 1. Nucleotide Diversity Estimated From Sequences of the First Hypervariable Region of the mtDNA Control Region

Taxon	Nucleotide diversity (π)
Western gorillas (*Gorilla gorilla*)	5.19%
Eastern gorillas (*Gorilla beringei)*	5.36%
Eastern mountain gorillas (*G. b. beringei*)	0.58%
Eastern lowland gorillas (*G. b. graueri*)	0.66%

split was more than twice as distant as that between western and eastern gorillas [Jensen-Seaman et al., 2001b]. This apparent difference emphasizes the point made earlier–that different genetic segments have different histories, and so only through comparison of multiple datasets can the molecular genetic evolution of a species be fully appreciated. The value of direct comparison of phylogenies derived from different genetic markers is illustrated by the example of the macaques, a genus in which it has been difficult to reconcile molecular and morphological phylogenies. MtDNA and Y-chromosome data produce discordant phylogenies, which apparently are best explained as being a result of strong female philopatry and male-mediated gene flow, along with potential past hybridization giving rise to new species [Tosi et al., 2000]. However, a more complete and confident resolution of macaque phylogeny awaits the use of multiple unlinked markers.

Molecular Ecology of Gorillas

The now-common use of noninvasive samples for DNA analysis has made it possible to conduct genetic analyses of all individual members of primate social groups without disturbing the behavior of the subjects [e.g., Constable et al., 2001; Gerloff et al., 1999; Launhardt et al., 2001; Vigilant et al., 2001]. Such approaches characterize individuals with the use of a suite of highly variable markers (e.g., microsatellites) in the nuclear genome that when compiled produce individually distinctive multilocus, or genotypes. Microsatellite analyses can reveal unexpected patterns of behavior–for example, the high frequency of extrapair mating in socially monogamous birds [reviewed in Griffin et al., 2002]. In animal groups, neither mating behavior nor social dominance rank always predict paternity, and genetic analyses have revealed the presence of alternative, unobserved male mating strategies that produce a significant proportion of offspring (e.g., in grey seals [Worthington Wilmer et al., 1999] and free-living sheep [Coltman et al., 1999]). One of the earliest attempts to use a molecular approach to assess the genetic mating system in wild apes was made in a study of mountain gorillas; however, the conclusions were limited [Field et al., 1998]. While the majority of gorilla social groups appear to be composed of a single mature silverback male along with adult females and offspring, some 40% mountain gorilla groups contained two or more silverback males [Robbins, 1999; Schaller, 1963; Weber & Vedder, 1983]. Furthermore, since male apes copulate and are fertile before growth and maturation are completed [references in Robbins & Czekala, 1997], blackback gorilla males must also be considered as possible fathers of group offspring. Preliminary results from paternity analyses in one mountain gorilla group indicate that subordinate males are successful in fathering offspring within the group [Bradley et al., 2001]. Further investigation of mating systems in gorillas, including testing of the assumption that the sole male in one-male groups fathers all of the offspring, is needed before we can evaluate the relative success of alternative male strategies. Microsatellite data can also be used to estimate the genetic relatedness of pairs of individuals, to address such questions as whether adult males in multi-male groups are close relatives, and whether female gorillas tend to disperse preferentially into groups in which they have female relatives.

The application of molecular methods to noninvasively collected samples appears to be a promising approach for ‘‘molecular tracking’’ of individuals, or ‘‘genetic censusing’’ of populations [Kohn et al., 1999; Mills et al., 2000; Palsbøll, 1999]. While microsatellite markers offer sufficient resolving power to distinguish

even closely-related individuals, two practical issues currently stand in the way of widespread application of molecular tracking of individuals. The first is the difficulty inherent in producing accurate genotypes using DNA derived from noninvasive samples [Morin et al., 2001; Taberlet et al., 1996, 1999]. Such DNA is low in quantity and quality, and results from ongoing work on numerous wild great ape populations (chimpanzees, gorillas, bonobos, and gibbons) indicate that on average only about 70% of samples yield extracts that contain sufficient nuclear DNA to produce an accurate genotype (Vigilant, 2002). However, it should be noted that noninvasive samples have been reported to be much less problematic in other species, such as elephants [Fernando et al., 2003] and even baboons [Vinson et al., 2003]. Although researchers observing habituated subjects can collect multiple samples per individual, this relatively high chance of failure to obtain usable DNA by current methodologies means that analyses for which nearly every sample must produce data (e.g., for a genetic census) are not feasible. The second difficulty with the large-scale application of genetic analysis of wild animals concerns time and costs. The production of accurate genotypes is a painstaking endeavor, and projects using noninvasive samples rarely exceed characterization of 100 individuals, and require at least 1–2 years for laboratory analysis. This slow pace is coupled with the high cost of laboratory reagents and supplies (an average researcher can spend about $10,000 on materials for such a project in 1 year). Improvements in the efficiency, speed, and price of genotyping, without loss of accuracy, are thus needed before genotyping of wild primate populations can occur on a large scale.

DISCUSSION AND CONCLUSIONS

One message from the research summarized here is that different genetic segments have different histories. This does not mean that contradictory information will necessarily be produced (for example, the idea that the human population has undergone a population reduction and subsequent recent expansion is supported by multiple data sets [e.g., Harpending et al., 1998; Jorde et al., 1997; Kaessmann et al., 2001; Yu et al., 2002]). It does mean that comprehensive understanding depends upon the analysis of a comprehensive amount of data–not of a limited number of individuals at several markers, or many individuals at a single genetic locus. Nonetheless, the single genetic locus that has offered the most insights into the evolutionary histories of a wide variety of animal taxa is the mtDNA molecule [Avise, 1998].

Unfortunately, of the limited amount of data available concerning genetic variation in gorillas, those concerning mtDNA variation are the most problematic. Attempts to analyze sequence variation at the mtDNA control region, the segment that is the most variable and hence the most useful for intraspecific analyses, typically yield multiple sequences for each individual. It has been presumed that one sequence represents the authentic mtDNA sequence, while the other(s) derive from amplification of numts, the nuclear copies of mtDNA mentioned above. Such data would be usable if the sequences could be sorted with high confidence into those that represent real mtDNA and those that are derived from numts. However, this is not possible in all cases, such as when insertions of mtDNA have occurred recently enough that the translocated and mtDNA source sequences are nearly identical and thus appear intermixed in a phylogenetic analysis. The prevalence of numts is not consistent across animal taxa, and it has been suggested that gorillas in particular have a high frequency of translocated mtDNA sequences in the nuclear genome [Bensasson et al., 2001]. Accordingly,

all previously reported and any newly generated mtDNA sequences from gorillas must be considered to be provisional in the absence of accompanying data that convincingly demonstrate the authenticity of the sequences. In principle, authenticity can only be directly demonstrated by 1) using a prepartion of highly purified mtDNA that excludes genomic DNA, or 2) using an amplification strategy that takes advantage of the circular structure of mtDNA, and does not permit amplification of segments located in the nuclear genome. Neither of these strategies can be applied to the low-quantity, low-quality DNA obtained from noninvasive samples, and it is the noninvasive samples obtained from gorillas living in wild populations that are of greatest interest. Assuming that a means can be devised to produce validated gorilla mtDNA sequences, the issues to be addressed with such data include the pattern of mtDNA variation in western gorillas, and a reevaluation of previous conclusions, such as the age of the divergence between the western and eastern gorilla lineages, which was based on earlier mtDNA analyses of gorillas. While analysis of the maternally-inherited mtDNA molecule provides information on female-mediated gene flow, the pattern of variation on the Y-chromosome can provide complementary information from the male side. A comparison of mtDNA and Y-chromosome variation is expected to be particularly interesting in the case of gorillas, where reproductive success among males is likely to vary much more than among females, resulting in a comparatively lower effective population size for males.

Analysis of variation at multiple microsatellite markers is useful not only for answering questions regarding individual identity and paternity on the level of the social group, but also for inferring long-term demographic parameters. For example, population differentiation can be estimated, and the effective number of migrants per generation [Balloux & Lugon-Moulin, 2002], as well as the effective population size, can be inferred [Luikart & Cornuet, 1999]. The effective size of a population is typically orders of magnitude smaller than the census size, and is a more relevant measure for considerations of long-term population viability [Frankham, 1995]. A difficulty with the application of these estimators is that in order to obtain reliable results, individuals must be analyzed at more microsatellite markers ($t > 20$) than are typically used for studies addressing questions of molecular ecology [Cornuet & Luikart, 1996; Luikart & Cornuet, 1999].

Finally, genetic analyses, even of species of conservation interest, can only be descriptive—not prescriptive. After a period of heavy reliance on trees exhibiting molecular monophyly, recent opinion calls for the integration of ecological and genetic data with an emphasis on population exchangeability and a more graded classification of conservation units [Crandall et al., 2000]. Researchers have often attempted to use the results from analyses of genetic variation in populations to produce management guidelines, such as those concerning maintenance of continuity or separation of populations [Frankham et al., 2002]. There are at least two difficulties in the application of genetic data to conservation practice that are worth mentioning. The first exists within the concept of an evolutionary significant unit (ESU) as a population unit with high conservation priority that requires separate management [Moritz, 1994]. The emphasis on maintaining isolation between distinct units contains an implicit conflict with the goal of maintaining the levels of genetic variation that are assumed to accompany adaptive flexibility [Crandall et al., 2000]. The second difficulty lies in the fact that despite the effort that has gone into assessments of molecular genetic diversity, the extent to which such measures relate to heritable variation in adaptive traits is unclear [Frankham, 1999; Hedrick, 1999; McKay & Latta, 2002; Reed & Frankham, 2001]. Thus, while genetic data undisputably add to our

knowledge concerning wild animals, such information is only one aspect among many, and much concerning the genetic variation in gorillas remains yet to be determined.

Several publications of particular relevance [Bradley et al., 2004; Clifford et al., 2004; Jensen-Seaman et al., 2004; Thalman et al., 2004; Yu et al., 2004] appeared after the revision of this manuscript.

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REFERENCES

Amos W, Balmford A. 2001. When does conservation genetics matter? Heredity 87:257–265.

Avise JC. 1998. The history and purview of phylogeography: a personal reflection. Mol Ecol 7:371–379.

Avise JC, Johns GC. 1999. Proposal for a standardized temporal scheme of biological classification for extant species. Proc Natl Acad Sci USA 96:7358–7363.

Avise JC. 2000. Phylogeography. Cambridge: Harvard University Press. 447p.

Balloux F, Lugon-Moulin N. 2002. The estimation of population differentiation with microsatellite markers. Mol Ecol 11: 155–165.

Barratt EM, Deaville R, Burland TM, Bruford MW, Jones G, Racey PA, Wayne RK. 1997. DNA answers the call of pipistrelle bat species. Nature 387:138–139.

Bensasson D, Zhang D-X, Hartl DL, Hewitt GM. 2001. Mitochondrial pseudogenes: evolution's misplaced witnesses. Trends Ecol Evol 16:314–321.

Bradley BJ, Doran–Sheehy DM, Lukas D, Boesch C, Vigilant L. 2004. Dispersed male networks in western gorillas. Curr Biol 14:510–513.

Bradley BJ, Robbins MM, Williamson EA, Boesch C, Vigilant L. 2001. Assessing male reproductive strategies in wild mountain gorillas *Gorilla gorilla beringei* using DNA from feces. Primate Report 60: 16–17.

Chen FC, Li WH. 2001. Genomic divergences between humans and other hominoids and the effective population size of the common ancestor of humans and chimpanzees. Am J Hum Genet 68:444–456.

Clifford SL, Anthony NM, Bawe-Johnson M, Abernethy KA, Tutin CEG, White LJT, Bermejo M, Goldsmith ML, McFarland K, Jeffery KJ, Bruford MW, Wickings EJ. 2004. Mitochondrial DNA phylogeography of western lowland gorillas (*Gorilla gorilla gorilla*). Mol Ecol 13:1551–1565.

Coltman DW, Bancroft DR, Robertson A, Smith JA, Clutton-Brock TH, Pemberton JM. 1999. Male reproductive success in a promiscuous mammal: behavioural estimates compared with genetic paternity. Mol Ecol 8:1199–1209.

Constable JL, Ashley MV, Goodall J, Pusey AE. 2001. Noninvasive paternity assignment in Gombe chimpanzees. Mol Ecol 10:1279–1300.

Cornuet JM, Luikart G. 1996. Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. Genetics 144: 2001–2014.

Crandall KA, Bininda-Emonds ORP, Mace GM, Wayne RK. 2000. Considering evolutionary processes in conservation biology. Trends Ecol Evol 15:290–295.

Dawood A, Channing A, Bogart JP. 2002. A molecular phylogeny of the frog genus Tomopterna in Southern Africa: examining species boundaries with mitochondrial 12S rRNA sequence data. Mol Phylogenet Evol 22:407–413.

Disotell TR, Tosi AJ, DiFiore A. 2003. X-chromosome phylogeny of the Platyrrhini. Am J Phys Anthropol S36:89–90.

Doran DM, McNeilage A. 1998. Gorilla ecology and behavior. Evol Anthropol 6:120–131.

Fernando P, Vidya NC, Rajapakse C, Dangolla A, Melnick DJ. 2003. Reliable noninvasive genotyping: fantasy or reality? J Hered 94:115–123.

Field D, Chemnick L, Robbins M, Garner K, Ryder O. 1998. Paternity determination in captive lowland gorillas and orangutans and wild mountain gorillas by microsatellite analysis. Primates 39:199–209.

Frankham R. 1995. Effective population size/adult population size rations in wildlife: a review. Genet Res Cambr 66:95–107.

Frankham R. 1996. Relationship of genetic variation to population size in wildlife. Conserv Biol 10:500–1508.

Frankham R. 1999. Quantitative genetics in conservation biology. Genet Res 74:237–244.

Frankham R, Ballou JD, Briscoe DA. 2002. Introduction to conservation genetics. Cambridge: Cambridge University Press. 617 p.

Garner KJ, Ryder OA. 1996. Mitochondrial DNA diversity in gorillas. Mol Phylogenet Evol 6:39–48.

Gerloff U, Hartung B, Fruth B, Hohmann G, Tautz D. 1999. Intracommunity relationships, dispersal pattern and paternity success in a wild living community of bonobos (*Pan paniscus*) determined from DNA analysis of faecal samples. Proc R Soc Lond B Biol Sci 266:1189–1195.

Goodman M, Tagle DA, Fitch DH, Bailey W, Czelusniak J, Koop BF, Benson P, Slightom JL. 1990. Primate evolution at the DNA level and a classification of hominoids. J Mol Evol 30:260–266.

Goodman M, Porter CA, Czelusniak J, Page SL, Schneider H, Shoshani J, Gunnell G, Groves CP. 1998. Toward a phylogenetic classification of primates based on DNA evidence complemented by fossil evidence. Mol Phylogenet Evol 9:585–598.

Griffin SC, Owens IPF, Thuman KA. 2002. Extra pair paternity in birds: a review of interspecific variation and adaptive function. Mol Ecol 11:2195–2212.

Harpending HC, Batzer MA, Gurven M, Jorde LB, Rogers AR, Sherry ST. 1998. Genetic traces of ancient demography. Proc Natl Acad Sci USA 95:1961–1967.

Hebert PDN, Cywinska A, Ball SL, deWaard JR. 2003. Biological identifications through DNA barcodes. Proc R Soc Lond B 270: 313–321.

Hedrick PW. 1999. Highly variable loci and their interpretation in evolution and conservation. Evolution 53:313–318.

Janzen FJ, Krenz JG, Haselkorn TS, Brodie ED. 2002. Molecular phylogeography of common garter snakes (*Thamnophis sirtalis*) in western North America: implications for regional historical forces. Mol Ecol 11:1739–1751.

Jensen-Seaman M. 2000. Evolutionary genetics of gorillas. Ph.D. dissertation, Yale University, New Haven, Connecticut.

Jensen-Seaman M, Kidd K. 2001. Mitochondrial DNA variation and biogeography of eastern gorillas. Mol Ecol 10:2241–2247.

Jensen-Seaman MI, Altheide TK, Deinard AS, Hammer MF, Kidd KK. 2001a. Nucleotide diversity at autosomal, Y-chromosomal, and mitochondrial loci of African apes and humans. Am J Phys Anthropol S32:86.

Jensen-Seaman MI, Deinard AS, Kidd KK. 2001b. Modern African ape populations as genetic and demographic models of the last common ancestor of humans, chimpanzees, and gorillas. J Hered 92:475–480.

Jensen-Seaman MI, Deinard AS, Kidd KK. 2003. Mitochondrial and nuclear DNA estimates of divergence between western and eastern gorillas. In: Taylor AB, Goldsmith ML, editors. Gorilla biology: a multidisciplinary perspective. Cambridge: Cambridge University Press. p 247–268.

Jensen-Seaman MI, Sarmiento EE, Deinard AS, Kidd KK. 2004. Nuclear integrations of mitochondrial DNA in gorillas. Am J Primatol 63:139–147.

Jorde LB, Rogers AR, Bamshad M, Watkins WS, Krakowiak P, Sung S, Kere J, Harpending HC. 1997. Microsatellite diversity and the demographic history of modern humans. Proc Natl Acad Sci USA 94: 3100–3103.

Kaessmann H, Wiebe V, Weiss G, Pääbo S. 2001. Great ape DNA sequences reveal a reduced diversity and an expansion in humans. Nat Genet 27:155–156.

Kingston T, Lara MC, Jones G, Akbar Z, Kunz TH, Schneider CJ. 2001. Acoustic divergence in two cryptic Hipposideros species: a role for social selection? Proc R Soc Lond B Biol Sci 268:1381–1386.

Kohn MH, York EC, Kamradt DA, Haught G, Sauvajot RM, Wayne RK. 1999. Estimating population size by genotyping faeces. Proc R Soc Lond B Biol Sci 266:657–663.

Launhardt K, Borries C, Hardt C, Epplen JT, Winkler P. 2001. Paternity analysis of alternative male reproductive routes among the langurs *Semnopithecus entellus* of Ramnagar. Anim Behav 61:53–64.

Luikart G, Cornuet JM. 1999. Estimating the effective number of breeders from heterozygote excess in progeny. Genetics 151:1211–1216.

McKay JK, Latta RG. 2002. Adaptive population divergence: markers, QTL and traits. Trends Ecol Evol 17:285–291.

Mills LS, Citta JJ, Lair KP, Schwarz MK, Tallmon DA. 2000. Estimating animal abundance using noninvasive DNA sampling: promise and pitfalls. Ecol Appl 10:283–294.

Morin PA, Chambers KE, Boesch C, Vigilant L. 2001. Quantitative PCR analysis of DNA from noninvasive samples for accurate microsatellite genotyping of wild chimpanzes *Pan troglodytes verus*. Mol Ecol 10:1835–1844.

Moritz C. 1994. Defining 'evolutionarily significant units' for conservation. Trends Ecol Evol 9:373–375.

Mourier T, Hansen AJ, Willerslev E, Arctander P. 2001. The Human Genome Project reveals a continuous transfer of large mitochondrial fragments to the nucleus. Mol Biol Evol 18:1833–1837.

Nei M. 1987. Molecular evolutionary genetics. New York: Columbia University Press.

Palsbøll P. 1999. Genetic tagging: contemporary molecular ecology. Biol J Linn Soc 68:3–22.

Reed DH, Frankham R. 2001. How closely correlated are molecular and quantitative measures of genetic variation? A meta-analysis. Evolution 55:1095–1103.

Richmond JQ, Reeder TW. 2002. Evidence for parallel ecological speciation in scincid lizards of the *Eumeces skiltonianus* species group Squamata: scincidae. Evolution 56:1498–1513.

Robbins MM, Czekala NM. 1997. A preliminary investigation of urinary testosterone and cortisol levels in wild male mountain gorillas. Am J Primatol 43:51–64.

Robbins MM. 1999. Male mating patterns in wild multimale mountain gorilla groups. Anim Behav 57:1013–1020.

Robbins MM, Sicotte P, Stewart KJ, editors. 2001. Mountain gorillas. Cambridge: Cambridge University Press. 431 p.

Ruvolo M. 1996. A new approach to studying modern human origins: hypothesis testing with coalescence time distributions. Mol Phylogenet Evol 5:202–219.

Ruvolo M. 1997. Molecular phylogeny of the hominoids: inferences from multiple independent DNA sequence data sets. Mol Biol Evol 14:248–265.

Satta Y, Klein J, Takahata N. 2000. DNA archives and our nearest relative: the trichotomy problem revisited. Mol Phylogenet Evol 14:259–275.

Schaller GB. 1963. The mountain gorilla. Chicago: University of Chicago.

Sibley CG, Comstock JA, Ahlquist JE. 1990. DNA hybridization evidence of hominoid phylogeny: a reanalysis of the data. J Mol Evol 30:202–236.

Sterner KN, Raaum RL, Tosi AJ, Noviello CM, Schienman JE, Collura RV, Stewart C, Disotell TR. 2003. Colobine molecular phylogeny. Am J Phys Anthropol S36:200

Stoneking M. 1993. DNA and recent human evolution. Evol Anthropol 2:60–73.

Taberlet P, Griffin S, Goossens B, Questiau S, Manceau V, Escaravage N, Waits LP, Bouvet J. 1996. Reliable genotyping of samples with very low DNA quantities using PCR. Nucleic Acids Res 24:3189–3194.

Taberlet P, Waits LP, Luikart G. 1999. Noninvasive genetic sampling: look before you leap. Trends Ecol Evol 14:323–327.

Tautz D, Arctander P, Minelli A, Thomas RH, Vogler AP. 2003. A plea for DNA taxonomy. Trends Ecol Evol 18:70–74.

Taylor AB, Goldsmith ML, editors. 2002. Gorilla biology. Cambridge: Cambridge University Press. 508 p.

Thalmann O, Hebler J, Poinar HN, Pääbo S, Vigilant L. 2004. Unreliable mtDNA data due to nuclear insertions: a cautionary tale from analysis of humans and other great apes. Mol Ecol 13:321–335.

Tosi AJ, Morales JC, Melnick DJ. 2000. Comparison of Y chromosome and mtDNA phylogenies leads to unique inferences of macaque evolutionary history. Mol Phylogenet Evol 17:133–144.

Tourmen Y, Baris O, Dessen P, Jacques C, Malthiery Y, Reynier P. 2002. Structure and chromosomal distribution of human mitochondrial pseudogenes. Genomics 80: 71–77.

Vigilant L, Hofreiter M, Siedel H, Boesch C. 2001. Paternity and relatedness in wild chimpanzee communities. Proc Natl Acad Sci USA 98:12890–12895.

Vigilant L. 2002. Technical challenges in the microsatellite genotyping of a wild chimpanzee population using faces. Evol Anthropol S1:162–165.

Vinson A, Packer C, Rogers J. 2003. An optimized panel of microsatellites for fecal DNA studies of wild baboons: preliminary analyses of genetic variation among Gombe baboons. Am J Phys Anthropol S36: 215.

Weber AW,Vedder A. 1983. Population dynamics of the Virunga gorillas: 1959–1978. Biol Conserv 26:341–366.

Woischnik M, Moraes CT. 2002. Pattern of organization of human mitochondrial pseudogenes in the nuclear genome. Genome Res 12:885–893.

Worthington Wilmer J, Allen PJ, Pomeroy PP, Twiss SD, Amos W. 1999. Where have all the fathers gone? An extensive microsatellite analysis of paternity in the grey seal *Halichoerus grypus*. Mol Ecol 8:1417–1429.

Xu X, Arnason U. 1996. A complete sequence of the mitochondrial genome of the western lowland gorilla. Mol Biol Evol 13:691–698.

Yamagiwa J. 1999. Socioecological factors influencing population structure of gorillas and chimpanzees. Primates 40:87–104.

Yu N, Fu Y-X, Li WH. 2002. DNA polymorphism in a worldwide sample of human X chromosomes. Mol Biol Evol 19:2131–2141.

Yu N, Jensen-Seaman MI, Chemnick L, Ryder O, Li W-H. 2004. Nucleotide diversity in gorillas. Genetics 166:1375–1383.

Zullo S, Sieu LC, Slightom JL, Hadler HI, Eisenstadt JM. 1991. Mitochondrial D-loop sequences are integrated in the rat nuclear genome. J Mol Biol 221:1223–1235.