Elephant Natural History: A Genomic Perspective

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Annu. Rev. Anim. Biosci. 2015. 3:139–67

First published online as a Review in Advance on December 8, 2014

The *Annual Review of Animal Biosciences* is online at animal.annualreviews.org

This article's doi:
10.1146/annurev-animal-022114-110838

Keywords

conservation, evolution, mammoths, mitonuclear discordance, taxonomy

Abstract

We review DNA-based studies of elephants and recently extinct proboscideans. The evidence indicates that little or no nuclear gene flow occurs between African savanna elephants (*Loxodonta africana*) and African forest elephants (*Loxodonta cyclotis*), establishing that they comprise separate species. In all elephant species, males disperse, whereas females remain with their natal social group, leading to discordance in the phylogeography of nuclear and mitochondrial DNA patterns. Improvements in ancient DNA methods have permitted sequences to be generated from an increasing number of proboscidean fossils and have definitively established that the Asian elephant (*Elephas maximus*) is the closest living relative of the extinct woolly mammoth (*Mammuthus primigenius*). DNA-based methods have been developed to determine the geographic provenance of confiscated ivory in an effort to aid the conservation of elephants.

INTRODUCTION

The parable of the blind men and the elephant is ancient and common to many cultures. The ageless parable is a fair description of the scientific process itself, and its application to elephant genetics is a good example of the cumulative power of science to expand and refine our understanding of nature. This review relates a modern version of the parable, in which molecular genetic methods were used to examine the population structure, systematics, taxonomy, mating system, and evolution of living and recently extinct elephant lineages, with results ultimately combining to produce a more comprehensive picture of the natural history of elephants.

What was known (and not known) about elephant mating systems, population structure, taxonomy, and systematics prior to the era of molecular genetics? In African savannas, elephant social behavior had been well characterized, with several long-term studies affirming a strictly matrilineal society in which females remain within and males disperse from their natal groups (1, 2). Core family groups temporarily associate with each other in a hierarchical fission-fusion process, but it was not known whether the frequency and duration of associations depended on degrees of relatedness among groups. Competition among males for reproductive success was shown to depend not only on body size and therefore age but also on a testosterone-fueled state of increased aggression called musth (3). How well this behavior translated into reproductive success was unclear. Social behavior of African forest and Asian elephants was not as well studied, but group sizes in both were known to be smaller, solitary individuals more common, and associations among groups less frequent compared with savanna elephants (4–6).

Prevailing views on the taxonomy of extant elephants were that they comprised two species, one in Asia (*Elephas maximus*), divided into several subspecies (4–6), and another in Africa (*Loxodonta africana*), sometimes divided into forest and savanna (or bush) subspecies (7–10). Opinions had varied for more than a century on these assignments, with intermittent reports of distinctions between elephants occupying the forests and savannas of Africa. In 1900, Matschie (11) described forest elephants as an additional species (*Loxodonta cyclotis*), whereas Frade (12, 13) was the first to hold that all of Africa's extant elephants fell into exactly two species. Despite persistent accounts of their morphological differences (13), reports that forest and savanna elephants hybridize (14) provided support for widespread recognition of only one species in Africa (7, 15).

The first truly quantitative study of morphological variation in African elephants was not published until 2000. A multivariate analysis of skull dimensions, featuring samples from widespread locations across the range, found two strikingly non-overlapping clusters corresponding to elephants from forest and savanna habitats, with a few intermediaries from habitat transition zones (15, 16). Therefore, results of the very first statistically compelling analysis with representation over much of the range of elephants in Africa were emphatically inconsistent with the single species classification. This review first discusses the substantial genetic evidence that has subsequently supported recognition that two elephant species are currently present in Africa. Other sections explore the unusual mitochondrial (mt)DNA patterns detected among elephantids, the conclusions reached by studies conducted using DNA from fossil proboscideans, and finally the role of DNA studies in promoting the conservation of extant elephants.

GENETIC EVIDENCE FOR SPECIATION IN AFRICAN ELEPHANTS

African Elephants Comprise Two Distinct Species

A year after the quantitative morphological study was published, an analysis of nuclear (intron) sequences, featuring samples from widespread locations in Africa, revealed that forest and

savanna elephant lineages are deeply divergent and reciprocally monophyletic (17), with a very limited degree of hybridization where the two types meet. A similar study using the same sample set but focusing on nuclear microsatellites gave the same result (18). These findings strongly supported species-level differences between elephants inhabiting forests and savannas in Africa. By contrast, the genetic distinctions between them were not as clear in studies that examined mtDNA patterns (19–22). Although this initially called into question the recognition of two species, it soon became evident that the unusual patterns found in mtDNA could be attributed to sex differences in dispersal (23–25). Male elephants disperse from their natal social group, whereas females do not (1, 2). Thus, unlike all other genetic markers, which are subject to substantial gene flow mediated by males, the mtDNA remains bound to the natal social group and is greatly circumscribed in terms of gene flow (26).

The role of gene flow in the delimitation of species boundaries has been reviewed by Petit & Excoffier (27), who examined the patterns of introgression among genetic markers following interspecies hybridization. Markers subject to a great degree of geographic dispersal were found to accurately delineate species boundaries, whereas markers transmitted only by the nondispersing sex showed patterns that did not delineate species boundaries. The centrality of gene flow in determining species boundaries that was noted by Petit & Excoffier (27) is also emphasized here to clarify any notion that divergence has been the primary or even the sole criterion for suggesting that African forest and savanna elephants comprise distinct species. The lack of gene flow between forest and savanna elephants (e.g., as reflected in the segregation of nuclear haplotypes) has been the critical evidence pointing to the existence of two African elephant species, ever since the initial report that first put forward comprehensive genetic evidence for a species boundary between forest and savanna elephants (17).

Widespread Nuclear Gene Flow Within but Not Between Species Boundaries

Some examples may help to demonstrate how the genetic data support the division of African elephants into forest and savanna elephant species. Among four intronic sequences used to initially examine forest and savanna elephants, indels (insertion-deletion variants) were present in two of the genes: *CHRNA1* and *VIM* (17). Indels are excellent markers for inferring gene flow, because the presence of the same indel in two individuals is evidence that they shared a common ancestor relatively recently. Both of the indels were common in two highly sampled forest localities (Lope in Gabon and Dzanga Sangha in the Central African Republic) in the western part of the Congolian tropical forest block of Central Africa (**Figure 1**) (17). To the east of the Congolian tropical forest block, in Garamba, were elephants showing signs of interspecies hybridization but with primarily forest elephant genotypes (17). In Garamba, both indels were also common (**Figure 1**) (17). The presence of the indels in elephants in the western and eastern edge of the Congolian Forest block (**Figure 1**) demonstrated that substantial gene flow had connected forest populations across the geographic extent of Central Africa. Gene flow across such long geographic distances would be consistent with forest elephants in the Congolian forest comprising a set of interbreeding populations, i.e., belonging to the same species (17, 23, 25, 28–31).

The same indels were completely absent among savanna elephants genotyped at localities north, east, and south of the Congolian tropical forest (**Figure 1**). This was true even though a much larger number of savanna individuals were sampled, the geographic extent of sampling was much greater than that for forest elephants, and less distance separated some of the savanna localities from nearby forest localities than separated the forest localities from each other (17). The absence among savanna elephants of indels common in forest elephants is an indication that nuclear genetic material from forest elephants was not being transmitted into savanna elephant populations. Some of the

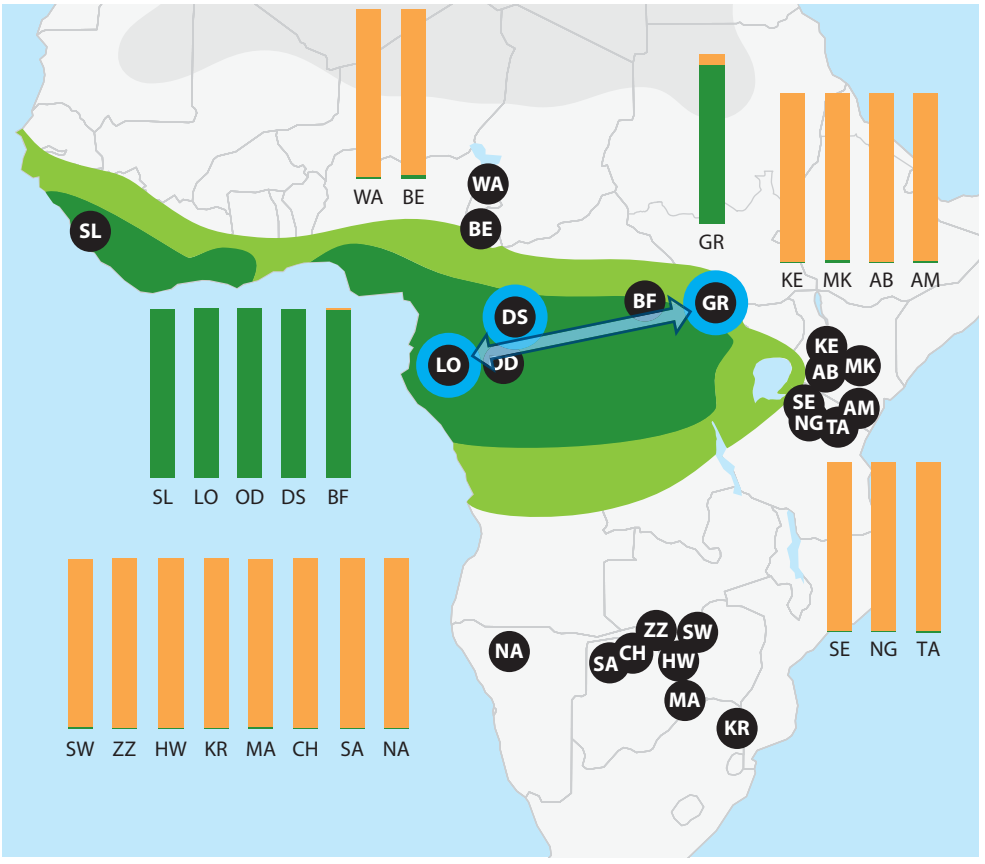

Figure 1

Nuclear genetic partitioning is consistent with forest and savanna elephants being distinctive species. The map portrays the historic extent of tropical forest habitat (*dark green*) and the forest-savanna transition zone (*light green*). Using data from 11 microsatellite loci, the software STRUCTURE identified two clusters among African elephants: Cluster 1 (*green*) comprised 99.5% of forest elephant populations and 0.6% of savanna elephant populations. Cluster 2 (*orange*) comprised 0.5% of forest and 99.4% of savanna populations. Note that no information was provided to the software as to whether the elephants at a locality were forest or savanna elephants. The bar plot for each locality depicts the proportion contributed to the genotypes of elephants by forest (*green*) or savanna (*orange*) elephant lineages, as estimated using STRUCTURE ($K = 2$). The blue double arrow represents gene flow across forest elephant populations, which could be inferred because the same insertion-deletion variants (indels) of the genes *CHRNA1* and *VIM* were detected among elephants in Lope (LO), Dzanga Sangha (DS), and Garamba (GR). Thus, gene flow had occurred across Congolian forest populations. Light blue circles on the map indicate the three localities in which indels were detected. Indels were not examined in the other Central African forest populations shown or in West Africa. Across all of the savanna localities shown, *CHRNA1* and *VIM* were sequenced in elephants. No indels were detected in any of the savanna elephant sequences, indicating that nuclear gene flow did not occur from forest into savanna elephant populations, consistent with a species barrier and with the microsatellite partitioning. The figure is adapted with permission from Reference 26. Abbreviations: AB, Aberdares; AM, Amboseli; BE, Benoue; BF, Bili; CH, Chobe; HW, Hwange; KE, Central Kenya/Laikipia; KR, Kruger; MA, Mashatu; MK, Mt. Kenya; NA, northern Namibia; NG, Ngorongoro; OD, Odzala; SA, Savuti; SE, Serengeti; SL, Sierra Leone; SW, Sengwa; TA, Tarangire; WA, Waza; ZZ, Zambezi.

elephants in Garamba had been found to be hybrids, with genetic markers typical of both species (17). But even though there was a hybrid zone, the indels present across forest elephant populations did not end up being transmitted into savanna elephant populations (17). This in turn suggested that hybrid elephants had been reproductively unsuccessful; otherwise, the indels would have spread to savanna populations (and it could later be inferred from mitonuclear patterns that male but not female hybrids had failed to reproduce). The genetic isolation of savanna elephants from forest elephants strongly supports their recognition as established parapatric species (17, 23, 25, 28–31).

Additional support for a species boundary between forest and savanna elephants was provided by the geographic distribution of haplotypes of the gene *GBA* (17). *GBA* sequences examined in elephants defined three haplotypes characterized by two polymorphic nucleotide sites (17). Haplotype C-C (cytosine nucleotide for the two polymorphic sites) was likely the ancestral haplotype, because it was present in all Asian elephants and both African elephant species and matched character states in the hyrax (17, 32). Haplotype T-C was found in 96% of chromosomes in savanna elephants and was also carried by some hybrid elephants in Garamba, but was not carried by any forest elephants (17). By contrast, many forest elephants sampled carried haplotype C-T, including forest elephants in Lope and Dzanga Sangha (17), populations geographically very close to savanna elephants in Cameroon (**Figure 1**). Yet no savanna elephants carried C-T. The observed distribution of *GBA* haplotypes was strong evidence of genetic isolation between savanna and forest elephant populations (20–24), despite the presence of a hybrid population carrying all three haplotypes in Garamba (17). It suggested that hybrids were not mediating gene flow from savanna elephants to forest elephants, indicative of a reproductive barrier between the two elephant species (17, 33).

Other Findings Consistent with Speciation Between Forest and Savanna Elephants

Lack of gene flow between the two species is now supported by similar patterns observed among additional indels and other polymorphic nuclear genes (23), from the sequencing of thousands of chromosomal segments (23, 34, 35), and from sampling by researchers of an independent set of savanna elephants in zoos (34, 35). The absence of nuclear gene flow in the face of parapatry and hybridization between forest and savanna populations provides the most compelling support for their separation as distinct species, yet it is supported by additional evidence, as follows:

Forest and savanna elephants are morphologically distinct (11–13, 15, 16, 21, 36). Relative to savanna elephants, forest elephants are smaller in stature and weigh less (15). Forest elephant tusks are thinner, straighter, and downward pointing (15). There are morphological distinctions in the cranium and mandible (15). A morphometric analysis of 295 African elephant skulls of known provenance by Grubb & Groves (15, 16) provided the first quantitative evidence supporting the division of forest and savanna elephants into two species.

Forest and savanna elephants generally reside in different habitats and display differences in behavior (15). The ranges of the two species have a limited overlap, with the range of forest elephants largely (although not entirely) coinciding with the range of tropical forest habitat in Central and West Africa (13, 15, 16, 37). Forest elephants are browsers and frugivores, whereas savanna elephants are browsers and grazers (15). Although forest elephants have been little studied, they may form smaller social groups than savanna elephants, and the males may be more solitary (15).

Most of the nuclear genetic diversity present in African elephants is due to differences between forest and savanna species. Genetic diversity has been examined within both forest and savanna elephants using both nuclear sequences and microsatellites (17, 18, 26). Diversity was much higher

within forest elephants than within savanna elephants (17, 18, 26), an indication that processes affecting the generation and loss of genetic diversity have distinct histories within the two species, and accumulated differences have been preserved by little genetic mixing between them (specifically, it can be inferred that male hybrids failed to reproduce). The degree of genetic mixing or of differentiation between populations can be estimated from genetic diversity by calculating Fst, which varies from 0 (indicative of no difference between two groups in allele presence or frequencies) to 1 (complete separation, with each group having distinct fixed alleles). High values of Fst (0.938 for intronic sequence data) between forest and savanna elephants, similar to the values obtained when Asian and African elephants are compared (0.902 forest–Asian, 0.997 savanna–Asian), indicate that forest and savanna elephants are strongly subdivided (17).

Elephant microsatellite data were also used to partition populations using a Bayesian approach (the software STRUCTURE) that relies on the deviation of allele frequencies from expectations under Hardy-Weinberg equilibrium (**Figure 1**) (26). This approach divided Africa's elephants into two groups or partitions, each of which corresponded to one of the species (**Figure 1**) (26). The partitions were determined without using prior knowledge of whether the populations or individuals were forest or savanna elephants (26). Although this analysis by itself would not have definitively established the presence of a species boundary (because many factors can affect Hardy-Weinberg equilibrium), the partitioning did correspond to the species division between forest and savanna elephants established using other criteria.

Forest and savanna elephant lineages are reciprocally monophyletic. When sequences of four nuclear introns were used to infer a phylogeny, forest and savanna elephants fell into distinct clades, regardless of tree-building method (17). Each clade had strong bootstrap support (which was even higher when the few hybrid individuals were excluded, because hybrids that mix gene sequences from both species tend to reduce support for distinctive clades). The two clades were reciprocally monophyletic: A clade consisting of all savanna elephants included no forest elephant individuals, and a clade consisting of all forest elephants included no savanna elephants (17). This was consistent with and further supported the division of forest and savanna elephants into separate species.

Forest and savanna elephants diverged millions of years ago. The most accurate estimates of divergence between forest and savanna elephant species have relied on comprehensive data sets and on multiple and well-calibrated dates from the fossil record (38). Based on 375 nuclear sequences totaling 39,763 bp, the split between forest and savanna elephants was estimated to have occurred at 2.6–5.6 Mya (with the older date more consistent with the fossil record when internal node date estimates are examined) (39). Another estimate used the complete coding region of the mitochondrial genome to estimate the timing of the split between F clade and S clade mitogenomes, which likely reflects the split between forest and savanna elephants (see below), at 5.5 Mya (**Figure 2**) (21, 40, 41). Although the mtDNA genome represents a single locus that is affected by female matrilocality (see below), estimated divergence dates were consistent with the higher end of the range estimated using nuclear markers, and date estimates for internal nodes calculated using mitogenomes were consistent with evidence from the fossil record (38, 40).

Species concepts allow for hybrid zones. It is important to note that the existence of hybrid elephants does not detract from recognizing forest and savanna elephants as distinct species, even under the biological species concept (BSC) as formulated by Ernst Mayr (29–31, 42). Under the BSC, species are defined as groups of interbreeding natural populations that are reproductively isolated from other such groups (42), which recognizes the role of gene flow in species delimitation (27). As extensive molecular data sets have been generated for elephants over the past fifteen years, under this definition forest and savanna elephants in Africa can be readily classified as distinct species under the BSC (17, 23, 25): Even though the two groups sometimes hybridize, nuclear gene

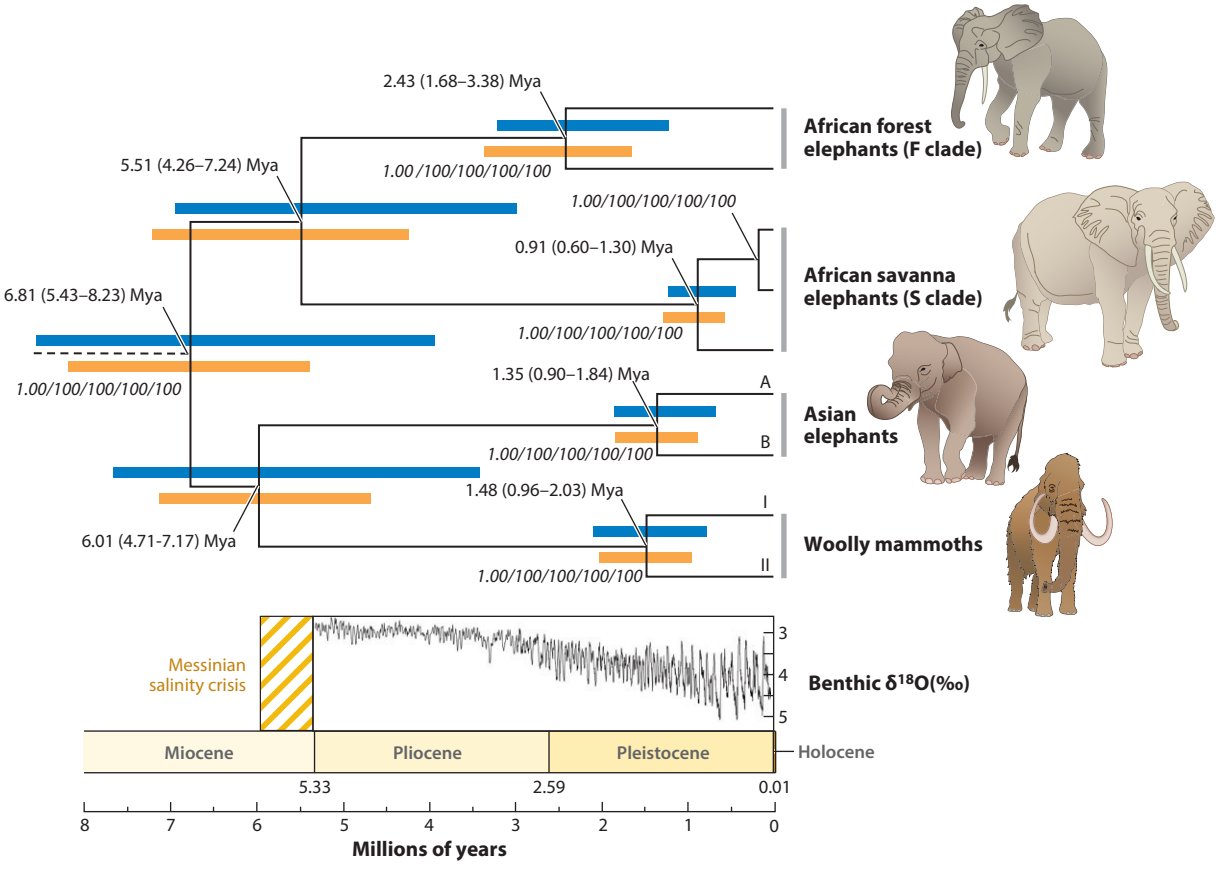

Figure 2

Phylogeny of elephantid species inferred from mitogenomic sequences. Note that the divergence date between forest elephant (F clade) and savanna elephant (S clade) mitochondrial genomes is comparable to the divergence between Asian elephant and mammoth mitochondrial genomes. Within each of the four lineages, the genomes analyzed represent the most basal within-lineage divergences; these indicated that F clade has the deepest within-lineage crown group coalescent date. Blue and orange bars indicate the 95% highest posterior density as determined by using two sets of calibrations. The lower bar relied on a narrower set of calibration dates that excluded fossils identified as being of questionable assignment to genera by Sanders et al. (38) and assumed the monophyly of the elephantid genera (6, 8, 39). A timescale and geological epochs are shown beneath the phylogeny: Lower values for the oxygen isotope curve correlate with lower estimated temperatures (163). The figure is modified from Reference 40 with permission.

flow is almost completely absent between them (17). It may be inferred from mitonuclear patterns that the absence of gene flow is due to a lack of reproductive success for male hybrids (23–25). In his definition of the BSC, Ernst Mayr noted that hybrid zones could exist between validly defined species as long as the genetic integrity of the parent species remained intact (31), which is true for nuclear loci in forest and savanna elephants (**Figure 1**) (17, 23, 26, 35). It is also important to note that the species status of forest and savanna elephants would be evident under any proposed species concept (43), because past debates regarding African elephant taxonomy were not due to the application of differing species concepts. The debates were initially driven by reports of forest-savanna elephant hybrids (14–16), although the genetic data have since shown that hybrid elephants have been reproductive dead ends. Later debates regarding elephant taxonomy were driven by the initial observation of mitonuclear incongruence among elephants, discussed next.

SEX DIFFERENCES AND MITONUCLEAR INCONGRUENCE IN ELEPHANTS

Interspecies Capture of mtDNA

In their review of interspecies hybridization patterns, Petit & Excoffier (27) noted that a defining feature of a species is that its constituent populations tend to be connected by gene flow. In reviewing both simulation studies and real-life examples of interspecies hybridization, they found a negative correlation between intra- and interspecific gene flow, an indication that markers associated with the most dispersing sex would better delimitate species (27). The delimitation of geographic boundaries between two hybridizing species was more effective when using genetic markers that experience a high level of gene flow, "a simple but not widely appreciated prediction" (27, p. 386).

Mitonuclear patterns in elephants conform to this expectation. In elephants, males are the dispersing sex, whereas adult females remain with their core social group. Genetic markers that can be transmitted by the dispersing males, such as autosomal, X-linked, and Y-chromosome loci, show deep separation between forest and savanna elephants and indicate that only a small number of hybrids exist where the current ranges of the two species meet (17, 18, 23, 26, 41). A different pattern is evident for the sole marker that is transmitted only by nondispersing females, the mtDNA. As is the case for nuclear loci, two deeply divergent mtDNA lineages have been detected (21, 23, 41, 44, 45). One of those lineages, called the S clade, has been found only among savanna elephants (21, 23, 41). Across thirty localities within the tropical forest zone, all forest elephants have been found to carry only mtDNA from the other lineage, called the F clade (21, 23, 41). However, approximately 20% of savanna elephants have also been found to carry F clade mtDNA (21, 23, 34, 35, 41), although the overwhelming majority of these F clade savanna elephants otherwise show no evidence of hybridization based on nuclear markers (24, 26, 34, 35, 41, 46).

Such highly dissimilar proportions of forest-typical and savanna-typical alleles among different genetic markers in the same savanna elephant populations and individuals cannot be accounted for by random hybridization (23–25). Rather, the observed pattern could appear only after multiple generations of unidirectional hybridization and backcrossing of forest or hybrid females to nonhybrid savanna elephant bulls (23–26, 47, 48). The mitonuclear patterns among elephants indicate that female but not male hybrids have been reproductively successful and that the inability of male hybrids to mate even after multiple generations of backcrossing is what keeps the two species from merging (23). Each backcross by a hybrid female to a nonhybrid male savanna elephant would dilute the proportion of forest nuclear alleles by half until the populations became overwhelmingly savanna-like in nuclear genes, while retaining the maternally inherited forest-typical F clade mtDNA haplotype (23, 24).

In effect, forest-savanna elephant hybrids of both sexes are evolutionary dead ends. The segregation of nuclear markers between forest and savanna elephants indicates that male hybrids are reproductively unsuccessful (17, 18, 23, 26, 41). The finding that 20% of savanna elephants carry F clade mtDNA derived from forest elephants (21) indicates that female hybrids do transmit their mtDNA successfully (21, 23, 34, 35, 41). But the female hybrid elephants have no way of effectively transmitting their nuclear DNA to future generations. Their daughters cannot transmit their nuclear alleles to other groups because they do not disperse; their sons can be inferred to be reproductively unsuccessful (23). The mtDNA that derived from forest elephants persists in the natal social group, but the nuclear DNA that derived from forest elephants is over time completely replaced by that of savanna elephants, maintaining the distinction between the two species (23–25, 41, 46).

What prevents the male hybrids from reproducing is unclear. There is no evidence of physiological sterility, but size and aggression likely play a role (3, 5, 47, 48). DNA-based paternity analyses in savanna elephants have established the strong dependence of male reproductive success on age (and therefore size) and musth status (49, 50). Because forest elephants are so much smaller than savanna elephants (15), fitness of hybrid males may be presumed to be lower than that of savanna males in regions where both species coexist. Other species isolation mechanisms may also be involved (24, 25, 33), especially because no nuclear or mitochondrial gene flow at all has been detected from savanna into forest elephant populations. Elephant males disperse from their core social groups, but females do not (1, 2, 5, 49, 50). Because males can transmit every locus except mtDNA, the mitochondrial genome is subject to a different evolutionary trajectory than other genetic loci (25, 26). MtDNA can develop a phylogeographic pattern that differs from other markers, and mtDNA signatures also tend to persist within a geographic region (23–26, 41, 51, 52). It should be noted that mitonuclear discordance has been reported to occur within and between species for many taxa (27, 53), not just elephants.

The discordant mitonuclear patterns in elephants may potentially affect the interaction of proteins coded by the two genomes. A recent study combined mtDNA genome sequencing with structural biology analyses to look for signatures of selection on residues in the mitochondrial DNA of S clade savanna elephants and F clade forest elephants (54). Signatures of positive selection acting on residues in mitochondrial proteins were detected (54). The residues showing signatures of selection were hypothesized to have played a role in the metabolic adaptation of forest and savanna elephants to their unique habitats (54). This would lead to further questions, such as how selection may play a role given that the mitochondrial genes are transmitted only by the nondispersing sex (27), or how the 20% of savanna elephants that carry F clade mitogenomes (21, 23, 25, 34, 35, 41) may be affected.

The local persistence of mtDNA variants was recently used to determine the source population for a group of savanna elephants that had recolonized community-owned land within the trans–Rift Valley region in Eastern Africa (55). The mtDNA (and to a lesser degree the microsatellite) allele frequencies of colonizing elephants differed from those in most of the potential source populations (55). However, the colonizing elephant genotypes showed no significant differentiation from elephants in the Serengeti/Masai Mara ecosystem, which was inferred to be their source population (55). Thus, connectivity and population viability were found to be possible outside of formally protected areas in the region (55).

Genetic Studies of the Elusive Forest Elephant

Most research on African elephants has involved the savanna species, although some genetic studies have focused specifically on forest elephants. Using multilocus microsatellite genotyping of noninvasively collected samples from forest elephants at Kakum National Park, Ghana, Eggert et al. (56) estimated population size, sex ratio, and genetic variability. The population size estimate for the park proved quite close to that obtained using nongenetic methods (56). Dung samples were also used to genetically examine the relatedness structure and historical demography of forest elephants at two sites in southwest Gabon (57). Pairwise relatedness values were significantly higher within spatially associated dung piles than between random pairings for one site (57). Pairwise relatedness estimates suggested that forest groups are largely composed of adult females, their sisters, and juvenile offspring (57). Based on the proximity of dung samples that were genotyped among forest elephants in Lope (Gabon), individuals within groups were significantly more related to each other than to individuals between groups, and most groups were composed of individuals of the same mtDNA matriline (58). Some individuals were inferred to have a larger

number of associates than observed from group sizes alone (58). Likewise, in Gabon genetic markers were used to detect the movements of forest elephants within and between seasons, which often involved travel to and from wetlands and habitats bordering lagoons (59). Multiple use areas bordering protected areas were found to provide year-round habitat for some elephants and additional habitat for others whose primary range was in parks, suggesting that areas not reserved for conservation were important for the wide-ranging forest elephants (59). Finally, there is evidence from a multispecies study that the ratio of mtDNA to nuclear coalescent dates is lower for forest elephants than for savanna elephants (40), consistent also with a high effective population size that has been detected for nuclear markers in forest elephants (39) (see sidebar, Mitochondrial Versus Nuclear DNA Diversity). This in turn suggests that male–male competition may not be as intense among forest elephants as among savanna elephants (39, 40), a prediction that awaits field studies for verification. An association of reproductive success with body size or musth is not well established in forest elephants, as it has been in savanna elephants (3, 5, 60–63).

Other Genomic Inferences

Genomic sequencing has been conducted on elephants (64–66) and their fecal microbiota (67). Several studies have identified genome components such as transposable elements or nuclear copies of mtDNA (numts) (68–71), and an expanded set of olfactory genes has been detected (72). Additionally, elephant immune system genes, including some genes within the major histocompatibility complex, have been examined, some in the context of diseases that affect captive elephants (73–75).

The elephant genome may provide insights into some aspects of human evolution. Elephants are one of the few mammalian lineages in which large brains evolved (24, 76, 77). One genomic study compared genes under selection in the lineage leading to humans (from mice to nonhuman primates to humans) to genes under selection in the lineage leading from tenrec to elephant (78). Nuclear genes that form complexes involved in mitochondrial function had high ratios of nonsynonymous to synonymous mutations (dN/dS) in both the human and the elephant lineages (78). A subsequent study examined genes under selection in dolphins, detecting high dN/dS ratios in 27 genes associated with the nervous system, including those related to human intellectual disabilities, synaptic plasticity, and sleep (79). As was the case with elephants, in the dolphin lineage a high

MITOCHONDRIAL VERSUS NUCLEAR DNA DIVERSITY

Do not confuse mtDNA diversity with nuclear genetic diversity when comparing forest and savanna elephants. African forest elephants show very high levels of genetic diversity when compared with the diversity present in other elephantids, including savanna elephants. This high diversity is evident among autosomal DNA sequences, X- and Y-chromosome sequences, and microsatellite markers (17, 23, 39). Among mtDNA, the forest elephant–derived F clade also shows greater diversity than the savanna elephant–derived S clade (40, 41). But the savanna species has greater mtDNA diversity than the forest species. Why? The species-isolating mechanisms that prevent nuclear alleles from flowing between the two elephant species also prevent S clade mtDNA from entering forest elephant populations. By contrast, F clade mtDNA (but not nuclear DNA) has been transferred from forest elephants into many savanna populations. Savanna elephants are therefore more diverse than forest elephants in mtDNA because they not only carry all of their own S clade mtDNA diversity but also have captured much of the F clade mtDNA diversity present in forest elephants.

mean dN/dS ratio was detected in nuclear genes coding for proteins that form mitochondrial complexes, suggestive of evolutionary changes in energy metabolism (79). Based on these findings, it has been suggested that parallel molecular trajectories may have occurred in cetaceans, elephants, and primates as the different lineages evolved large brains (78, 79). Subsequent studies may help to determine the functional roles of these genes, or to examine whether polymorphisms in the genes may affect behavior (80).

EVOLUTIONARY HISTORY

Proboscidean Relationships

Elephants and related extinct forms are classified within the mammalian order Proboscidea, which diverged approximately 64 Mya from lineages leading to hyraxes (order Hyracoidea) and to manatees and dugongs (order Sirenia) (6, 13, 38, 81–83). Based on morphological criteria, it had long been recognized that these three orders of placental mammals were related, and they were grouped together as the Paenungulata. However, the relationship of these groups to other mammalian clades was resolved only after large data sets of DNA sequences revealed that the Paenungulata were related to golden moles, elephant shrews, tenrecs, and aardvarks (83, 84). The superordinal clade containing these related orders was designated Afrotheria (African beasts) because this group, which diverged from other mammals approximately 103 Mya, likely originated in Africa during a time when it was isolated from other continents (84, 85).

Within the order Proboscidea, the three living species of elephant are similar enough to be classed as members of the family Elephantidae, which first appears in the fossil record in the late Miocene (6, 8, 38). Many other now-extinct proboscidean species survived as late as the end of the Pleistocene or into the Holocene (6, 8, 38, 86). These included nonelephantid species belonging to other proboscidean families, such as the American mastodon (*Mammut americanum*) in North America, classed into the family Mammutidae, which diverged from elephants some 24–30 Mya (6, 38, 87–89); elephantid species such as *Elephas (recki) iolensis*, a cousin of the extant Asian elephant that predominated in the African savannas until the Late Pleistocene (8, 10, 38); and mammoths (genus *Mammuthus*) living in North America and Eurasia (6, 8, 90). The late survival of these and other species and families of proboscideans suggests that some of their fossils may be amenable to ancient DNA studies.

The late survival of *Elephas* in Africa may be of particular interest since it may inspire speculation as to whether forest and savanna *Loxodonta* may have been geographically isolated from each other by *Elephas*, especially as *Elephas* in Africa was a meter taller than the modern savanna elephant and survived until at least 35,000 years ago (8, 10, 38). The reasons for the disappearance of *Elephas* in Africa also remain speculative, as does any potential role for humans in their extinction (8, 10, 38).

Mammoth Undertakings

One question definitively settled by ancient DNA studies is the relationship of the woolly mammoth (*Mammuthus primigenius*) to living species of elephant (**Figure 2**). Morphological studies tended to place mammoths closer to Asian elephants than to African elephants (6, 8, 38). However, initial studies relying on short mtDNA sequences or limited nuclear DNA sequencing tended to give disparate results. Many studies found woolly mammoths to be closer to Asian than African elephants, but some placed mammoths closer to African elephants, whereas a few placed them outside of an Asian-African elephant clade (32, 91–94). The question was resolved as complete

mitochondrial genome sequences were generated not just for elephants and mammoths (95–98) but for the extinct American mastodon, which served as an appropriate outgroup (99). This established that mammoth mitogenomes were closer to Asian elephant than to African elephant mitogenomes (99). A later study added mitogenomes from the forest elephant (40) and additional fossil calibration dates (**Figure 2**) (38). The mammoth and Asian elephant divergence date was estimated as 6.0 Mya (versus 5.5 Mya for forest F and savanna S elephant clades) (**Figure 2**). The four elephantid lineages began differentiating 6.8 Mya, in the late Miocene (**Figure 2**). This may have been driven by drier climates in Africa (40), as *Loxodonta*, *Elephas*, and *Mammuthus* all originally evolved there (8, 38). Within species, coalescent estimates were oldest for the F clade, at 2.4 Mya, suggesting that decreases in tropical forest cover during Pleistocene glacial cycles had geographically isolated distinct African forest elephant mitochondrial lineages (**Figure 2**) (19–21, 40, 41, 44, 45).

Given that mitochondrial DNA may demonstrate a distinctive evolutionary trajectory from that determined using nuclear DNA (100), the relationship of the mammoth to extant elephants was also examined using nuclear sequences (39, 101, 102). Random genomic sequences were generated using American mastodon DNA, and the reads were compared with the African savanna elephant genome, which was used to design flanking primers for PCR (39). Sequences of 375 nuclear DNA segments totaling 39,763 bp were generated for the African savanna elephant, African forest elephant, Asian elephant, and extinct American mastodon and woolly mammoth (39). The Asian elephant was found to be the closest living relative of the extinct mammoth (39). Once again, savanna and forest elephants proved to be about as divergent as mammoths were from Asian elephants (39).

The woolly mammoth had several morphological adaptations that allowed it to survive in its circumpolar distribution. Some genes responsible for these adaptations have been examined. In the woolly mammoth hemoglobin (Hb) protein, the heat of oxygenation was found to be lower in mammoth than in elephant Hb, which appears to be an adaptation to the harsh high-latitude climates of the Pleistocene glacial periods (103). The loci responsible for the long hair of the woolly mammoth have been searched for but not yet identified (104).

In addition to the periglacial woolly mammoth, Late Pleistocene North America was also home to the Columbian mammoth (*Mammuthus columbi*), which had a physically larger size and ranged into more temperate regions (8). Although paleontologists consider the two mammoth species to be highly divergent, the mtDNA sequences of *M. columbi* proved to fall within a highly derived subclade previously established for the woolly mammoth (105). It appears that interspecies capture of mtDNA genomes had occurred between Columbian and woolly mammoth populations via introgression (105), much as savanna elephant populations have captured F clade mtDNA (23) that originally derived from forest elephants (21). This explanation would have to be confirmed by nuclear DNA, e.g., by showing that the genetic integrity of Columbian and woolly mammoths remained intact despite what appears to be evidence of mitochondrial interspecies capture. The finding that Columbian and some woolly mammoths carry similar mtDNA was a dramatic confirmation of previous reports of mtDNA incongruence and mitochondrial transfer across species in elephantids (23). It may also prepare ancient DNA researchers to expect the unexpected when comparing mtDNA patterns in taxa that can be clearly distinguished morphologically among extinct proboscideans (25, 100, 105).

The Future of Ancient DNA

The continued improvement in ancient DNA methods (98, 101, 106–112) may help to resolve the relationships among an increasing number of fossil taxa. Future studies may examine nuclear

DNA from woolly and Columbian mammoths to establish their (nuclear) genetic distinctiveness (105, 111), or to provide additional insights into within-species population structure and into genetic changes associated with their extinction (86, 90, 110, 113–116). Nuclear DNA studies of woolly mammoths would resolve whether two distinctive mtDNA clades detected in woolly mammoths (clades I and II; **Figure 2**) correspond to individuals of the same or different species (117, 118). Gene flow from North American to Eurasian mammoths has been proposed based on temporal patterns detected using mtDNA (119). Given the mitonuclear incongruence detected in elephantids, the availability of additional nuclear DNA sequences from extinct species will draw fuller pictures of intercontinental gene flow and other ancient events (see sidebar, Ancient African War Elephants).

For fossils in which the DNA has completely degraded, information may be acquired from their protein remains. In one study involving specimens of a 43,000-year-old woolly mammoth and a more recent Columbian mammoth, 126 unique accessions were identified, mostly low-abundance extracellular matrix and plasma proteins. The Columbian mammoth fossil was from temperate latitudes, indicating the potential of this approach beyond subpolar environments (120). Protein and DNA analyses were recently used to definitively show that an elephant fetus referred to by Carl Linnaeus in his description of *Elephas maximus* was in fact an African elephant (64). Through some sleuthing, an Asian elephant skeleton in a Florence museum was identified as a more relevant potential type specimen among the exemplars of *E. maximus* that Linnaeus mentioned (64, 121).

GENETICS AND ELEPHANT CONSERVATION

Evolutionary History and Conservation of Asian Elephants

Several studies have extensively sampled Asian elephants from across the continent or looked at zoo elephants believed to derive from different Asian populations (122–126). Asian elephant mtDNA haplotypes were found to cluster into two distinctive clades, sometimes designated A and B (**Figure 2**) (122–126). Based on full mtDNA coding sequences, the clades are estimated to have diverged approximately 1.35 Mya (**Figure 2**) (40). The geographic distribution of the two clades

ANCIENT AFRICAN WAR ELEPHANTS

In the ancient world, Asian and African elephants were used in warfare. The Ptolemaic pharaohs of Egypt trained African elephants for their armies, and Hannibal and the Carthaginians may have used African elephants from a now-extirpated Maghreb population. The Greek historian Polybius mentioned in one account that African war elephants were smaller than Indian war elephants. This was likely based on a misconception regarding the size of Indian elephants on the part of Polybius and other ancient writers. But some modern readers have taken the statement by Polybius to mean that ancient war elephants must have been forest rather than savanna elephants. A series of conjectures, assumptions, and interpolations, magnified by repetition and by the attempt to reconcile disparate accounts into a coherent picture, have resulted in a mythological corpus suggesting that forest elephants had historically ranged across the Atlantic coast of the Sahara into the Maghreb and east to the Horn of Africa. Several rather confused taxonomic designations across this geographic expanse have been proposed based on this view. The modern conjectures and beliefs surrounding the purported use of forest elephants in ancient warfare are astounding, given the complete lack of evidence (142). Should samples of the extinct North African elephant become available, ancient DNA studies may be able to lay this issue to rest.

shows incomplete geographic partitioning, suggesting allopatric divergence and secondary admixture (**Figure 3**) (122). The allopatric divergence may have occurred in different glacial refugia, with one clade in the Myanmar region and the other clade possibly in southern India–Sri Lanka, with a later isolation in the Sunda region (125). Examination of large numbers of elephants in a regional population in southern India revealed that population bottlenecks, social organization, and biogeographic barriers have shaped regional distributions of nuclear and mtDNA genetic variation (127), and genetic markers are increasingly being used to examine the population size, age structure, and social organization of Asian elephants (128, 129).

Currently, three subspecies of Asian elephant are generally recognized (*Elephas maximus maximus*, *E. m. indicus*, and *E. m. sumatranus*), although more than a dozen have been proposed (130, 131). The mainland subspecies, *E. m. indicus*, includes all Asian elephants except for several island populations. However, mainland elephants in the Malay Peninsula are said to have somewhat distinctive DNA patterns (**Figure 3**) (123) and are purported to display morphological differences (131), suggesting that fast-evolving nuclear markers, such as microsatellites, should be used to compare elephants in the Malay Peninsula to others across mainland Asia. Among zoo elephants, nuclear genetic patterns do not show partitioning between elephants that carry the two deep clades of mtDNA (126), suggesting that, as in African elephants (26), mtDNA may not be suitable for inferring overall population structure, and suggesting the need for a comprehensive survey involving microsatellite markers for elephants from across all of Asia to determine whether the typological criteria used historically to name the subspecies are supported by genetic differences across populations (132).

The Asian elephant was first named scientifically by Linnaeus, based on a specimen believed to be from Ceylon (Sri Lanka) (64), and the Sri Lanka elephant is still recognized as a distinct subspecies, *E. m. maximus* (131). Sri Lanka was historically a center for trade in domesticated Asian elephants, and there is debate as to the degree that this trade may have affected mtDNA patterns on the island (122, 123, 125, 133). Interestingly, significant genetic differentiation of mtDNA has been reported between the mainland and Sri Lanka and between northern, mid-latitude, and southern regions in Sri Lanka (122). Although within-island population structure would be consistent with the claims by earlier naturalists that several morphologically different subspecies existed on the island (130), it seems unlikely that the island would harbor multiple distinct populations of Asian elephants. Male-mediated gene flow would tend to erase nuclear genetic distinctions, so any persistent mtDNA geographic partitioning may not reflect nuclear population structure (26). Whether Sri Lankan elephants are different enough from mainland forms to merit subspecies status, and whether any nuclear population structure persists on the island, are questions that remain to be answered by nuclear genetic analyses across Sri Lankan populations.

The other widely recognized subspecies is *E. m. sumatranus*, the Sumatran elephant (131). This appears to be a valid subspecies that should be treated as a separate evolutionarily significant unit or conservation unit, because there are distinctive mtDNA haplotypes on the island (**Figure 3**) as well as diagnostic morphological traits (notably, a difference in the number of ribs), and given the isolation of elephants on the island from other populations since at least the end of the last glacial cycle (123, 125, 131). The population of Sumatran elephants is low, and their habitat continues to be destroyed, making them a priority for conservation efforts among the living elephants (5).

The rare and inbred (65) elephants on Borneo are often grouped into the Sumatran elephant subspecies (5). However, their mtDNA shows molecular divergence, and all Borneo elephants have a haplotype unique to the island (**Figure 3**) (124). Whether the Borneo elephants represent the remnants of an endemic population (124), or whether they represent a historic release of elephants from a Javan population that was subsequently extirpated (134), it would seem that they preserve

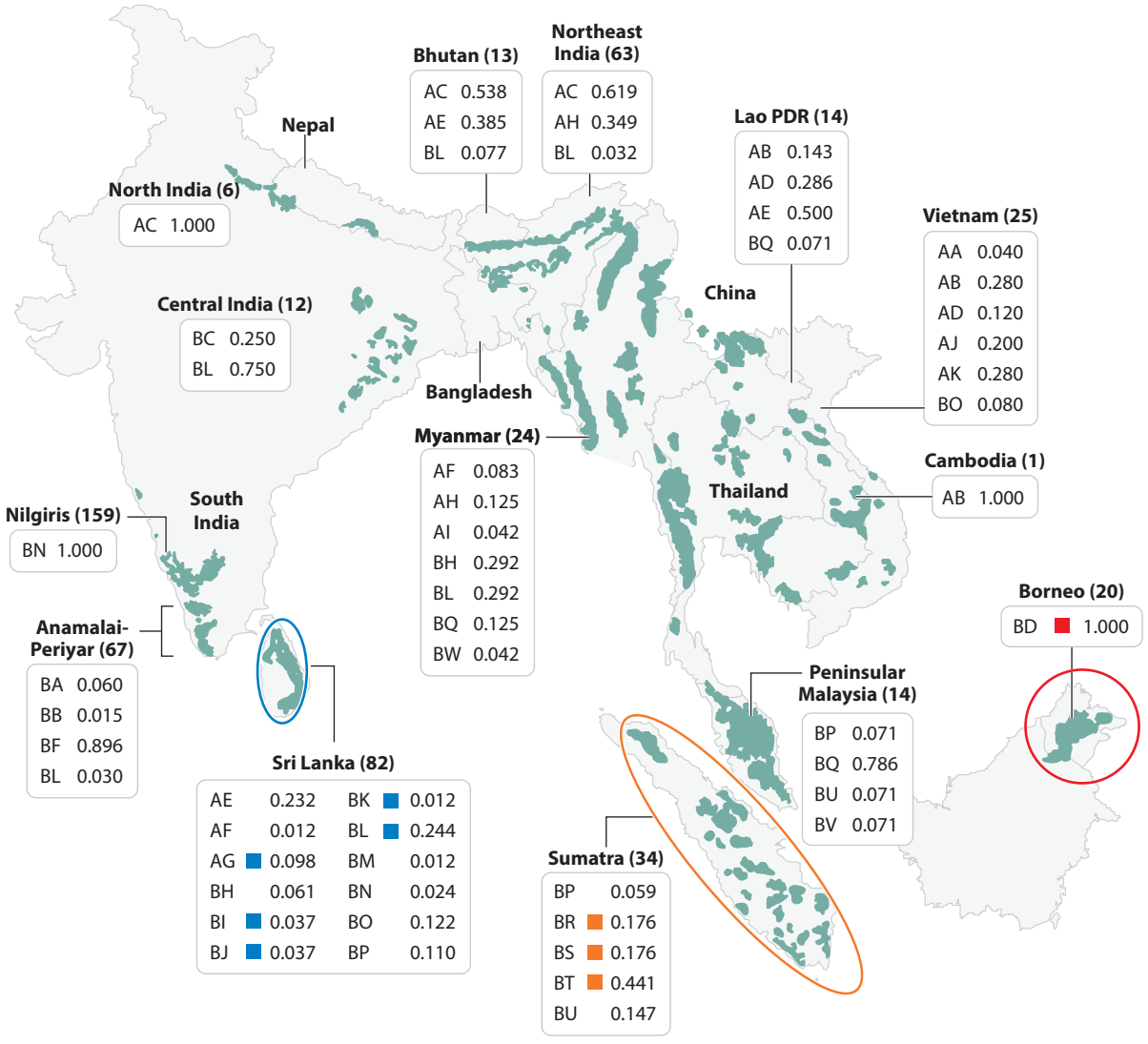

Figure 3

Map showing the current range (*teal*) of Asian elephants along with their mitochondrial DNA haplotypes. Haplotypes fall within two major clades (122–126), estimated to have diverged approximately 1.35 Mya (40). A and B clades are indicated as the first letter of each haplotype designation. For each country, the number of individuals sampled is indicated within parentheses, along with proportions of different haplotypes. Colored circles indicate islands with Asian elephant populations: Sri Lanka (*blue*), Sumatra (*orange*), and Borneo (*red*). Haplotypes unique to any one of the islands are indicated by a box next to the haplotype name (given the same color as the circle around the island). Note that Borneo has a single haplotype (BD) unique to the island. Sumatran and highly diverse Sri Lankan elephants carry some unique haplotypes and some shared with mainland populations. The figure is adapted with permission from Reference 125.

a distinctive lineage and merit treatment as a separate conservation unit. Their potential classification as a discrete subspecies, *E. m. borneensis* (130), awaits a detailed examination of morphology and nuclear markers relative to other populations (124, 131).

Are There Within-Species Conservation Units for African Elephants?

As discussed above, the evidence that African elephants comprise two distinct species is compelling, and they must be treated as distinct units for conservation. Currently, some conservation agencies continue to classify African elephants as a single species (135), but this appears to be due to concerns unrelated to the scientific evidence. Within each species, subdivisions among African elephants have never been shown convincingly. Measurements of the sizes of elephants have suggested that savanna elephants in populations across southern and eastern Africa attain similar shoulder heights (136). Putative geographical differences in morphology have been based largely on anecdotal assertions. Historically proposed subspecies designations were based almost entirely on typological rather than population genetic approaches, were proposed based on a few or even just one specimen, reflected nationalistic rather than scientific concerns, and often did not account for age differences in body size (15, 137). Likewise, attempts to interpret mtDNA patterns as informative for taxonomy have sometimes failed to recognize that mtDNA patterns may be incongruent with overall population structure (23–26, 41).

For forest elephants, there are five described mitochondrial subclades with distinct but overlapping geographic distributions (41), but there is no evidence that these correspond to nuclear genetic partitions in the forest elephant. Rather, the nuclear indels (in *CHRNA1* and *VIM*) indicate extensive gene flow across the Congolian forest (**Figure 1**) (17, 23). The Congolian forest block of Central Africa is not continuous with the Guinean forest block of West Africa (138); however, the degree to which the intervening Dahomey/Benin Gap may have limited gene flow between West and Central African forest elephants has not been established. Forest elephants are sometimes found in West African savanna habitats far from tropical forest habitats (26, 37), which may suggest that the Gap may not act as much of a barrier to gene flow, although it is also unclear whether recent anthropogenic effects played a role in current West African forest elephant distribution. Surveys of forest elephant nuclear alleles have suggested that West African and Central African forest elephants may be genetically similar (26, 32, 139), whereas the large effective population size for forest elephants (39) would have limited genetic drift as a factor in separating the two groups. A prudent approach to conservation would be to treat West and Central African forest elephants as distinct management units until nuclear genetic comparisons are conclusive. One caveat is that the currently small isolated populations in West Africa (135) may show genetic differences owing to drift, inbreeding, or hybridization induced by anthropogenic effects rather than by naturally occurring factors.

Among savanna elephants, there is little differentiation across populations in southern and eastern Africa (18). There are biogeographic reasons for considering whether savanna elephants in West Africa should be treated as a separate conservation unit. In West Africa, savanna elephants historically inhabited the Sahelian/Sudanian savanna habitat belts that extended from east to west, south of the Sahara and north of the tropical forests (13). Whereas savanna elephant habitats in southern and eastern Africa were continuous and unrestricted, Sudanian zone savanna elephants may have been genetically less connected to other savanna populations owing to the long and narrow geography of the Sahelian/Sudanian savanna belts. Genetic studies of elephants in Cameroon have established their similarity to savanna elephants in the rest of Africa (17, 26), although they are different enough that nuclear markers could accurately assign the provenance of Cameroon elephants to a greater degree than they could determine whether a savanna elephant is eastern or southern African (18). A conservative approach to conservation may be to manage Sudanian zone elephants separately until the question is settled. Again, a caveat must be added that future studies are likely to detect differences between West African and other savanna elephants owing to genetic drift, inbreeding, or hybridization induced by anthropogenic effects in small and

isolated populations (135), and that care must be taken to distinguish the effects of genetic drift consequent to anthropogenic factors from those allele differences that resulted from natural historical patterns of gene flow or isolation (see sidebar, The Elephants of West Africa).

Are Genetic Patterns the Result of Natural History or Anthropogenic Effects?

Human activities can affect genetic patterns present among populations, but it may be possible to distinguish patterns owing to natural history from anthropogenic effects. One notable study used 16 microsatellite loci to estimate historical and recent gene flow among African savanna elephant populations across seven protected areas in Tanzania (140). Because these elephant populations remain large (135) and continuity had been disrupted only recently, Fst was expected to still reflect historical gene flow (140). More recent gene flow was examined by using assignment-based tests that are more sensitive to events of the past few generations (140). Historically, gene flow had been affected by persistent landscape features; notably, elephants had avoided regions with steep slopes (140). By contrast, contemporary connectivity was influenced most by human settlement (140). A similar historical pattern was also detected in Kenya, where the steepness of the landscape may have affected the distribution of elephants (52, 55, 141). These findings can inform conservation efforts on large and complex landscapes (140), especially when considering long-term efforts toward widespread restoration of natural areas to reflect historical connectivity and gene flow. One caveat is that nuclear rather than mtDNA markers may be more effective at examining connectivity and gene flow, because mtDNA phylogeography may reflect the persistence of even older patterns that had been determined by climate and habitat during global glacial cycles (24, 41, 142), which may be of little relevance to patterns of connectivity subsequent to the last glacial period.

Elephants have a fission-fusion social organization, whereby stable groups of individuals coalesce into higher-order groups or split in a predictable manner (143, 144). Their hierarchical complexity is rare among animals and offers the opportunity to study the evolution of social behavior (143, 144), potentially providing insights into human evolution. In Amboseli, genetic relatedness was positively correlated with group fission and fusion (49). Adult females remained with their first-order maternal relatives when core groups fissioned temporarily, and core groups that shared mtDNA haplotypes were significantly more likely to fuse than groups that did not share mtDNA (49). Thus, associations between core social groups appear to persist long after the

THE ELEPHANTS OF WEST AFRICA

Did Frade assert that West African elephants are morphologically intermediate? The Portuguese naturalist Fernando Frade consistently argued that Africa's elephants fall into two and only two species. In one publication (13), Frade reproduced a map of Africa listing the many subspecies that had been proposed for African elephants, ending with the category of "indeterminable," which is assigned to places like eastern South Africa, which Frade knew was home to the savanna elephant, but where the subspecies of savanna elephant was uncertain (13). The "indeterminable" category was also given to West Africa, presumably because no one had suggested subspecies trinomials for any elephant specimens west of Cameroon. Recently, it has been asserted that by using "indeterminable," Frade must have meant that West African elephants could not be assigned to forest or savanna species on morphological grounds. This is not consistent with statements made by Frade, within the same publication, assigning to *Loxodonta cyclotis* the elephants of Ghana, Ivory Coast, Liberia, and Sierra Leone (13). Frade also assigns to *Loxodonta africana* the elephants of the "*Soudan occidental, central et oriental*," i.e., the full east-west range of the Sudanian savanna belts immediately south of the Sahara (13). Frade thus placed West African elephants unequivocally into the two species that he recognized.

original maternal kin have died, and inclusive fitness (mediated via the individual and kin) is believed to crystallize elephant hierarchical social structuring along genetic lines when populations are undisturbed (49, 50, 144). By contrast, human depredation, leading to social disruption, is believed to have altered the genetic underpinning of social relations in a different study population in Kenya (144). However, such analyses must take into account the breakdown that occurs over time (owing to male-mediated nuclear gene flow) in the correlation between overall relatedness and similarity of mtDNA (26). Owing to the temporal turnover of dominant males, some individuals of similar age across social groups may share the same sire and be more closely related genetically than individuals of different ages within a single social group (62, 63).

In Mikumi National Park, Tanzania, the population of savanna elephants had been reduced by 75% owing to poaching. In a genetic study conducted 15 years subsequent to the period of highest poaching, it was found that group size, group relatedness, and female social bonds all continued to be heavily disrupted (145). Females would form groups with other non-kin females, join established groups, or remain alone, unable to form stable adult female bonds (145). In Uganda, where the elephant population was decimated in the 1970s and 1980s, females with differing mtDNA haplotypes were found to have come together to form new social groups (146). These disrupted patterns detected using molecular methods may reflect widespread and increasing changes in elephant behavior that are attributable to human activities (147).

Nuclear Markers and Illegal Ivory

Following a decade in which the number of African elephants may have been cut in half, the international trade in ivory was outlawed in 1989. Initially, the ban seemed successful, but poaching has been increasing recently. Owing primarily to demand from China, more than 20,000 African elephants are now killed every year for their ivory (**http://www.traffic.org**). Between 2002 and 2011, illegal poaching across Africa reduced the number of forest elephants by 62% (148). Although illegal ivory may be confiscated during shipment or after delivery, this often occurs far from the range countries where the poaching occurred (149). It may be impossible to determine the source population of elephants, because ivory can be smuggled across multiple international borders and along numerous trade routes, making the poaching hot spots and trade routes difficult to identify (149). Thus, genetic methods are being developed in an attempt to use DNA to track the provenance of confiscated ivory.

A protocol has been developed for the isolation of DNA from elephant ivory involving pulverization, decalcification, and DNA extraction (150). The method appears to be robust, effective even for tusks that had been stored at room temperature for long periods of time (150). A genetic and statistical method to determine the origin of poached ivory was developed that used a smoothing method to estimate geography-specific allele frequencies over the range of African elephants, using fast-evolving microsatellite loci (139). The method was tested with samples of known origin, and initially 50% of elephants could be located by this method to within 500 km of their actual locality (139). This innovative DNA assignment method of Wasser and colleagues was subsequently improved using a Voronoi tessellation approach to examine genetic similarities across tusks to simultaneously infer the origin of multiple samples that could have been poached in one or in multiple localities (151). When tested using elephant DNA of known origin, the joint analysis of many tusks was found to perform better at geographic assignment than methods based on analyzing samples individually (151). A very large ivory seizure was found to derive from savanna elephants estimated to have originated from a narrow east-to-west band of southern Africa, possibly from a single range country (151). In a subsequent analysis involving two sets of seizures that included large volumes of elephant ivory, all ivory seized in each set had common origins, indicating that

crime syndicates had targeted specific populations for intense exploitation (152). This result would be contrary to a view that dealers were consolidating ivory from across Africa for their shipments (152). Instead, it appeared that ivory from one source was being placed in multiple shipments, passing through intermediate countries before being sent to Asia (152). Thus, a risk-reduction strategy of smuggling that would distance ivory dealers from the poaching locality was detected through DNA forensic information (152). Poaching appears to initially have a bias toward male elephants, which declines as the larger males are killed (153). Population assignment methods do not involve matching tusks to the carcass of a poached individual elephant (although, as noted above, DNA can be used to establish the taxon from which ivory was derived). Rather, the findings can enable conservation and law enforcement officials to better understand strategies used for smuggling and allow limited resources to be focused on areas determined to be under poaching pressure (149). This would potentially enable interventions to stop wildlife trafficking before the animals are actually poached.

Additionally, several approaches have been proposed to improve the utility of nuclear markers for use with DNA from ivory. DNA from ivory may be degraded and may exist only in short fragments, as is DNA from dung samples, which are often collected for elephant genetic studies. By reducing the size of the region targeted for amplification, a greater success rate can be achieved with degraded DNA (110, 154). Amplicon sizes have been reduced for microsatellite markers (154, 155), and primers have been developed to amplify short regions of DNA that nonetheless can identify the species (46, 142) or the sex (156, 157) of the elephant. Because microsatellite diversity varies by locus, as does the geographic information provided by each marker, the information content of a marker can be determined, indicating how useful each genetic marker is for distinguishing among elephants from different geographic localities (26). By testing large numbers of loci for their information content, it may be possible to develop and use a set of markers that would be especially effective at identifying the provenance of elephant samples (26). Microsatellite markers may be combined in a multiplex PCR for analyses of savanna elephants (158). Before this strategy can be applied to populations across Africa, the presence in many microsatellite markers of allelic size differences and indels in the forest elephant species (18) must be considered.

Mitochondrial Sequences and Illegal Ivory

MtDNA may also be useful for forensic purposes (but see sidebar, GenBank Taxonomic Assignments Are Not Completely Reliable). MtDNA can determine whether ivory derives from

GENBANK TAXONOMIC ASSIGNMENTS ARE NOT COMPLETELY RELIABLE

GenBank tags for proboscidean sequences may be wrong or incomplete in several ways. First, the taxonomic labeling of forest elephant and savanna elephant sequences in GenBank is inconsistent. Many authors have placed all African elephant sequences under the taxonomic category *Loxodonta africana*, including their sequences of forest elephants. The forest elephant (*Loxodonta cyclotis*) entry in GenBank may include sequences from savanna elephants. Second, some studies have deposited haplotype sequences but without an adequate indication of the names of all localities at which the haplotype is found, or the number of individuals at each locality carrying each distinct haplotype. This makes it difficult to use these sequences for geographic comparisons. Finally, several ancient DNA sequences likely represent contamination or other artifacts. Thus, when the true mtDNA sequence of an American mastodon was first published (99), several previously deposited sequence entries for this species in GenBank proved to be spurious.

a mammoth, an Asian elephant, or an African elephant; however, because many savanna elephants carry F clade mtDNA, it cannot clearly distinguish between African species. Raw and carved ivory has been assigned to a taxon by using mtDNA (159–162); for example, two ivory carvings depicting Lord Krishna were found to derive from Asian elephant tusks (160). The effectiveness of mtDNA for assigning the regional or local provenance of African elephants (or their ivory) has been examined using 653 savanna and forest elephants from 22 localities in 13 countries (41). Using 4,258 bp of mtDNA sequences, eight well-supported mtDNA subclades have been identified, of which seven have regionally restricted distributions (**Figure 4**) (41). Among 108 distinct

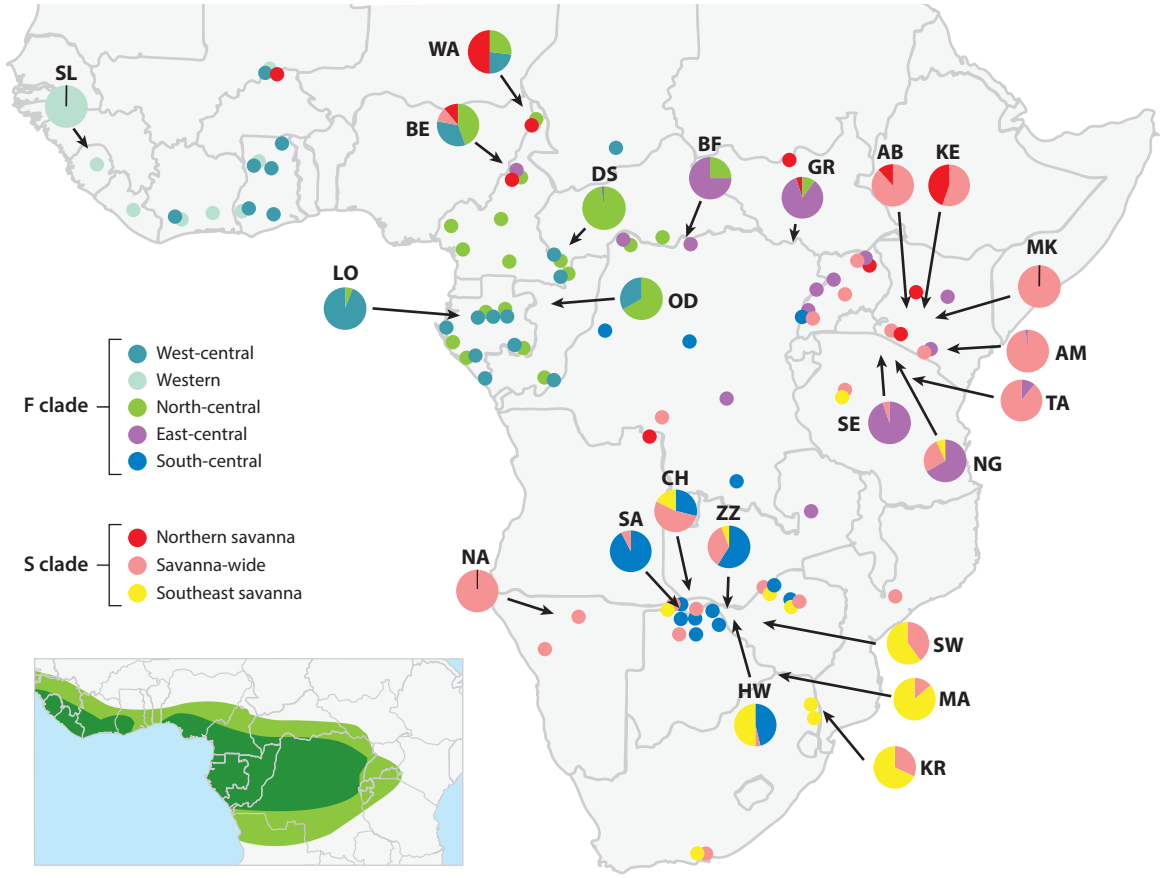

Figure 4

Map showing the geographic distribution of elephant mitochondrial DNA (mtDNA) subclades across Africa. The large pie charts show the frequency at each locality of eight deep well–supported mtDNA subclades (see the color key). The small circles represent the presence of a subclade at a locality (without indicating frequencies). The inset map shows tropical forest (*dark green*) and mixed (*light green*) habitat zones (138), which correspond approximately to the range of the forest elephant. Note the regional distribution of each of the mtDNA subclades. The subclades in turn group into deeper F and S clades: F clade is believed to have originated in the forest elephant, whereas S clade originated in savanna elephants; note that many savanna populations now carry F clade mtDNA, whereas no forest population carries S clade mtDNA. This figure is from Reference 41 and is reproduced under the terms of the Creative Commons Attribution, Noncommercial License. Abbreviations: AB, Aberdares; AM, Amboseli; BE, Benoue; BF, Bili Forest; CH, Chobe; DS, Dzanga Sangha; GR, Garamba; HW, Hwange; KE, Central Kenya/Laikipia; KR, Kruger; LO, Lope; MA, Mashatu; MK, Mt. Kenya; NA, northern Namibia; NG, Ngorongoro; OD, Odzala; SA, Savuti; SE, Serengeti; SL, Sierra Leone; SW, Sengwa; TA, Tarangire; WA, Waza; ZZ, Zambezi.

haplotypes identified, 72% were found at only one locality, and 84% were country specific, whereas 44% of individuals carried a haplotype detected only at their sampling locality (41). The eight subclades of mtDNA included three subclades within S clade, which were found only at savanna localities, and five subclades within F clade, which were carried by forest elephants and by some savanna elephants (**Figure 4**) (41). However, the nuclear partitioning clearly separated the two species and did not follow mtDNA subclade distributions (41), consistent with the separation of African elephants into two distinct species, and consistent with the mitonuclear incongruence detected by other studies (**Figures 1** and **4**) (23, 26, 34, 35).

The control region sequences were then combined with those generated by previous transnational surveys of African elephants, which had sequenced mtDNA from dung or museum samples from across Africa (19–22, 44, 51, 137). The combined data set included control region sequences across 81 localities spanning 22 countries (**Figure 4**) (41). Of 101 distinct control region haplotypes in the combined data set, 62% were present in only a single country (41). Because elephant nuclear and mtDNA markers differ in phylogeographic patterns (**Figures 1** and **4**) (23, 41), combining the two markers would be ideal for triangulating the origins of confiscated ivory (41).

Forensic methods for use with ivory are likely to improve. Future advances are likely to build upon the methods that have led to recent breakthroughs, including more rigorous mathematical approaches, more extensive and intensive sampling, the combination of mitochondrial with nuclear markers, the use of a greater number of markers and of markers with high information content, and improvements in laboratory techniques. DNA forensics can play an important role in conserving elephants, although this must be combined with more serious efforts to curb the demand for ivory, especially by the government of China.

SUMMARY POINTS

1. The genetic evidence overwhelmingly indicates that savanna elephants (*Loxodonta africana*) and forest elephants (*Loxodonta cyclotis*) in Africa comprise separate species.
2. Because female elephants do not disperse from their natal social group, gene flow is male mediated, resulting in distinctive mitochondrial and nuclear phylogeographic patterns.
3. Any conclusions about elephants derived from mtDNA patterns should be subject to strong scientific scrutiny and to verification using other genetic or morphological markers.
4. The ratios of mtDNA to nuclear coalescence among elephant species suggest that savanna elephants and mammoths may have displayed the highest degree of male–male competition, whereas Asian elephants displayed intermediate degrees and forest elephants displayed the lowest. This hypothesis must be confirmed for the extant taxa by field studies.
5. Molecular studies have shown that, in addition to hyraxes and manatees, elephants have a very ancient affinity with golden moles, elephant shrews, tenrecs, and aardvarks. All of these mammalian orders are grouped together into Afrotheria, with a Mesozoic origin on an isolated African continent.
6. Ancient DNA studies have firmly established that the closest living relative of the woolly mammoth is the Asian elephant.
7. Molecular dating suggests that the lineages that gave rise to savanna elephants, to forest elephants, to Asian elephants, and to woolly mammoths all began diverging from each other in Africa in the late Miocene, a time of increasing aridity on the continent.
8. The evolution of large brains in primates, cetaceans, and elephants may have involved similar sets of genes, notably nuclear genes involved in mitochondrial metabolism.

9. Genetic patterns among elephants reflect the effects on gene flow of natural landscape features such as steep slopes and, increasingly, of anthropogenic impacts, such as isolation of populations, reduced population size, and disruption of social bonds.
10. DNA forensics shows promise as a means of establishing the provenance of confiscated illegal ivory and of revealing strategies used by poachers.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

For enabling much of the primary research reviewed here, we thank M. Gadd and R. Ruggiero at the US Fish and Wildlife Service African Elephant Conservation Fund, and specifically Grant AFE-1446-F14AP00464 to A.L.R. We thank T.N.C. Vidya for permission to use a figure from one of her publications. We thank S.J. O'Brien for helpful comments on an earlier version of the manuscript.

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Annual Review of
Animal Biosciences

Volume 3, 2015

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