

Defining 'Evolutionarily Significant Units' for conservation

Craig Moritz

Craig Moritz is at the Dept of Zoology and Centre for Conservation Biology, The University of Queensland, Qld 4072, Australia.

Writing in the first issue of *TREE*, Ryder¹ brought the term 'Evolutionarily Significant Unit' (ESU) to the attention of a broad audience of ecologists and evolutionary biologists. The ESU concept was developed to provide a rational basis for prioritizing taxa for conservation effort (e.g. captive breeding), given that resources are limited and that existing taxonomy may not adequately reflect underlying genetic diversity². With the explicit recognition of the genetic component of biodiversity in conservation legislation of many countries and in the Convention on Biological Diversity, the ESU concept is set to become increasingly significant for conservation of natural as well as captive populations.

However, the ESU remains poorly defined, both conceptually and operationally. Most definitions suggest that an ESU should be geographically discrete, but genetic criteria range from significant divergence of allele frequencies³ through some level of genetic distance¹ to congruently structured phylogenies among genes⁴. Several authors have argued that an ESU should display concordant divergence for both molecular and non-molecular traits^{1,5,6}. Although all are trying to achieve the same end, it seems that the operational definitions vary according to the biological and legislative context. The purpose of this essay is to revisit the ESU concept in relation to recent developments in molecular population genetics. The suggested definitions and criteria are not supposed to be proscriptive – rather, the intention is to promote debate on the purpose and practice of using genetic information to define conservation units.

Conservation goals: what do we mean by 'significant'?

The overriding purpose of defining ESUs is to ensure that evolutionary heritage is recognized and protected and that the evolutionary potential inherent across the set of ESUs is maintained. For a given set of populations we cannot predict future outcomes, but we can make

inferences about the evolutionary past. Thus, the term 'significant' in ESU should be seen as a recognition that the set of populations has been historically isolated and, accordingly, is likely to have a distinct potential. According to this view, the emphasis is on historical population structure rather than current adaptation. This departs from the more usual concern that we should seek to maintain the full array of differently adapted geographic variants within a species^{3,5}. I suggest that to focus on maintaining the full array of locally adapted variants is not only difficult in practice, but also negates the evolutionary process that we seek to maintain, insofar as preservation of variants adapted to previous conditions may retard the response to natural selection. There may, of course, be other non-evolutionary reasons (e.g. ecological, economic, aesthetic) for ascribing conservation value to a particular population.

The recognition of ESUs is primarily relevant to long-term management issues, that is, defining conservation priorities and setting strategy, although in the short term it may be prudent to avoid translocating individuals between ESUs^{7,8}.

Criteria for recognizing an ESU

Defining an ESU as a historically isolated set of populations leads to a qualitative criterion based on the distribution of alleles in relation to their phylogeny (Fig. 1). Simulation studies suggest that it takes about $4N$ generations from the time that two populations separate for there to be a high probability of their having reciprocally monophyletic alleles⁹. Because of its relatively low effective population size and high substitution rate, animal mitochondrial DNA (mtDNA) is expected to achieve this condition more rapidly than nuclear alleles. Indeed, well-differentiated sister species may have reciprocally monophyletic mtDNA but phylogenetically unsorted alleles at nuclear loci (e.g. northern versus southern elephant seals¹⁰). To require reciprocal monophyly for both nuclear and mtDNA genes (as required for genealogical concordance⁴) seems overly restrictive. Nonetheless, significant divergence in nuclear allele frequencies should be required to avoid misclassifying populations linked by nuclear, but not organellar, gene flow.

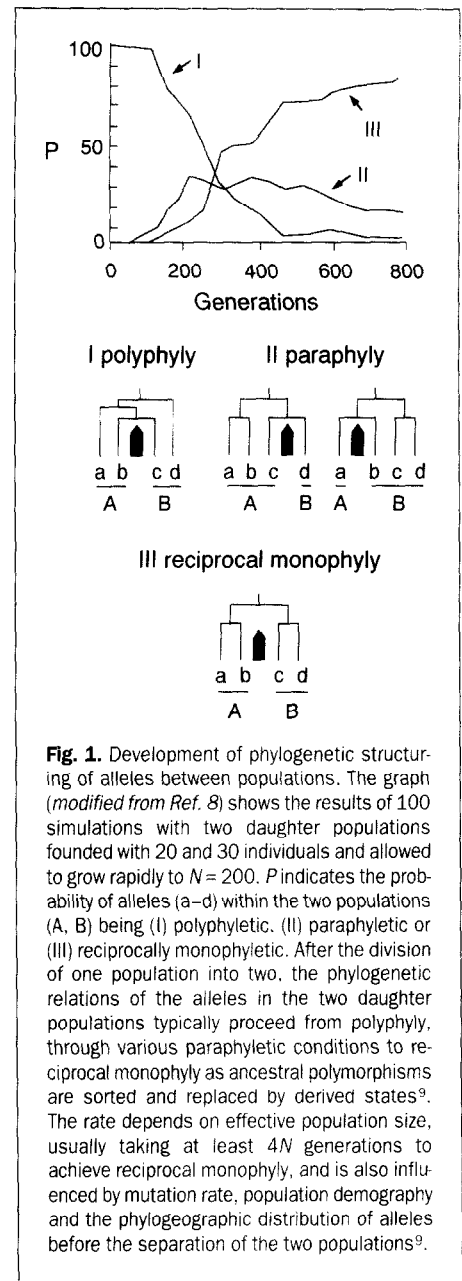


Fig. 1. Development of phylogenetic structuring of alleles between populations. The graph (modified from Ref. 8) shows the results of 100 simulations with two daughter populations founded with 20 and 30 individuals and allowed to grow rapidly to $N = 200$. P indicates the probability of alleles (a–d) within the two populations (A, B) being (I) polyphyletic, (II) paraphyletic or (III) reciprocally monophyletic. After the division of one population into two, the phylogenetic relations of the alleles in the two daughter populations typically proceed from polyphyly, through various paraphyletic conditions to reciprocal monophyly as ancestral polymorphisms are sorted and replaced by derived states⁹. The rate depends on effective population size, usually taking at least $4N$ generations to achieve reciprocal monophyly, and is also influenced by mutation rate, population demography and the phylogeographic distribution of alleles before the separation of the two populations⁹.

The above theory suggests a genetic criterion for recognizing an ESU: *ESUs should be reciprocally monophyletic for mtDNA alleles and show significant divergence of allele frequencies at nuclear loci.*

Although such a definition may seem to be overly restrictive in some cases (see below), it has the advantages of being theoretically sound and of avoiding the issue of 'how much divergence is enough?' that plagues quantitative criteria such as allele frequency divergence and genetic distance. It considers the pattern rather than the extent of sequence divergence, as it is not the intention to ascribe conservation value to an ESU in relation to mtDNA distance.

Contrast with 'management units' and 'stocks'

In practice, genetic analyses often reveal differences between sampled populations ranging from reciprocal mon-

ophyly, through substantial but incomplete phylogenetic separation, to minor but statistically significant differences in allele frequency. Populations that do not show reciprocal monophyly for mtDNA alleles, yet have diverged in allele frequency, are significant for conservation in that they represent populations connected by such low levels of gene flow that they are functionally independent. The recognition of such 'Management Units' (MUs) is fundamental to proper short-term management of the more inclusive ESUs, in that MUs are the logical unit for population monitoring and demographic study.

MUs are therefore recognized as populations with significant divergence of allele frequencies at nuclear or mitochondrial loci, regardless of the phylogenetic distinctiveness of the alleles. The distinction between ESUs and MUs is important, as it affects ways in which genetic evidence is obtained and interpreted¹¹.

To use genetic information effectively, we should therefore distinguish between two types of conservation units, both important for management: ESUs, concerned with historical population structure, mtDNA phylogeny and long-term conservation needs; and MUs, addressing current population structure, allele frequencies and short-term management issues. The concept of discrete 'stocks' as used for marine species^{5,12} sometimes

combines the two types of unit. Dizon *et al.*⁵ attempted to clarify the definition of stocks using a hierarchy of phylogeographic pattern in conjunction with other evidence. Although their scheme was explicit, it remained unwieldy and did not recognize the different conservation goals. I suggest that the term 'stock' be restricted to short-term management issues (e.g. monitoring harvests, etc.) and, in relation to genetics, be treated as synonymous with MUs as defined above.

Application and limitations

The foregoing treatment was written with animal populations undergoing predominantly divergent evolution in mind. In practice, ESUs will usually complement rather than replace 'species' defined under traditional, predominantly morphological criteria (although ESUs and species would be synonymous under some species concepts^{13,14}). Given the shortage of resources for managing major ecosystems, let alone previously described species, it is logical to focus genetic studies on species of greatest concern. However, an exciting extension is to apply these principles to whole communities – using comparative phylogeography to define geographic areas where component species have evolutionary histories separate from their conspecifics¹⁵. This could have considerable significance for planning of regional reserve systems.

The identification of ESUs as defined above requires information on the distribution and phylogeny of mtDNA alleles and on the distribution of nuclear alleles. In contrast, only information on allele frequency is directly pertinent to the delineation of MUs, although for small samples and loci with high substitution rates, sequence information may provide more power for detecting population subdivision¹⁶. These types of data are accumulating rapidly for threatened or exploited species^{11,15,17}. For green turtles (*Chelonia mydas*), the genetic data suggest two ESUs – one in the Atlantic Ocean and the other in the Indo-Pacific – each consisting of numerous MUs (Fig. 2). Here, the black turtle (*C. m. agassizi*) represents just one MU within the larger Indo-Pacific ESU. The humpback whale (*Megaptera novaengliae*), another intensively studied species, appears to represent a single ESU with numerous MUs, many of which correspond to major stocks recognized from migration routes¹⁸.

As with any evolutionary property of populations or species, the definition of the ESU needs to be applied with common sense. In some circumstances it may seem overly restrictive. For example, where there has been rapid speciation or recent hybridization, mtDNA alleles may not yet be sorted between otherwise discrete taxa. However, the failure to define these as separate ESUs should

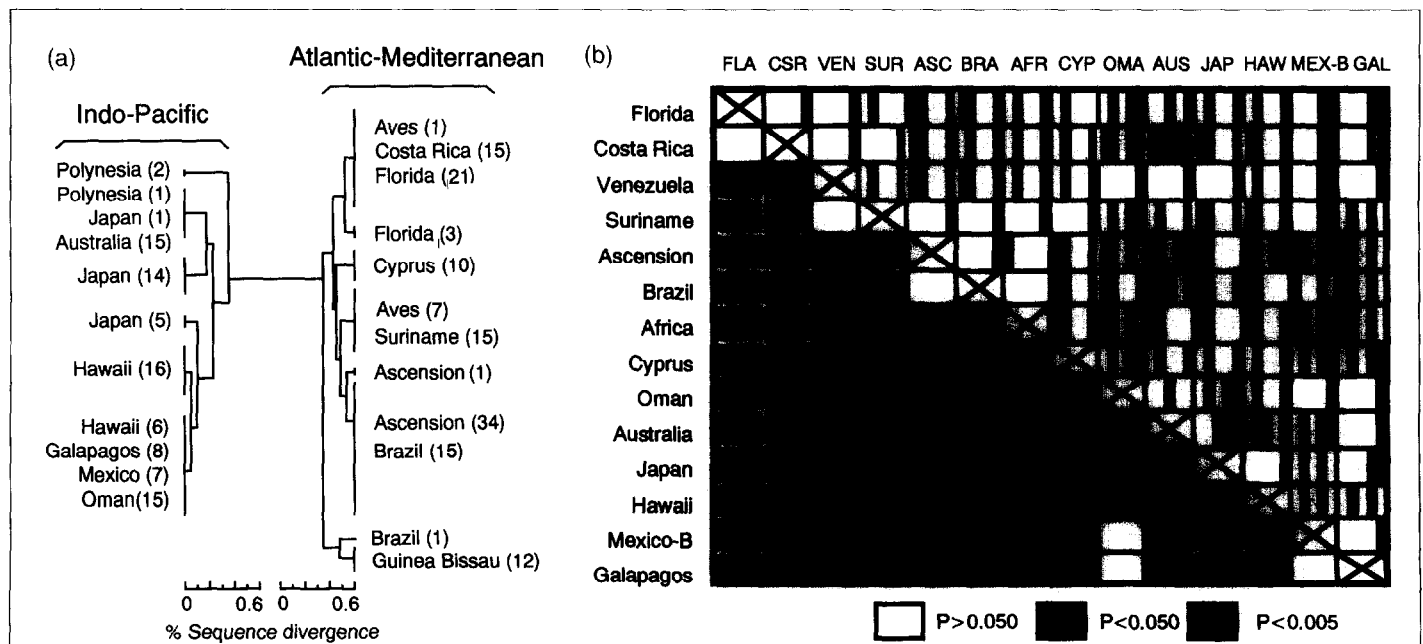


Fig. 2. Defining conservation units for green turtles (*Chelonia mydas*). (a) UPGMA dendrogram of mtDNAs analysed from 15 rookeries of green turtle spanning most of the global distribution (redrawn from Ref. 20). (b) Tests of heterogeneity of allele frequencies at mtDNA (below) and five nuclear loci (above) among the same 15 rookeries. The first eight rows or columns are the Atlantic-Mediterranean rookeries (redrawn from Ref. 21). Rookeries throughout the global distribution of the green turtle have been screened for restriction fragment length polymorphisms of mtDNA²⁰ and anonymous single copy nuclear sequences²¹. A major phylogeographic break is evident between mtDNAs from Atlantic-Mediterranean and Indo-Pacific rookeries (a), supported by slight, but significant variation in nuclear genes. Structuring of allele frequencies among rookeries within either area was substantial for mtDNA and less marked, but still significant for the nuclear loci (b). Accordingly, the species should be managed as two ESUs (Atlantic-Mediterranean and Indo-Pacific) each consisting of multiple MUs. Reanalysis of mtDNAs by sequencing of control region sequences can greatly increase the resolution of MUs (e.g. from 3 to 9 in the Australasian region²²) but has not altered the perception of ESUs.

not affect conservation priorities because the taxa in question are probably already recognized as species on broader biological criteria. A group of populations, such as North Atlantic humpback whales, which shows substantial but incomplete phylogenetic sorting of mtDNA alleles¹⁸, would not be classified as a separate ESU, but still warrant conservation attention as separate management units. Conversely, the criteria may be oversensitive in some cases in species of very low vagility where most local populations are strongly differentiated for mtDNA and nuclear genes (e.g. *Ensatina*¹⁹). In this circumstance, the genetic differences need to be interpreted in the context of the total variation within the species. An additional caveat is that the identification of ESUs and MUs is susceptible to error because of insufficient sampling: the analysis of too few individuals or populations could lead to the false recognition of ESUs; sampling too few nucleotides or too few nuclear loci could lead to failure to recognize important genetic patterns.

Future directions

The concepts and criteria for ESUs and MUs expounded above seem logical and theoretically valid, but it remains to be seen whether they are practical. Pertinent data are expanding rapidly, but there is

a need for further theoretical study of the dynamics of allele distribution and phylogeny in demographic contexts relevant to threatened and exploited species. This is certainly a field where close interaction between experimental and theoretical biologists would pay off.

Acknowledgements

Thanks to John Avise, Brian Bowen, Peter Dwyer, Peter Hale, Shane Lavery, Nancy Fitzsimmons and Steve Palumbi for critical reviews of the manuscript, to Anita Heideman and Lyn Pryor for preparation of figures and to the inmates of the Conservation Genetics lab for inspiration. Supported by grants from the Australian Research Council.

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The early evolution of life: solution to Darwin's dilemma

J. William Schopf

William Schopf is at the Dept of Earth & Space Sciences, Center for the Study of Evolution and the Origin of Life, Institute of Geophysics and Planetary Physics, and Molecular Biology Institute, University of California, Los Angeles, CA 90024, USA.

In 1859, Charles Darwin stated the problem:

'If the theory [of evolution] be true it is indisputable that before the lowest Cambrian stratum was deposited long periods elapsed ... and that during these vast periods the world swarmed with living creatures [However], to the question why we do not find rich fossiliferous deposits belonging to these earliest periods ... I can give no satisfactory answer. The case at present must remain inexplicable ...' (Ref. 1, Ch. X)

Surprisingly, it was not until more than a century later, with publication of three pivotal papers in 1965^{2–4} and of a major monograph in 1968⁵, that search for the 'missing' Precambrian fossil record was demonstrated to be a fruitful area of scientific inquiry. Since that time – in a scant three decades – more than 3000 taxonomic occurrences of microscopic fossils have been discovered in nearly 400 Precambrian geological formations^{6,7}; the new field of Precambrian paleobiology has emerged, matured and become established worldwide as a viable sub-discipline of the natural sciences⁸; and most recently, two mammoth compendia, prepared by international groups of respected experts, have summarized the status of this interdisciplinary area of science^{6,7}. To a major extent, Darwin's dilemma has been resolved – much of the missing fossil record has been uncovered. What has been learned? Where does this young field go from here?

What has been learned?

As in any emerging area of science, numerous new generalizations have been drawn, four of which stand out as being particularly significant.

(1) *Life originated very early in Earth history, much earlier than had been assumed.* Before the discovery of the Precambrian fossil record, few imagined that the well documented history of Phanerozoic life – the familiar progression from seaweeds to flowering plants, from trilobites to humans – was merely the tip of the evolutionary iceberg. Indeed, the recent discovery of diverse cellularly preserved microorganisms in the 3465 ± 5-million-year-old Apex chert of Western Australia⁹ indicates that the Phanerozoic temporally encompassed less than 15% of all of evolution, and that living systems have existed for more than three-quarters of the history of the planet (Fig. 1). Moreover, because most of the 11 species described from this earliest known fossiliferous deposit are comparable to extant (oscillatorian) cyanobacteria⁹ – oxygen-producing photoautotrophs that are among the most highly evolved of all eubacteria¹⁰ – it seems certain that life must have originated substantially (and probably hundreds of millions of years) earlier.