Considering evolutionary processes in conservation biology

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Conservation biologists assign population distinctiveness by classifying populations as evolutionarily significant units (ESUs). Historically, this classification has included ecological and genetic data. However, recent ESU concepts, coupled with increasing availability of data on neutral genetic variation, have led to criteria based exclusively on molecular phylogenies. We argue that the earlier definitions of ESUs, which incorporated ecological data and genetic variation of adaptive significance, are more relevant for conservation. Furthermore, this dichotomous summary (ESU or not) of a continuum of population differentiation is not adequate for determining appropriate management actions. We argue for a broader categorization of population distinctiveness based on concepts of ecological and genetic exchangeability (*sensu* Templeton).

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The evolutionarily significant unit (ESU) is a population unit that merits separate management and has a high priority for conservation[1]. The ESU has been associated with distinct population segments that receive protection under the US Endangered Species Act and is also used in a variety of less formal contexts around the world. However, the general concept of ESUs has evolved over time (Box 1) and the implementation of the concept has been inconsistent across different cases. ESUs were first described as population units that 'actually represent significant adaptive variation' based on 'concordance between sets of data derived by different techniques'[1]. Later, Waples[2] described them as 'reproductively separate from other populations and have unique or different adaptations'. Thus, the concept has two components: reproductive and historical isolation, and adaptive distinctiveness. These components are not necessarily correlated. Adaptive differences can arise under gene flow and might depend upon it for continued variability, but gene flow is prevented under reproductive isolation. Therefore, within the ESU concept an implicit tension exists between maintaining isolation between independently evolving units and maintaining adaptive variability within such units.

Operationally, efforts to document ESUs have emphasized reproductive isolation rather than the maintenance of adaptive differences. This focus is apparent in the progression of definitions since the concept was first developed in 1986 (Box 1). Moritz's[3] more recent rationale for a focus on the evolutionary past is that long isolated populations have distinct potential, presumably to develop into populations, population groups or ones that are uniquely adapted to existing environmental conditions. Therefore, recognizing ESUs as reciprocally monophyletic groups ensures that populations will be managed separately and that the evolutionary heritage within species will be maintained. We agree that the maintenance of historical population structure, as defined by molecular genetic techniques, should be one goal of species management. However, there are several conceptual and practical problems with this approach.

First, although populations with a history of reproductive isolation should perhaps be managed separately, this goal is overemphasized. In general, the potential for species evolutionary success is maximized through the maintenance of adaptive diversity[4–9], by preserving the maximum diversity of functionally divergent gene copies across the geographic range of a species. Moritz[3] asserts that maintaining adaptive variants is not only difficult in practice but also negates the evolutionary process, because preserving variants adapted to previous conditions might retard the response to contemporary selective pressures. However, as long as maintenance of evolutionary processes in populations is a goal of management, rather than the maintenance of variants alone, natural selection should eliminate those adapted to previous conditions. Functionally divergent populations might not be those with a long history of isolation; in fact, substantial functional divergence and reproductive isolation can occur despite high levels of gene flow[10].

Second, the widespread use of molecular genetic markers in the context of Moritz's ESU concept (Table 1) has led many investigators to regard these data as essential to conservation management, often to the exclusion of other data. However, ESUs, as defined by Moritz, are unlikely to be found in species characterized by high levels of gene flow (e.g. many species of birds and large mammals). Furthermore, the reciprocal monophyly criterion presents difficulties for populations with paraphyletic histories (Box 2)[11]. Conversely, populations designated as ESUs might not be functionally distinct from one another. With the increasing resolution of molecular techniques, significant differentiation can be found at very small scales, even down to the individual, and this can lead to inappropriate diagnosis of ESUs within functionally equivalent populations.

Finally, the conceptual framework of the ESU designation forces just two categories (ESU or not) from the continuous distribution of genetic diversity, habitat types and selective pressures across populations. In practice, it would be more useful for conservation management if multiple categories were used to represent these continua. Furthermore, both genetic and ecological information should be used, with an emphasis placed on exchangeability instead of genetic distinctiveness. This approach will provide better insights into the conservation units that can best maintain evolutionary processes and the potential for evolutionary change in the future.

Diagnosing distinct populations

To become useful in conserving species, theoretical concepts must be practical in application. Similar to species concepts[12], ESU concepts are often troubled by a lack of applicability to the real world[3,13,14]. The Moritz definition is appealing in this regard because the ESU is defined primarily by reciprocal monophyly, a characteristic that can be deduced using established phylogenetic techniques[15]. However, within species, reciprocal monophyly is a common characteristic only of animal mitochondrial genes, because they are rapidly evolving

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Box 1. Definitions of evolutionarily significant units over time

Concepts of population distinctiveness have changed over the past 15 years, with the latest definition emphasizing genetic distinctiveness to the point of reciprocal monophyly. We argue that the previous definitions of evolutionarily significant units (ESUs) appropriately incorporated adaptive differences, as evidenced by genetic and ecological data. Here, we present operational criteria for determining the genetic and ecological distinctiveness of populations, in terms of rejection criteria for genetic and ecological exchangeability. In the past, ESUs have been defined in the following ways:

Ryder 1986: populations that actually represent significant adaptive variation based on concordance between sets of data derived by different techniques[1].

Waples 1991: populations that are reproductively separate from other populations and have unique or different adaptations[2].

Moritz 1994: populations that are reciprocally monophyletic for mtDNA alleles and show significant divergence of allele frequencies at nuclear loci[3].

Rejection criteria for different classes of exchangeability

Ecological exchangeability: the factors that define the fundamental niche and the limits of spread of new genetic variants through genetic drift and natural selection[17].

- Exchangeability is rejected when there is evidence for population differentiation owing to genetic drift or natural selection.
- Representative evidence includes differences in life history traits, morphology, habitat, QTLs and allozymes under selection – such differences would ideally be demonstrably heritable.

Genetic exchangeability: the factors that define the limits of spread of new genetic variants through gene flow[17].

- Exchangeability is rejected when there is evidence of restricted gene flow between populations.
- Representative evidence could be obtained from microsatellites, nucleotide sequences (mtDNA, cpDNA and nDNA) and allozymes.

and are presumed to be nonrecombining (but see Ref. 16). Thus, plants and other life forms will be proportionately less well represented as ESUs under Moritz's concept. Our diagnosis of distinct populations avoids this problem because it emphasizes variation in phenotypes, thus allowing preservation of important adaptive characters and their associated underlying genetic variation. This variation can be shaped by gene flow, genetic drift and natural selection, with the effects of gene flow falling under genetic exchangeability, and the effects of genetic drift and natural selection falling under ecological exchangeability[17,18]. Exchangeability serves as a null hypothesis[19,20]. From both recent and historical perspectives, the rejection of, or failure to reject, exchangeability (Box 1) forms the foundation of population distinctiveness and management practices (Fig. 1).

Ecological exchangeability

The central idea of ecological exchangeability is that individuals can be moved between populations and can occupy the same ecological niche or selective regime. Ecological exchangeability arises from the shared fundamental adaptations of a population[18], such as similar life history traits, ecological requirements, morphologies and demographic characteristics. Ecologically exchangeable individuals perform similar functions in each ecological community. Characters used to demonstrate ecological exchangeability should be demonstrably heritable to avoid confusing environmentally and genetically based differences. The null hypothesis of ecological exchangeability is tested by overlaying the ecological data (either categorical or continuous) on the underlying genealogy of the population, and by using the statistical procedures outlined in Templeton and Sing[21]. Such tests of ecological exchangeability are straightforward to perform and examples are given by Templeton[19,20]. In the absence of genealogical data only current ecological exchangeability can be tested, but in a straightforward fashion, using standard statistical tests for morphological variation (t-tests and principle components analyses, etc.).

Genetic exchangeability

Individuals from different populations are genetically exchangeable if there is ample

Table 1. Example studies surveyed for evolutionarily significant units (ESU) usage[a]

Taxon	Data source	Management conclusion	Crosshair classification[b]	Refs
Puritan tiger beetle (*Cicindela puritana*)	mtDNA (RFLP haplotypes)	Connecticut River and Chesapeake Bay are independent units	+ + + −	40
	Ecology (habitat parameters)	Chesapeake Bay is a single unit	+ − + −	
African elephants (*Loxodonta africana*)	mtDNA (ND5-6 restriction fragment haplotypes)	Single, albeit subdivided population	+ − − −	42
Black rhinoceros (*Diceros bicornis*)	mtDNA (restriction maps)	Maintain current conservation units (equivalent to subspecies), although mixing could be permissible	− − − −	38
Cryan's buckmoth (*Hemiluca* spp.)	Allozymes	ESU based on ecology (despite no reciprocal monophyly)	− + − −	39
	mtDNA (CO-II sequence phylogeny) ecology (larval–host plant performance)			
Coho salmon (*Oncorhynchus kisutch*)	Microsatellites	Two populations form an ESU with a third	+ + − −	43
	Heritable characters (morphology, swimming ability and age at maturation)			

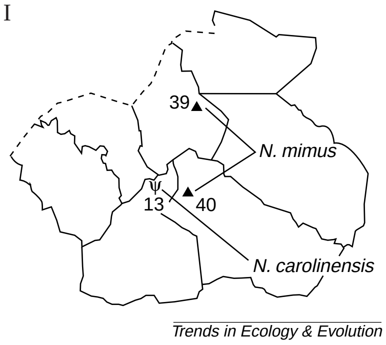
[a] Full details of all references and a summary of the studies can be found at http://www.nceas.ucsb.edu

[b] Details of crosshair classification can be found in Fig. 1. Upper versus lower rows correspond to a temporal comparison with upper being recent and lower being historical. Left and right correspond to genetic versus ecological exchangeability, respectively. A plus sign indicates a rejection of that null hypothesis (e.g. populations are not genetically exchangeable) and a minus sign is a failure to reject the null hypothesis.

Box 2. Nonmonophyletic evolutionarily significant units

The reciprocal monophyly criterion of Moritz is problematic because it does not incorporate important information about adaptive differences between populations and because these differences (and populations) might not be partitioned in a monophyletic fashion. Monophyly implies that all the members of that population share a more recent common ancestor with each other than with individuals from outside the population. However, the process of population subdivision and speciation is known to produce polyphyletic relationships that slowly progress over time to paraphyletic and then to monophyletic relationships[44–46]. Thus, the criterion of reciprocal monophyly is overly stringent.

I



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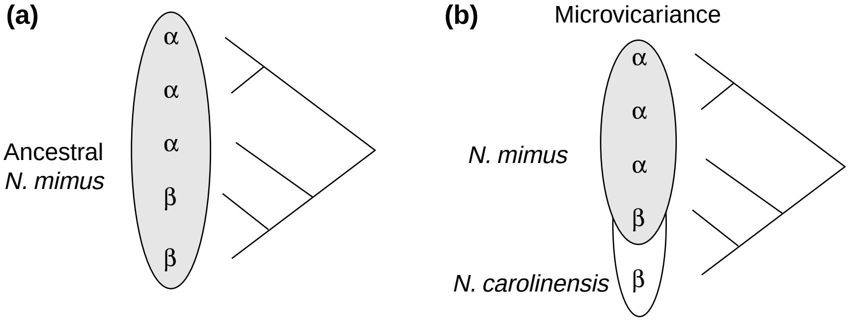
We demonstrate this idea with an example (Fig. I) from cave and surface spiders studied by Hedin[47]. Our example consists of three populations of spider (*Nesticidae: Nesticus*), two sampled from mountain tops (*Nesticus mimus*) and one from a cave (*Nesticus carolinensis*) in North Carolina, USA (populations 13 and 40 are found in the vicinity of Linville Mountain, whereas population 39 is from the vicinity of Grandfather Mountain, North Carolina). During the Pleistocene, these cryophilic spiders apparently lived at lower elevations and were more continuously distributed; however, climatic changes led to their current fragmented distribution in mountain top populations. By contrast, the cave population was able to survive by entering the cave habitat. This population shows evidence for morphological change consistent with a cave-limited existence, including a reduction in pigmentation and eyes, and an elongation of legs.

II



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The evolutionary history of these three populations has been estimated using both mtDNA and nDNA (Fig. II; alpha indicates the geographic region around population 39, whereas beta represents the region around populations 13 and 40), and it has been shown that the mountain top populations are paraphyletic with respect to the cave population. However, as far as we know, the surface forms are ecologically exchangeable, whereas the cave form, restricted to deep cave environments, is not. Thus, the surface forms are genetically nonexchangeable but ecologically exchangeable (Case 7, Fig. 1), which leads to the conclusion that they should be treated as a single population. Compared with the surface forms, the cave form leads to a rejection of both genetical and ecological exchangeability (Case 1, Fig. 1) (here, we are considering the significant mtDNA divergence as evidence for historical separation), which leads to the conclusion that they are separate species (indeed, this is how they have been treated with the cave form called *N. carolinensis* and the surface populations called *N. mimus*). Fig. IIa shows the ancestral geographic distribution (over regions alpha and beta) of ecologically similar spiders, whereas Fig. IIb indicates the ecological and morphological divergence of the cave populations through microvicariance. However, the relationship of the cave form to the two surface lineages is one of paraphyly, not reciprocal monophyly. Thus, populations classified under our categorization as distinct species (owing to reproductive isolation and a suite of adaptive differences) would not even be classified as ESUs under the Moritz criterion of reciprocal monophyly. *Figures reproduced, with permission, from Ref. 47.*

gene flow between populations. Unique alleles, low gene flow estimates ($Nm < 1$, where Nm is the effective number of migrants per generation)[22] or phylogenetic divergence concordant with geographic barriers[23] provide criteria for rejecting genetic exchangeability. Levels of gene flow can be estimated from microsatellites, allozymes, nucleotide sequences (mtDNA, nDNA and cpDNA), AFLPs, RAPDS and so on[24,25]. Various molecular techniques have differential strengths and weaknesses depending upon the historical time frame involved[13,24,25].

Historical versus recent exchangeability

Both ecological and genetic exchangeability can be partitioned along a timescale. We consider three approaches for deducing such temporal divisions. The first is to evaluate historical data directly. For many plant species, historical community structure, geographic distribution and associated ecological variables (e.g. climate) can be reconstructed through pollen analyses[26]. Genetic data have been sampled directly from historical samples[27]. Historical data on distributions of species and habitat types can also be obtained from expedition notes and museum collections[28,29].

The second approach uses different molecular markers to obtain information from different timescales. For example, microsatellite data can identify sibling relationships within a pedigree, whereas mtDNA sequences typically have a more distant range of inference on the order of thousands to millions of years. Thus, the combination of different types of data with different underlying mutation rates allows partitioning of historical from recent phenomena.

The third approach examines phylogenetic relationships. A phylogenetic tree allows temporal partitioning by the relative position on the topology[30]. Both nuclear and mitochondrial data tend to form nonbifurcating relationships at the population genetic level[31]; thus, network approaches are more applicable to population genetic data. Inherent in such an approach is a nested statistical design that corresponds to partitions in evolutionary time. This nested design can be effectively used to partition historical from recent genetic[32,33] and ecological[18] events.

Although these approaches offer only operational criteria for establishing different time frames, the dichotomy of historical versus recent (current) must be made in the context of a conservation assessment of a particular species and a particular set of molecular markers. What is historical for microsatellite data can be recent for mtDNA and what is historical for *Escherichia coli* (after 5000 generations) is recent to a mammal species. Thus, the division must be made relative to the question and data at hand.

Sampling strategy

Central to population-based analyses is an appropriate sampling strategy, in terms of both numbers of individuals and the geographic coverage. Inferences about ecological and genetic exchangeability can change drastically depending on the sampling scheme. For example, limited sampling can lead to the erroneous diagnosis of distinct populations when sampling intermediate populations would show ongoing gene flow. Conversely, limited sampling can have the opposite effect if data are inadequate to reject the null hypothesis of exchangeability. Guidelines can be obtained from coalescent theory; for biologically relevant levels of genetic diversity, samples of between 20 and 50 individuals (for low to high levels of diversity, respectively) will sample 95% or more of the existing haplotypes in a population[34].

Management and restoration of populations

Three general principles should govern management actions. First, management should aim to preserve adaptive diversity and evolutionary processes across the geographic range of a species[9]. A lack of evidence for nonexchangeability should not be used to promote homogenization or other alterations of the gene pool, or to allow contraction or changes in the geographic distribution of populations. Failure to reject the null hypothesis does not imply that the null hypothesis is true, but could simply be a result of the lack of relevant data. To preserve evolutionary processes, the goal of management should be to preserve the natural network of genetic connections between populations, rather than just distinct populations within that network. This ensures that the processes that maintain adaptive diversity and evolutionary potential are conserved. To preserve adaptive diversity, the population network that best samples functional diversity within the species should be given high priority for conservation.

Second, management actions might depend on the severity and nature of recent disturbance. In general, restoration (either of separate or single populations) should be attempted more often for populations that have become disjunct or mixed as a result of recent anthropogenic activities (e.g. habitat degradation, unnatural barriers to dispersal and introductions of nonnative forms). Attempts at restoration should rarely be considered if isolation or mixing result from more natural processes, such as postglaciation environmental change[35].

Finally, when possible, management recommendations should be made on the basis of adequate sampling and appropriate analyses[12]. We suggest that managers use techniques that explicitly consider the statistical power of alternative hypotheses[36]. In practice, managers tend to assume that maintaining separate management units is always the conservative action because it is potentially reversible, whereas mixing distinct units is not. However, this action is not conservative in the sense that continued isolation could compromise the future viability and evolutionary potential of a population[37].

Relative strength of evidence	Evidence of adaptive distinctiveness	Recommended management action
Case 1	$\begin{array}{c\|c} + & + \\ \hline + & + \end{array}$	Treat as long separated species
Case 2	$\begin{array}{c\|c} + & + \\ \hline - & + \end{array}$ $\begin{array}{c\|c} + & + \\ \hline + & - \end{array}$	Treat as distinct species
Case 3	$\begin{array}{c\|c} - & + \\ \hline + & + \end{array}$	Treat as distinct populations (recent admixture and loss of genetic distinctiveness)
Case 4	$\begin{array}{c\|c} + & - \\ \hline + & + \end{array}$	Natural convergence on demographic exchangeability – treat as single population
Case 5	(a) $\begin{array}{c\|c} + & + \\ \hline - & - \end{array}$ (b) $\begin{array}{c\|c} - & + \\ \hline - & + \end{array}$ (c) $\begin{array}{c\|c} - & - \\ \hline + & + \end{array}$	Anthropogenic convergence on demographic exchangeability – treat as distinct populations. (a) and (b) Recent ecological distinction, so treat as distinct populations; and (c) allow gene flow consistent with current population structure
Case 6	$\begin{array}{c\|c} - & + \\ \hline - & - \end{array}$	Allow gene flow consistent with current population structure; treat as distinct populations
Case 7	$\begin{array}{c\|c} + & - \\ \hline + & - \end{array}$	Allow gene flow consistent with current population structure; treat as a single population
Case 8	$\begin{array}{c\|c} + & - \\ \hline - & - \end{array}$ $\begin{array}{c\|c} - & - \\ \hline - & + \end{array}$ $\begin{array}{c\|c} - & - \\ \hline + & - \end{array}$ $\begin{array}{c\|c} + & - \\ \hline - & + \end{array}$ $\begin{array}{c\|c} - & + \\ \hline + & - \end{array}$ $\begin{array}{c\|c} - & - \\ \hline - & - \end{array}$	Treat as a single population; if inexchangeability is a result of anthropogenic effects, restore to historical condition; if inexchangeability is natural, allow gene flow

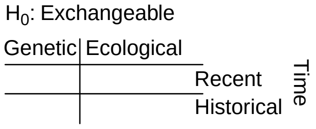

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Fig. 1. Categories of population distinctiveness based on rejection (+) or failure to reject (−) the null hypotheses (H$_0$) of genetic and ecological exchangeability, for both recent and historical time frames. As the case numbers increase (from Case 1 to Case 8) there is decreasing evidence for significant population differentiation.

Applications

Methodology and management implications

Null hypotheses of exchangeability are scored as rejected (+) or not rejected (−) in each of four categories: genetic, ecological, recent and historical (Fig. 1). Sixteen outcomes are, therefore, possible; however, some are biologically implausible or unlikely. For example, rejection of only recent genetic and historical ecological exchangeability (or the converse) is implausible and would cast doubt on the accuracy of information.

Management implications follow from our concern to preserve adaptive diversity and evolutionary processes within species (Fig. 1). In general, we stress evidence for recent ecological nonexchangeability because it is indicative of the adaptive divergence necessary for population persistence. In Case 1 (Fig. 1), exchangeability is rejected in all four categories; consequently, the units under comparison represent separate species. Where only historical information for either genetic or ecological exchangeability is not rejected (Case 2, Fig. 1), the evidence is still strong that these are distinct units and we suggest that they should be managed as separate species.

Evidence for historical adaptive divergence only indicates divergence that is now not essential for population persistence, although it might have been in the past. Similarly, evidence for rejection of recent or historical genetic exchangeability alone is not sufficient to warrant separate priority conservation unless it is accompanied by adaptive divergence (Cases 4 to 7, Fig. 1). In these

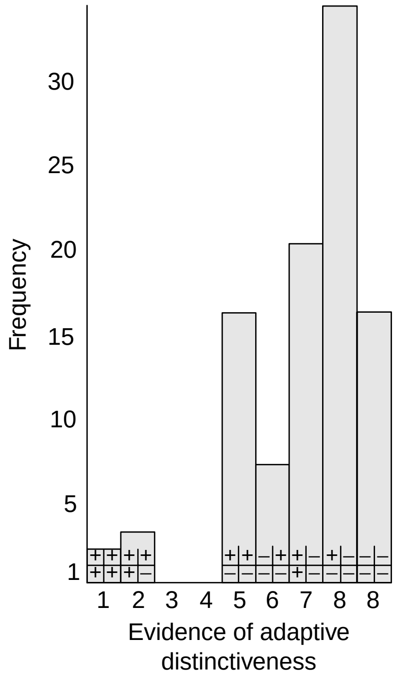

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Fig. 2. A survey of the use of the evolutionarily significant units (ESU) concept from 98 population genetic studies. Each study was categorized using the exchangeability rejection criteria discussed in the text, with + for rejection of exchangeability and − for failure to reject exchangeability. Based on the classification of each study using the scheme in Fig. 1, we tallied the occurrence of each of the eight cases presented. ESUs were claimed under each of the eight cases.

instances, as well as in the straightforward result where there is no evidence to reject exchangeability (Case 8, Fig. 1), we recommend that the units be treated as populations connected by various degrees of gene flow. The goal of management should be to maintain levels of gene flow, as inferred from molecular data. Thus, reciprocally monophyletic populations would represent one end of a continuum in a network of populations connected by different degrees of gene flow. However, they would not receive special conservation concern unless there was evidence for adaptive divergence. Furthermore, if recent genetic divergence is a result of anthropogenic causes, we recommend that restoration to the natural condition of interchange be attempted through habitat restoration, translocation or other appropriate methods.

To explore the empirical distribution of cases in this continuum, we surveyed 98 studies from the literature and scored their results according to our four tests of exchangeability. We present a representative set of studies in Table 1 (references and a summary of all studies surveyed can be found at http://www.nceas.ucsb.edu/). In many cases, failure to reject the null hypothesis arises because the evidence for some categories was not presented or was inadequate to constitute an effective test. Indeed, ecological data was frequently lacking. Our survey shows that the overwhelming majority of analyses fall within Case 8 (Fig. 1), either rejecting just recent genetic exchangeability or failing to reject any exchangeability (Fig. 2). Interestingly, the authors assigned the term ESU to every category represented in Fig. 2, even when there was no evidence against exchangeability.

A few examples highlight the weaknesses of the ESU as it is currently applied. First, researchers often hesitate to consider geographically distinct populations as indistinguishable for fear that their endangered status be lessened. For example, ecological and genetic exchangeability could not be rejected for the black rhino (*Diceros bicornis*); however, the management recommendation by O'Ryan *et al.*[38] was for two distinct subspecies. Second, reciprocally monophyletic relationships might not always indicate historical isolation. Significant adaptive differences might be partitioned in a nonmonophyletic fashion (Box 2).

Two studies exemplify the joint application of phenotypic and genetic data. Legge *et al.*[39] found no evidence to reject the null hypothesis of genetic exchangeability among populations of Cryan's buckmoth (*Hemiluca* spp.); however, there was significant evidence to reject ecological exchangeability. Thus, these populations fell under Case 6 (Fig. 1) in our scheme with recent ecological nonexchangeability. Here, the authors argue, as we do in general, for the adaptive significance of ecological differentiation and that this differentiation provides better evidence for divergence relevant to population persistence and conservation. Finally, a study of tiger beetles (*Cicindela puritana*) shows how both ecological and genetic data can be used to determine population differentiation. Populations were found to be genetically nonexchangeable both recently and historically, and were ecologically nonexchangeable[40]. Thus, this example falls into our Case 2 (Fig. 1), showing strong evidence for adaptive distinctiveness.

However, studies using both adaptive characteristics and molecular markers are rare. In some cases, especially in the plant literature, information on life history variation and morphology are available to be incorporated in evaluation of population status. Recently, quantitative genetic techniques have been introduced for the study of phenotypic variation in the wild, thus suggesting that the genetic component of variation can be precisely defined[7,9,41]. Lynch[8] offers an approach that uses relatedness data based on molecular markers to estimate the heritable component of character variation in populations. Such new approaches highlight the importance of molecular genetic techniques to assess relatedness and population history. By contrast, measurement of adaptive diversity requires analysis of ecologically relevant heritable traits.

Prospects

The ESU concept has evolved over the past 15 years, and with the increasing sophistication of molecular techniques and genetic data analysis, ESUs now rely on measures that reflect genetic isolation rather than adaptive diversity. This is not appropriate for the overall goal of maintaining evolutionary potential, and, in certain cases, might limit the options for gene flow and adaptation via natural selection. Population units for conservation should be explicitly defined on traits that enhance the potential for species survival. Thus, there needs to be a focus on preservation of functional diversity rather than of historical legacy. Genetic techniques are also essential because they provide estimates of gene flow between populations and thus guide efforts to maintain historical levels of genetic exchange between populations. The network of genetic connections among populations is the web that maintains the potential for evolution. Rather than a focus on long-isolated populations, efforts should be directed at maintaining networks that capture the adaptive diversity within species. The necessity for genetic and phenotypic data provides a mandate for collaboration and consensus between molecular and ecologically oriented biologists.

In this context, the application of a specific terminology (ESU) hardly seems necessary or appropriate. The removal of this dichotomous terminology from its conceptual foundations in evolutionary biology might have been responsible for the frequent misdiagnosis of conservation units (Fig. 2). We suggest that the terminology is abandoned and replaced with a more holistic concept of species, consisting of populations with varying levels of gene flow evolving through drift and natural selection.

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