The role of subspecies in obscuring avian biological diversity and misleading conservation policy

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Subspecies are often used in ways that require their evolutionary independence, for example as proxies for units of conservation. Mitochondrial DNA sequence data reveal that 97% of continentally distributed avian subspecies lack the population genetic structure indicative of a distinct evolutionary unit. Subspecies considered threatened or endangered, some of which have been targets of expensive restoration efforts, also generally lack genetic distinctiveness. Although sequence data show that species include 1.9 historically significant units on average, these units are not reflected by current subspecies nomenclature. Yet, it is these unnamed units and not named subspecies that should play a major role in guiding conservation efforts and in identifying biological diversity. Thus, a massive reorganization of classifications is required so that the lowest ranks, be they species or subspecies, reflect evolutionary diversity. Until such reorganization is accomplished, the subspecies rank will continue to hinder progress in taxonomy, evolutionary studies and especially conservation.

Keywords: mitochondrial DNA; subspecies; reciprocal monophyly; conservation

1. INTRODUCTION

The Linnaean rank of subspecies became prevalent during the mid-twentieth century with the emergence of the biological species concept. Under this paradigm, many prior-named species were 'demoted' to subspecies status. For example, the number of bird species dropped by 50% when ornithologists adopted this view of species (Mayr 1970). Although some viewed this as an advancement, others (Wilson & Brown 1953, p. 100) viewed the subspecies rank sceptically: '… the subspecies concept is the most critical and disorderly area of modern systematic theory…'. In retrospect, subspecies have functioned as units in at least three roles, namely in classifications, evolutionary theories and, more recently, conservation plans, without strong tests of how well they function in these roles.

2. MATERIAL AND METHODS

(a) *Molecular tests*

Molecular systematic studies below the species level provide tests of the value of named subspecies. Analysis of mitochondrial DNA (mtDNA) sequence variation within and among subspecies reveals whether subspecies are evolving independently, are freely exchanging breeding individuals or are at some intermediate stage of isolation. In particular, if a subspecies has been evolving independently for $2N_{ef}$ generations (on average), where N_{ef} is the inbreeding effective size of the female population, an mtDNA gene tree should show that all sequences from a subspecies share a common ancestral sequence not found in individuals from any other subspecies, a pattern termed reciprocal monophyly (Avise 2000). This expectation, that subspecies will be monophyletic, provides a way to evaluate named subspecies. Subspecies should be judged to fail as meaningful units if they do not predict the evolutionary history of the populations they represent.

(b) *Dataset*

The taxonomy of birds provides a powerful empirical test of the predictive value of subspecies. Avian taxonomy is in a mature state because nearly all taxa have been described as species or subspecies (Sibley & Monroe 1990). Numerous studies of mtDNA variation in continentally distributed species exist (Ball & Avise 1992; Zink *et al.* 2000), some of which are summarized in electronic Appendix A (available on The Royal Society's Publications Web site). Although the use of a single molecular marker such as mtDNA might result in mistaken intraspecific histories (Avise & Wollenberg 1997), mtDNA gene trees routinely recover significant divisions in avian species; rarely have nuclear markers shown that such divisions are incorrect (Avise 2000). Furthermore, because of longer coalescence times, nuclear markers such as microsatellites are unlikely to capture genetic diversity not also evident in mtDNA gene trees (Palumbi *et al.* 2001). However, it will be important to confirm the findings reported here with nuclear loci.

3. RESULTS

Analyses of mtDNA data reveal historical divisions in many species, which have an inconsistent relationship with subspecies boundaries. For example, analysis (Zink *et al.* 2001) of the cactus wren (*Campylorhynchus brunneicapillus*), a wide-ranging species in the Chihuahuan and Sonoran deserts and Baja California, showed that only two mtDNA groups exist (figure 1), although six named subspecies were sampled. No individual subspecies yields a monophyletic set of mtDNA sequences. The only consistency between the mtDNA gene tree and subspecies taxonomy is that the two major mtDNA groups correspond to groups of subspecies.

The results for the cactus wren are not atypical. In a survey of 41 species (see table 1 in electronic Appendix A), only 3% of avian subspecies qualify as distinct evolutionary entities. Some island and tropical subspecies are well differentiated (Seutin *et al.* 1994; Tarr & Fleischer 1995) but, in general, continental subspecies are not

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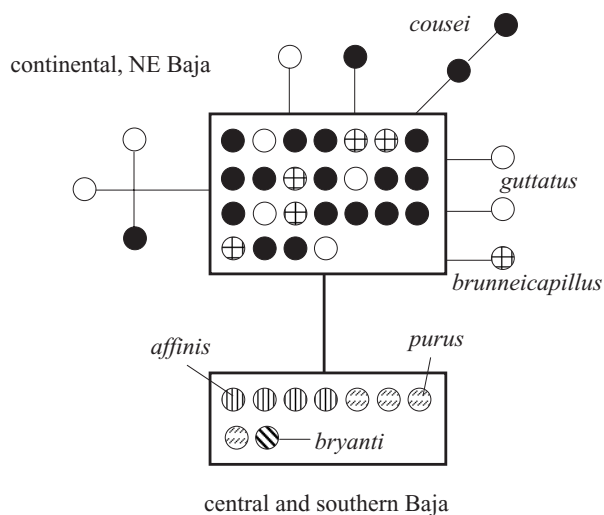DOI 10.1098/rspb.2003.2617

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Figure 1. Results of phylogenetic analysis of mtDNA sequences of the cactus wren (Zink *et al.* 2001). Each circle corresponds to an individual bird, and patterns denote subspecies membership (names in italics). Two main clades of haplotypes were found: central and southern Baja California versus the rest of the range. Within each clade, individuals of the same putative subspecies are not genetically distinct and often individuals of different putative subspecies designation share the same haplotype. The two clades constitute groups that merit conservation attention, but each subspecies does not.
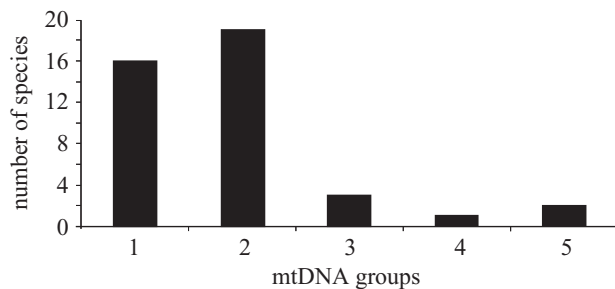

Figure 2. Distribution of the number of monophyletic sets of mtDNA haplotypes in avian species. The majority of species consist of either one or two mtDNA clades.

(Ball & Avise 1992; Zink *et al.* 2000). However, this does not imply that avian species are genetically uniform. On average, biological species of birds contain 1.9 independently evolving groups (figure 2) irrespective of the number of named subspecies, which for the species surveyed (see table 1 in electronic Appendix A) averages 5.5. Unfortunately, there is no way to discern from subspecies nomenclatures whether species include multiple evolutionarily significant groups and, if they do, which subspecies belong to which groups. For example, one would not discover the pattern of evolutionary diversity that exists in the cactus wren (figure 1) from its subspecies nomenclature. This is unfortunate, because it is the historically significant groups that merit the highest attention.

The patterns observed in non-threatened species extend to threatened and endangered species. Twenty-one temperate continentally distributed subspecies are identified as threatened or endangered in North America (http://ecos.fws.gov/; 12 February 2003). Thirteen of these have been surveyed for mtDNA variation (see

electronic Appendix A), of which 12 lack mtDNA reciprocal monophyly (*Grus canadensis pulla, Empidonax traillii extimus, Polioptila californica californica, Charadrius alexandrinus nivosus, Tympanuchus cupido attwateri, Glaucidium brasilianum cactorum, Rallus longirostris obsoletus, Rallus longirostris levipes, Rallus longirostris yumanensis, Ammodramus savannarum floridanus, Sterna antillarum browni*) and two subspecies are monophyletic (both of spotted owl, *Strix occidentalis lucida* and *S. o. caurina*; Barrowclough *et al.* 1999). It is difficult to compare this result with that for subspecies in general because some studies of endangered subspecies do not include the entire species' range. At the species level, six out of 41 (14.6%) non-endangered species have at least one subspecies that is monophyletic, whereas for the endangered forms, only one out of 11 (9.1%) does.

4. DISCUSSION

Ball & Avise (1992) and Zink *et al.* (2000) called attention to the lack of support in mtDNA gene trees for avian subspecies. What was not apparent in these surveys was that considerable genetic structure does exist, and that it is inconsistently related to subspecies boundaries. This latter aspect is explored below.

(a) *Subspecies and conservation*

Because subspecies are rarely historically independent units and do not represent the evolutionary entities that do exist in bird species (figure 2), focus on subspecies could misdirect conservation effort. For example, significant resources were focused on a subspecies of the seaside sparrow (*A. maritimus*), the now extinct dusky seaside sparrow (*A. m. nigrescens*), whereas mtDNA analyses subsequently showed that it was a routine example of genotypes found on the Atlantic coast (Avise & Nelson 1989). Significant funds have been allocated to the preservation of the California subspecies of the California gnatcatcher (*P. c. californica*), a small songbird that lives in a habitat (coastal sage scrub) threatened by development. This subspecies, however, is not supported by patterns of morphological and mtDNA variation, making this subspecies a misleading flagship for biodiversity and conservation (Zink *et al.* 2000).

The notion that subspecific variation represents local adaptation (Mayr 1970) fostered the use of subspecies as proxies for conservation units. Thus, an alternative view (Crandall *et al.* 2000) is that subspecies might reflect adaptive variation, important to species' survival, irrespective of the pattern of mtDNA reciprocal monophyly. Many species that lack reciprocally monophyletic groups do exhibit a geographical pattern in morphology (Zink *et al.* 2000), which might reflect local adaptations, important for the species' future survival. Thus, preservation efforts based on putative adaptive variation could conflict with the historical approach advocated here (Moritz 2002). However, phenotypic variation, whether adaptive or not, can evolve rapidly. For example, the house sparrow (*Passer domesticus*) developed geographical differences in size and shape in approximately 100 generations (Johnston & Selander 1971). By contrast, reciprocally monophyletic groups often take tens of thousands of years to evolve. Hence, restoration of historical groups would take much

longer than restoration of the phenotypic variation characteristic of subspecies (Mooers & Atkins 2003). Preservation of reciprocally monophyletic groups will preserve both significant bouts of independent history and adaptive phenotypic variation included in such groups (Crnokrak & Merila 2002).

If the goal is to direct limited resources to protect historically unique segments of biological species, which is contentious, mtDNA data (see table 1 in electronic Appendix A) argue against considering subspecies as proxies for units of conservation. Currently, decisions on which populations to preserve are based on demographic information, such as evidence indicating marked population declines. However, decisions about species' management should be informed by the historical status of populations as well. For example, if three subspecies of the cactus wren were threatened, two in one major group, one in the other, but funds were available for conservation of only two groups, the overriding goal ought to be to ensure the long-term viability of the two major units revealed by genetic data. This would downgrade the priority of one of the two subspecies from the same group (one would want to try to preserve the one with the greatest demographic potential to survive). Other important considerations exist, such as cases in which rare but non-historical populations function ecologically as keystone species. Nevertheless, an assessment of historical significance ought to play a greater role in decisions to preserve populations; such assessments can be made relatively rapidly with modern genetic methods.

(b) Are subspecies bad or are there just bad subspecies?

One way to interpret this study is to conclude that the subspecies rank has been badly enacted, and that taxonomists need to 'clean their house'. Based on mtDNA studies, one would expect 1.9 subspecies per avian species. Most subspecies, thus revised, would consist of what are today groups of subspecies. Thousands of currently named subspecies would be eliminated because they are based on arbitrary divisions of single morphological character clines. Alternatively, one could argue that each evolutionarily distinct unit should be recognized as a species and that the subspecies rank should be abandoned. In this case, there would be approximately twice as many species of bird as are currently recognized (and no subspecies). Debates about species concepts are ongoing (De Queiroz 1998); however, it is clear that currently named subspecies do not reveal where or whether historical diversity exists within species (figure 2). Thus, subspecies cannot serve as an effective proxy for units of conservation.

(c) Subspecies and gene trees

Subspecies might indeed be isolated, but for an insufficient period (less than $2N_{ef}$ generations) for mtDNA monophyly to have evolved (Avise 2000). If subspecies represent an incipient stage of differentiation (prior to reciprocal monophyly), then grouping populations from different geographical localities into subspecies will result in a higher F_{ST} than ignoring them (F_{ST} is the variance component that represents the fraction of genetic variation distributed among populations). For example, in a wide-spread European species, the bluethroat (*Luscinia svecica*), F_{ST} for 20 population samples across the range (*ca.* 10 000 km) was 0.29, whereas grouping the samples into the seven represented subspecies reduced this value to 0.24 (Zink *et al.* 2003). This general result is also true for other species (Fry & Zink 1998; Zink & Blackwell-Rago 2000), suggesting that subspecies are not simply too evolutionarily young to be monophyletic for mtDNA trees.

The apparent conflict between mtDNA gene trees and subspecies limits might reflect differing rates of morphological and molecular evolution. Morphological traits on which subspecies are based are probably polygenic (Schluter 1984). It is possible that, prior to the evolution of monophyletic mtDNA gene trees or significant F_{ST}-values, morphological characters could provide evidence of isolation because of the increased additive genetic variance associated with multiple genetic loci. However, to validate a subspecies nomenclature, subspecies names should be predictive of geographical patterns evident in multiple morphological characters (Barrowclough 1982). If a subspecies has been evolving independently, one expects congruent character variation. Relatively few such studies exist and in general they indicate that few subspecies predict multivariate morphological patterns (Johnson 1980; Zink 1986; Rising 2001). Instead, subspecies limits are more often based on divisions of single, arbitrarily chosen characters.

(d) Subspecies and nomenclature

Formal taxonomic names ought to have a consistent meaning. A meaning that is both consistent and biologically significant is that a taxon has had an independent evolutionary history. Taxa at higher ranks, such as genera and families, are not accepted without historical significance, and it is illogical that, at the subspecies level, formal taxonomic names can be applied to trivial non-historical patterns of variation. Only taxa defined by the congruence of multiple morphological or molecular characters should be recognized at some rank. Over 90% of continental avian subspecies fail this test. Thus, avian taxonomists must revise classifications by eliminating thousands of subspecies names so that the formal names that remain coincide with known patterns of biodiversity. Only then will these taxa, whether ranked as species or subspecies, fulfil roles in classification, evolutionary studies and conservation plans.

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