To split or not to split: the case of the African elephant

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There has been a spate of discoveries of new floral and faunal species since the 1990s (ref. 1). Although one would naturally expect the majority of such discoveries to be microbes, arthropods, molluscs and other speciose taxa, mammals figure reasonably high on this list. Recent discoveries of new mammalian species are, surprisingly, a full 10% of the ~4800 previously described ones, and mammalian subspecies recently recognized as species constitute another 10% at least[2]. In the latter category, falls the possible species recognition of the African forest elephant. The taxonomic designation of the African elephant has been much debated. Ansell[3] divided the genus *Loxodonta* into two groups based on morphology: the forest elephants, including extant and extinct forms, and the savannah elephants, including four different forms. However, both forest and savannah groups were classified as a single species, *Loxodonta africana*, under this scheme, with the six forms being different subspecies[3]. Subsequently, the two groups have been considered as just two subspecies, the African forest elephant, *L. africana cyclotis*, found in the forests of West and Central Africa, and the African savannah elephant, *L. africana africana*, found in savannah areas across Africa (Figure 1). The typical savannah and forest elephants are morphologically distinct: forest elephants are smaller, with rounded and smaller ears, longer and straighter tusks, a large number of toenail-like structures[4], and distinct skull features (Figure 1).

The first mitochondrial DNA (mtDNA) sequence from a forest elephant was significantly different from that of savannah elephants[5], suggesting species differences between the two forms. Skull measurements on a large number of savannah and forest elephants also led to their being classified as two distinct species[6,7]. The first major genetic study that demonstrated species-level distinction between the African savannah and forest elephants was that of Roca *et al.*[8]. Based on four slow-evolving nuclear gene introns from three populations of forest elephants and 15 populations of savannah elephants from southern, eastern and north-central Africa, Roca *et al.*[8] found significant genetic divergence between the two forms, amounting to almost 60% of the Asian–African elephant divergence at the same genes, and therefore, a divergence time between the savannah and forest elephants of 2.63 ± 0.94 million years ago (Mya). In the Garamba area of Central Africa that could not be classified a priori as a forest or savannah population, there was evidence for limited hybridization in the past, but no ongoing hybridization[8].

Although the study by Roca *et al.*[8] did not sample elephants from West Africa, Eggert *et al.*[9] examined mtDNA and nuclear microsatellite DNA in West and Central African populations, but did not find clear genetic separation between forest and savannah elephants. Instead, they found three deep, divergent lineages corresponding to (i) the forest elephants of Central Africa, (ii) the forest and savannah elephants of West Africa, and (iii) the savannah elephants of Central, eastern and southern Africa, and suggested that these lineages arose as a result of allopatric divergence and secondary admixture of lineages due to major climatic changes during the Pliocene and Pleistocene in Africa. Accordingly, Eggert *et al.*[9] proposed that three taxa be recognized within the African elephants. Groves[10] had also previously reported that forest and savannah elephants coexisted and interbred in West Africa, although they were two species according to him.

Another study based on mtDNA control region and nuclear microsatellites found population differentiation between savannah elephants in southern, eastern,

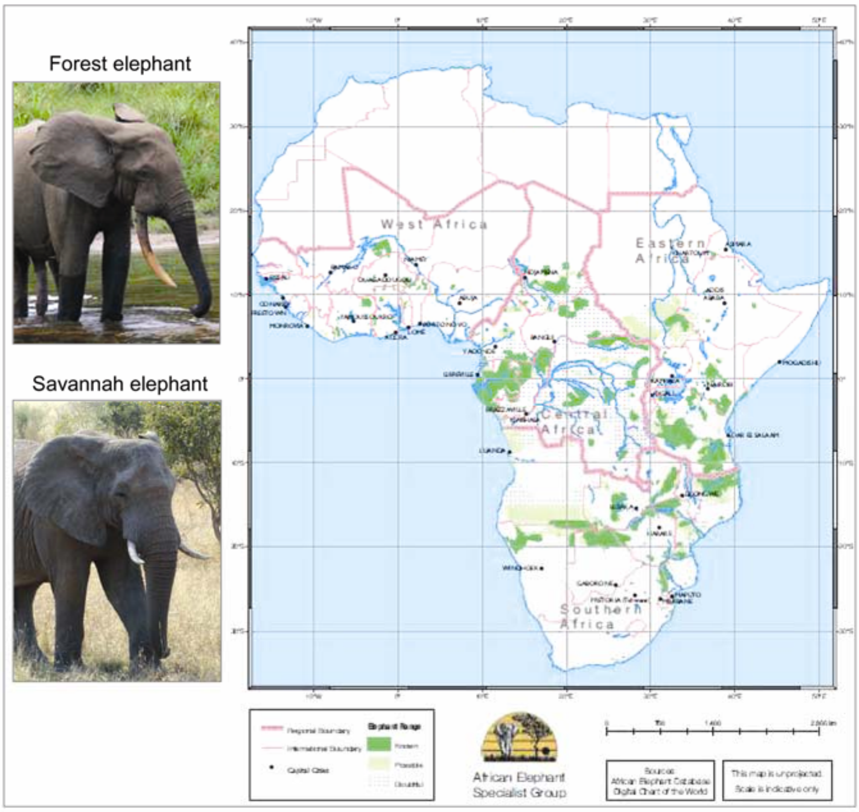

Figure 1. (Right) Map showing elephant distribution in the different regions of Africa. Reproduced from Blanc *et al.*[20], © 2007, International Union for Conservation of Nature and Natural Resources. (Left) A forest elephant male (photo: © Peter Wrege/Elephant Listening Project) and a savannah elephant male (photo: T.N.C.V.).

and West Africa[11]. Although the West African clade showed reciprocal monophyly, haplotypes from the other two divergent clades were found to coexist in eastern and southern Africa, possibly due to allopatric fragmentation during glacial periods and subsequent secondary contact due to expansions from glacial refugia[11]. These results were roughly in keeping with the findings of Eggert *et al*.[9] about savannah elephants. On the other hand, Comstock *et al*.[12], using microsatellite loci, reported species-level differentiation between forest and savannah elephants (using African elephant–Asian elephant differentiation as the comparison), with the latter showing lower genetic diversity, possibly as a result of a population bottleneck. A problem with using fast-mutating microsatellites, however, is that levels of homoplasy (identity by state as opposed to identity by descent) are high, leading to an underestimate of deep divergence, such as that between African and Asian elephants.

A few years later, Roca *et al*.[13] expounded on the observed disparity between the results based on mtDNA and nuclear DNA by analysing X-linked, Y-linked, and mitochondrial genes across savannah, forest and Garamba (that had savannah, forest and morphologically intermediate elephants) populations. They found genetic divergence between forest and savannah elephants based on X and Y chromosomal DNA, and cyto-nuclear genome dissociation (cytoplasmic mtDNA and the nuclear DNA showing different patterns) in African savannah elephants from the mixed forest–savannah habitats, Tanzania, Botswana and Zimbabwe, with forest-typical mtDNA haplotypes and savannah-typical nuclear genes. The absence of forest-typical nuclear genes from populations of savannah elephants with high proportions of forest-typical mtDNA haplotypes was inferred to have resulted from many generations of unidirectional hybridization of forest females or hybrid females to savannah males. The presence of cyto-nuclear genome dissociation in savannah elephants explained the absence of a clear forest–savannah elephant split in studies based on mtDNA and also suggested that mtDNA may not represent true species phylogeny. However, elephants from West Africa were still not sampled by Roca *et al*. in this[13] or in their subsequent[14] study. The divergence time between the savannah and forest elephants was revised to 3.2 Mya by Roca *et al*.[13] because of new *Loxodonta* fossils.

Debruyne[15] carried out a genetic study after first examining the morphotypes of the elephants sampled and also found divergent clades in mtDNA, but while one clade comprised of only savannah elephant haplotypes, the other comprised of all the forest elephant and some savannah elephant haplotypes. This was taken as support for a single species with four evolutionarily significant units[16] approach to the conservation of African elephants[15]. This study was followed by Roca *et al*.[14] re-examining the morphological data of Groves and Grubb[6], and Grubb *et al*.[7], along with several genetic datasets, to examine how forest and savannah elephant morphotypes, mtDNA haplotypes, and nuclear DNA alleles were geographically distributed. The results of Debruyne[15] were reinterpreted to indicate cyto-nuclear genomic dissociation because of an incongruence in characteristics between forest and savannah elephants based on morphology, which would be mostly affected by nuclear DNA and mtDNA, and a congruence between morphology and nuclear gene-based phylogeography. Therefore, the debate seemed settled at the two-species scheme.

However, Johnson *et al*.[17] re-examined African elephant phylogeography based on mtDNA and many additional samples from Central Africa, and declared that the two-species model was unsuitable, especially because elephants in West Africa were morphologically indeterminate but had forest-typical haplotypes. They also opined that there was continued hybridization between the two ecotypes, and stressed the need for using faster evolving markers for elephants in the hybrid zone. Unlike the findings of Eggert *et al*.[9] based on mtDNA, Johnson *et al*.[17] did not find evidence for the West African elephants to form a third distinct lineage, but instead found two divergent lineages within Central Africa, suggesting the presence of at least two glacial refugia and subsequent admixture.

A paper by Rohland *et al*.[18] would seem to have put to rest the subspecies/species debate by finding as deep a divergence between African savannah and forest elephants as between woolly mammoth and Asian elephants. This study was based on 39,763 base pairs of nuclear DNA from 375 loci from the extant African savannah, African forest, and Asian elephants, and the extinct woolly mammoth and American mastodon. Nuclear DNA sequences from extinct taxa are difficult to obtain and nuclear gene sequences in Asian elephants were largely unavailable previously. Shotgun sequencing, bioinformatics analysis to identify homology with African elephant sequences and multiplex PCR protocols[19] were employed to make sequencing of such a large number of nuclear DNA loci from extinct species possible. Analysis of population demographic history using Bayesian methods revealed a conservative divergence time estimate of 1.9–7.1 Mya and a best estimate of 2.6–5.6 Mya between the forest and savannah elephants, both of which were similar to the estimates of divergence between Asian elephant and woolly mammoth. Although studies based on mtDNA suggest the occurrence of more recent gene flow between the forest and savannah elephants, this would also imply deeper initial divergence[18].

Does this recent paper[18] finally ring the death knell for the subspecies/species debate? The discovery of the ancient divergence emphatically supports the existence of two species, *L. africana* and *L. cyclotis*. Although the study has been carried out using only one or two samples per taxon, since the current results are based on hundreds of loci, marked deviations from these results are not expected if other typical forest or savannah elephants are compared. It would be reassuring, however, to have more individuals sequenced. But what of the morphologically indeterminate elephants of West Africa? It would be desirable to sample and sequence such individuals; however, the set of loci used[18] is not likely to uncover another similarly divergent species in this region, since such a large genetic divergence between the West African and typical forest elephants as between forest and savannah elephants would probably not have resulted in the level of morphological similarity observed. However, if the West African elephants are found to group within the *L. cyclotis* clade based on the current set of loci, present or recent hybridization cannot be ruled out, since faster evolving markers will be required to examine more recent time-frames. At present, the one-species designation is ruled out, but it remains to be seen if

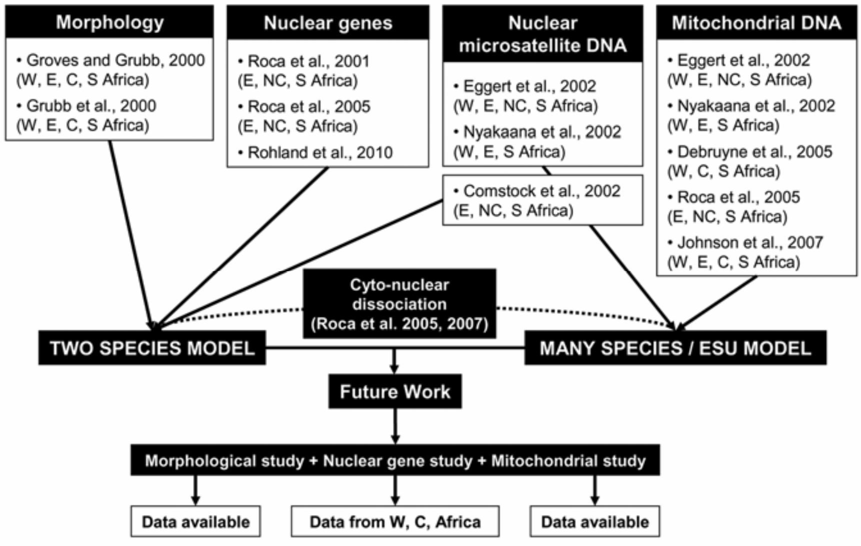

Figure 2. A schematic listing of the studies carried out based on different methods, the regions sampled, and the results found. Cyto-nuclear dissociation found in Roca *et al.*[13] would explain the results found from studies based on mtDNA and is shown by the dotted line. Nuclear microsatellite DNA may show different results than nuclear genes since the former are fast mutating, and should typically be used to examine fine structure. The study of Barriel *et al.*[5], which examined only one forest elephant sequence and, while finding high divergence between forest and savannah elephants, desisted from making inferences about species status, is not included in the figure. Abbreviations: W: West, E: East, C: Central, NC: North-Central, S: South.

there are two or more species of elephants in Africa (a summary of the different studies is shown in Figure 2). Although exploration of new areas has been responsible for the recent discovery of a large proportion of new species[2], this is one of the increasing instances in which technology is helping delineate species.

But why does the number of African elephant species matter at all? Conservation biologists would like to preserve as much of the existing biodiversity as possible, and one of the tenets of conservation biology is to preserve distinct evolutionary lineages. Therefore, if the two forms are accepted as two different species, they would be separately managed and conserved. The number of African elephants (savannah and forest) is estimated at approximately 500,000, with the number of elephants in Central and West Africa being approximately 67,000 (based on the 'definite' and 'probable' categories in Blanc *et al.*[20]). The latter includes savannah elephants also and, therefore, the number of forest elephants is a subset of this. With increasing inroads into Central Africa for purposes of logging, forest elephants are being gravely threatened by poaching for ivory[21]. If species status were accorded to the forest elephant, it could possibly enjoy better protection due to it likely being given an 'Endangered' status based on the relatively small population size, as opposed to the 'Vulnerable' status that the African elephant as a single species carries at present (http://www.iucnredlist.org/). In the absence of consensus amongst scientists previously about the number of species of African elephant, the African Elephant Specialist Group of IUCN–SSC had decided to continue to treat the African elephant as a single species, especially because of the uncertain status of possible hybrids if a two-species split were to be made[20]. With the publication of the work of Rohland *et al.*[18], it is hoped that the forest elephant will get separate species recognition and priority in conservation.

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