Conservation genetics: from species to habitats

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Conservation genetics - and indeed conservation in general - falls into the two broad areas of the identification and preservation of (1) endangered species and (2) habitats with high biodiversity. Most research has been towards the first aim, with hard-won findings on the importance of genetic variation in population management, but genetics promises to be at least as important in the second aim. To this end, molecular phylogenies have long been proposed as an important approach that can capture conservation worth and evolutionary distinctiveness better than simple species richness and avoid common problems in defining species identity and boundaries. Progressively faster and cheaper DNA sequencing and the rise of DNA barcoding are making the phylogenetic approach to habitat conservation widely applicable. Barcoding was seen initially solely as a means to species discovery and to species identification, but it now holds promise as a resource-efficient means of rapidly estimating the evolutionary history preserved by different sets of reserves. Initial indications are that biodiversity assessment, using the short *cox1* sequence standard for barcoding animals, reliably reflects the picture from longer sequences. The phylogenetic approach, assisted by barcoding, not only infers evolutionary history but also in synergy with morphology will speed species discovery and the subsequent expansion of general biological knowledge.

Keywords
Species preservation, phylogenetic biodiversity, DNA barcoding, mitochondrial DNA, Madagascar ants.

Two kinds of conservation genetics

The application of genetics to conservation has two broad aspects, the preservation of individual endangered species and the preservation of endangered communities. Of these, by far the most effort has gone into the first approach and indeed, the second is usually not mentioned in textbooks of conservation genetics. Given the diversity of life on earth (Odegaard 2000), the preservation of communities leading to the preservation of very large numbers of species, is the major aim of conservation biology, so that the second approach should come increasingly into its own.

Conservation genetics, as applied to relatively well known single species, has made great progress (Frankham *et al* 2002). The chief findings have been that, for a very great many species, the loss of genetic variation and a consequent occurrence of inbreeding depression not only weakens individuals (Brown and Brown 1998; Hedrick and Kalinowski 2000; Spottiswoode and Møller 2004), but also increases the risks of population extinction (Frankham 2005; Saccheri *et al* 1998). Immigration can reverse the decline of inbred populations (Madsen *et al* 1999; Saccheri and Brakefield 2002; Vila *et al* 2003; Vrijenhoek 1998). Not all species suffer from inbreeding depression, for example, inbreeding hymenopteran parasitoids do not (Hamilton 1967), but very many charismatic species with public appeal do, so that the hard-won findings from conservation population genetics are highly relevant, not only to policy for wild populations but for management of zoo stocks.

Conservation genetics - as applied to the selection of policies to best preserve mass biodiversity - is best embodied in the weighting of species by phylogenetic distinctiveness, rather than by counting them all equally in the traditional approach maximising species richness. Species richness has been recognized as inadequate as a conservation currency by some for a long time, for example Wilson (1992) argued against relying on rapid speciation to replace extinct species because the new species would be similar to each other and lack the depth of evolutionary history lost by extinction. A favorite example of differential weighting was given by May (1990), namely that the tuataras constitute the long-divergent sister group to all other lizards, so that losing them would entail a vastly greater loss of evolutionary history than a pair of, say, skink species.

Phylogenetic approaches to conservation weighting

The idea that evolutionary distinctiveness should be taken into account stems from the fact that longer periods of evolutionary divergence are expected to yield more new features of organisms than short ones (Crozier 1997; Wilson 1992). Initial approaches to differential weighting of species according to phylogenetic distinctiveness concentrated on node-counting on dendrograms (reviewed by Crozier (1997)), but attention to the degree of change along branches became the norm from 1990 (Crozier 1992; Faith 1992; May 1990; Pamilo 1990; Witting *et al* 1994) (see Crozier (1997)). Use of the branch lengths gives a better indication of the length of time evolution has had to produce differences than the number of perceived speciation events.

The distinction between using species richness as the sole criterion and using evolutionary history is shown in figure 1. Protecting habitat α would preserve most species (four), but while protecting habitat β would preserve only two species, the portion of the tree that joins them (shown in green) is 20 units long as against 14 for the species in habitat α (assuming a rooted-tree approach, see below). Given that more new features evolve if there is a longer evolutionary time for them to do so, more novel features, including ones currently unseen, would be preserved by protecting habitat β than habitat α. While habitat β would preserve as many species as habitat γ, it preserves more evolutionary history (20 as against 18 units) and so, other factors being equal, it would be the preferred choice. Similarly, if two sites can be protected, although sites including α always preserve more species, more tree length is preserved if the sites protected are β and γ.

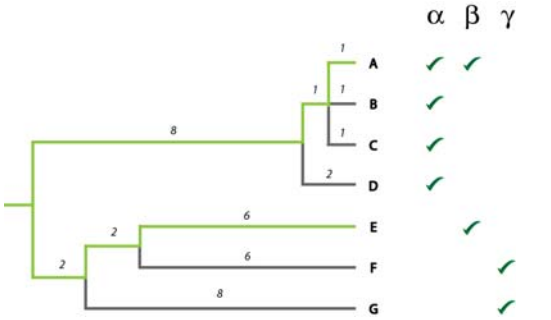

Figure 1. Optimal selection of sites α, β, or γ, according to the phylogeny of the included species A--G. While site α has the highest species richness it would preserve less of the evolutionary history of the group than either of the other two sites. Green lines trace the phylogenetic diversity (PD) of site β.

Distance measures specifically aimed at estimating the genetic variation preserved by different combinations of sites (Crozier 1992; Witting *et al* 1994) seem well suited to closely-related species, but Faith's (1992) simple measure of total tree length (phylogenetic diversity, PD) has become that most generally applied and underlies the approach in Figure 1. There is uncertainty about when this became the current consensus view (Crozier *et al* 2006; Crozier *et al* 2005; Faith and Baker 2006; May 1994), but is now generally agreed that the tree's root is best included when calculating the amount of evolutionary history preserved. Pardi and Goldman's (2007) notation suggestion of using rPD for rooted PD and uPD for unrooted PD should be followed if the measure used is not otherwise made clear. Strictly speaking, the branch connecting the species under consideration to the rest of life should also be included and should become increasingly easy to do.

As more species are added to a study, does phylogenetic diversity yield different answers to the use of species richness? Nee and May

(1997) in a simulation study found that randomly removing 95% of species still preserved 80% of the tree, leading to the conclusion that when large enough assemblages are considered, there is no advantage to considering evolutionary distinctiveness in selecting sites for preservation. A similar conclusion was reached by Rodrigues et al. (2005). Various lines of evidence suggest that this conclusion is too optimistic. The assumption of random extinction is often violated, with phylogenetically divergent mammal and bird species tending to be at greatest risk (Johnson *et al* 2002; Purvis *et al* 2000). The assumption of random distribution of species to sites, implicit in the simulation models, did not hold for a study of the Cape flora (Forest *et al* 2007), because the degree of phylogenetic dispersion varies between one part of the region and another. Further, the very meaning of "species" seems to vary more between groups of animals than would [relatively] objectively inferred trees, especially molecular ones (Agapow 2005; Agapow *et al* 2004).

In the absence of a well-founded phylogeny, still now and for the near future the most likely situation, the systematics of a group can be used as a surrogate (Crozier *et al* 2005; Faith 1994; Strahan 1989; Warwick and Clarke 1995). Forest et al. (2007) found that such surrogacy led to results reflecting the more precise picture derivable from the molecular phylogeny.

The tree alone

Using species, even within a phylogenetic framework, still entails potential difficulties from the state of taxonomy differing between groups and such problems as unrecognised cryptic species. These problems have been clearly recognized for a long time in microbial studies, where many organisms were first discovered from their sequences and many remain only known in that form. A study using eubacterial rDNA sequences from the Oklo region of Gabon to estimate evolutionary history found significant differences in the phylogenetic dispersion of sequence diversification between sampling sites at different depths (Crozier *et al* 1999). Mace et al. (2003) suggested that for multicellular organisms, use of the tree of individuals could avoid problems of species boundaries. Others are now making the same point (Faith and Williams 2005; Faith and Williams 2006; Forest *et al* 2007).

Using a tree of individuals would provide a speedy estimate of biodiversity, but as a first estimate. Links to the rest of biology and possible refinements to the biodiversity estimate, would follow through more traditional processes, as argued below. Maximization of the preservation of evolutionary history as an aid to preserving functional communities has been suggested for some time (Cattin *et al* 2004; Warwick and Clarke 1995; Webb *et al* 2002) and draws experimental support (Maherali and Klironomos 2007).

Statistical sufficiency

We hope that very often the results of an analysis between different proposed preservation schemes will be very clear cut, but even in such cases it is desirable to be able to tell policy makers that the best choice not only appears clear but actually is statistically significantly better. Under this logic, the same level of statistical rigor should be applied to biodiversity estimates as to other scientific endeavours. As part of this process, the best methods of phylogenetic analysis should be used. We hope to no longer see the use of suboptimal methods justified by the 'size of the dataset'. Programming virtuosos have rushed to provide programs capable of estimating PD for hundreds of thousands of sequences 'in seconds' (Levy *et al* 2006; Minh *et al* 2006; Pardi and Goldman 2007; Steel 2005) and Bayesian methods able to provide estimates of uncertainty of phylogeny can also handle very large numbers of sequences in tolerable lengths of time (Hebsgaard *et al* 2007).

Two sources of variability in estimates can be identified – uncertainty in tree construction and incompleteness of sampling. Uncertainty in tree construction can be taken into account by using trees from bootstrap sampling of the character data (Crozier *et al* 1999), or those derived after stationarity following Bayesian procedures. Uncertainty stemming from incomplete sampling can be investigated using bootstrap samples from the complete array of sequences

(Crozier *et al* 2005) and standard statistics such as from sample coverage theory (Chao and Lee 1992; Shen *et al* 2003). So far, programs taking uncertainty into account have looked at one source or the other, but not both, but a combined examination presents no serious methodological or conceptual obstacles.

Other external constraints, such as the budget available and the long-term preservation costs of sites under consideration (Hartmann and Steel 2006; Pardi and Goldman 2007; Weitzman 1993) and weights, such as those favouring the inclusion of adjacent sites, could be readily included.

For large numbers of sequences or potential reserve sites, we suggest that an optimization procedure, rather than an exact solution search strategy, be used. Simulated annealing has been used, in combination with weights favouring contiguous sites, for species richness maximization (McDonnell *et al* 2002).

DNA barcoding

DNA barcoding, the use of a short piece of DNA to characterize a specimen, has become extremely popular (Dasmahapatra and Mallet 2006; Hebert *et al* 2003; Herre 2006) as well as controversial (Hickerson *et al* 2006; Rubinoff 2006). We will not dwell on technical aspects, but will note that although a region of the *cox1* gene is the standard sequence for most animals, other sequences are required for plants (Taberlet *et al* 2007) and bacteria (which lack mitochondria) and the use of a particular sequence should be separated from the concept of rapid characterization. Thus, rDNA has also been used for DNA barcoding (Page *et al* 2005). DNA barcoding has so far been seen in terms of specimen identification (the identification of an unknown specimen (Armstrong and Ball 2005), or part thereof, using the match to known sequences in a database (Ratnasingham and Hebert 2007)) and species discovery (finding that a new sequence falls sufficiently far from known ones as to indicate the likely existence of a new species, e.g., Hebert et al. (2004)).

Specimen identification and species discovery are vital contributions to biodiversity and general biological research; we now propose that DNA barcoding be used directly for the initial rapid estimate of biodiversity, using the sequences found from a set of sites. There is no practical alternative for assessing bacterial biodiversity and probably no real alternative for other microbes. For most other groups, we argue that DNA barcoding is now becoming the most practical means for rapid biodiversity assessment. Given current throughput rate at leading DNA barcoding centers of 2000 specimens per week, results of even quite extensive surveys should be obtained and analysed within months or weeks of return from the field. Indeed, given access to the molecular technology, May's (2004) remark that the field component is the rate-limiting step for surveys remains true.

Although DNA barcoding as the starting point for biodiversity surveys is demonstrably practical for many groups, further work is needed before truly general analyses involving large fractions of the biota can be routinely implemented. One question for example, pertains to differences in *cox1* evolutionary rate between groups – a bias in preserving species with more rapidly evolving mtDNA should be avoided! However, the question is not whether differences in rates occur, but to what extent would they compromise biodiversity assessment. A further question that should be explored is the extent to which tree-reconstruction errors using a short DNA barcoding sequence compromise phylogenetic biodiversity assessment. Trees built using the *cox1* sequence bear a surprisingly close resemblance to those constructed using much longer sequence, although they do show occasional marked differences (Hebert *et al* 2003; Smith *et al* 2005b). Such differences do not necessarily mean that phylogenetic biodiversity assessment using a short DNA barcoding sequence differ very much from assessment using longer sequences, but the extent to which this does should be examined. The finding that comparative analyses are quite robust to errors in phylogeny inference (Symonds 2002) gives grounds for optimism that the same will be so for biodiversity estimation using DNA barcoding.

Barcoding to taxonomy

A reliable taxonomy and systematic nomenclature give us the framework on which to organize biological knowledge of life on earth. DNA barcoding yields a rapid assessment of biodiversity, but does not reduce the need for well-trained specialists able to move knowledge from the initial survey to more refined stages of taxonomic knowledge (Figure 2). Thus, the second stage of the recognition of molecular operational taxonomic units (MOTUs) absolutely requires taxonomist participation to see whether the sequences grouped in the tree correspond to one or more already known species. The setting of the similarity level between sequences of the same MOTU (e.g., 2% or 3%) requires the collaboration of both taxonomist and molecular ecologist. Once MOTUs are established, a second estimate of biodiversity can be made in terms of the number of these and of course using the phylogeny relating them.

Finally, the use of other sources of information, such as from other loci (especially nuclear ones), behaviour, morphology and other sources of taxonomic knowledge, will lead to the firm discovery of new species or the inference that some species have deep phylogenetic splits inside them (step G above). Of course, such knowledge of the genetic structure of single species can assist in their individual management (Avise 2005; Moritz 1994) if desired, but in terms of conserving whole habitats, considerations of variation within single species are likely to be important for particularly charismatic or culturally important ones. This discovery of new species then allows further estimates of biodiversity and allows the regular pursuit of biological knowledge, enlightened by better understanding of what is there.

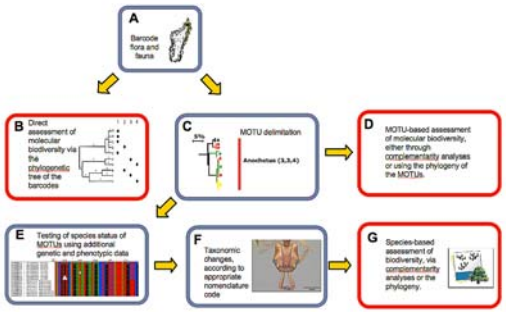

Figure 2. Stages in the uses of DNA barcoding for biodiversity assessment. Red-bordered boxes denote stages where biodiversity assessment can be made: step B rapid assessment using the tree alone, step D using molecular operational taxonomic units and step G using species defined and described using a wide range of data.

A test case of the method is provided by a survey of ants in Madagascar, involving a partnership between a barcoder and an ant taxonomist (Smith *et al* 2005a). Calculating the loss of biodiversity in terms of either morphospecies lost or PD lost if a site is not preserved, from the four northernmost studied, yields the same order of preference. Recording the Genetic Diversity (Crozier 1992) preserved by different combinations of 4, 3, 2, or 1 sites yields a clear priority order for preservation. Within this group the study of the related genera *Anochetus* and *Odontomachus* has yielded several new species, made new associations of sexuals with workers (by identity of *cox1* sequences), highlighted species whose deep phylogenetic splits may yet prove to be several species and yielded diagnostic *cox1* nucleotide positions for each (Fisher and Smith 2008).

Our suggestion is to cut the Gordian Knot linking biodiversity assessment to species counts, yielding a method of rapid biodiversity assessment. DNA barcoding does NOT replace traditional taxonomy and systematics, but rather hastens its progress in a time when conservation biology is a "discipline with a deadline" (Wilson 2000).

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