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Biodiversity at the Molecular Genetic Level: Experiences from Disparate Macroorganisms

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CONTENTS

SUMMARY

Genetic variation is the basis of adaptive flexibility in populations and is the ultimate evolutionary basis of much species and community–level diversity. Accordingly, the preservation and maintenance of genetic diversity has a high priority in many conservation programmes. This paper discusses how genetic diversity is measured at the molecular level, including some newer measures made possible with restriction site or DNA sequence data as well as the development of a phylogenetic approach to assessing the significance of genetic variation within a species. These measures of genetic diversity are then used to re-examine the validity of the 50/500 rule of conservation biology; a rule that states that populations should have no fewer than 50 individuals for short-term maintenance of genetic variation and no fewer than 500 individuals for long-term maintenance. Both the 50 and 500 parts of this rule are found to be invalid and frequently misleading. Instead of invoking 'universal' rules, conservation biologists should recognize the role of biodiversity in management policies. Not all species are the same, and we need more research and a willingness to try novel approaches rather than naively apply a 'rule' that has no demonstrable generality.

1. INTRODUCTION

Biodiversity can be defined at several levels of biological organization, including the genetic, the species, and the community levels. Genetic diversity is at the lowest hierarchy in this sequence, but this enhances – not diminishes – its importance. Without genetic diversity, a population cannot evolve and it cannot adapt to environmental change. Environmental change is now occurring on a global scale due to human activities, and many species will have to adapt to this change or experience an ever increasing chance of extinction. Moreover, as is common with many hierarchical systems, genetic diversity has an impact on the higher levels of biodiversity. Species, in their most basic sense, are evolving lineages, and the maintenance of their capacity to evolve requires the existence of genetic diversity. Their current array of adaptations and ecological requirements are the product of this past evolutionary history, and hence species diversity emerges from genetic diversity over evolutionary time. Among these evolved adaptations of a species are its habitat needs and its responses to other species, and these adaptations in turn are the basis for much of community structure. Hence, the impact of genetic diversity percolates through all levels of biodiversity via the evolutionary process.

Given the importance of genetic diversity for both short-term (to an evolutionary biologist) adaptation to environmental change and long-term impact on species and communities, the preservation of genetic diversity has been a high priority in many conservation programmes. Genetic diversity can also be used to monitor or infer other historical or demographic processes that are essential for making wise decisions related to biodiversity management at the species level and above. This paper will focus on the preservation of genetic diversity in conservation programmes. The indirect roles of genetic diversity as a probe for learning about other aspects of populations, species or communities are summarized elsewhere (Templeton 1991*a*, 1993).

Our ability to survey for genetic diversity at the molecular level has increased by orders of magnitude over the past few years. In the mid 1960s, protein electrophoresis became the first widely used genetic survey technique for detecting molecular level variants, but it was limited to protein coding genes (and usually, those coding for soluble enzymes) and had poor resolution in the sense that most types of molecular genetic changes were not detectable by this technique (indeed, only a minority of all amino acid changes could be detected by this technique). Today, such techniques as DNA fingerprinting, the polymer-

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ase chain reaction (PCR), restriction site mapping, and DNA sequencing are becoming increasingly common tools for performing molecular genetic surveys. All of these techniques have greater resolution than protein electrophoresis, and some can be applied to both coding and non-coding DNA regions, thereby opening up the entire genome to survey techniques rather than a small subset as with protein electrophoresis. These newer molecular techniques not only have the advantage of greater and broader resolution, but some of them provide qualitatively new information as well. Protein electrophoresis could tell us how many alleles there were at a locus, their frequencies within a population, and their geographical distribution over the species' range. Techniques such as restriction site mapping and DNA sequencing can also provide historical information and/or quantitative assessments of how diverse alleles are from one another. That is, they can tell you not only how many alleles or haplotypes exist at a locus, but also how those alleles are related to one another evolutionarily and/or by how many nucleotides the alleles differ. This paper will focus upon the use of this new molecular genetic historical information in conservation biology as the use of non-historical genetic surveys has already been discussed (e.g. Nevo 1991). Before discussing the lessons to be learned from modern molecular genetic surveys on the preservation of genetic diversity, it is first necessary to discuss how genetic diversity is measured.

2. MEASURES OF GENETIC DIVERSITY

With the advent of protein electrophoresis, several measures of genetic diversity have been measured in a variety of organisms (e.g. Nevo 1978, 1988). Although these measures do not take full advantage of the information present in modern molecular genetic surveys, they are still useful and have the benefit of being applicable to a broad array of genetic data sets. Perhaps the simplest measure of genetic diversity is the 'percent of polymorphic loci' or P. Although there are many different criteria for determining whether a locus is polymorphic or not, one of the most common criterion is that a locus is polymorphic if the frequency of its most common allele is less than 0.95. To calculate the percent of polymorphic loci, a genetic survey on several loci or DNA regions must be performed, and then each locus is categorized as being polymorphic or not according to the stated criterion. If p loci out of a total of n loci are categorized as being polymorphic, the percent of polymorphic loci is simply $P = 100(p/n)$. With protein electrophoretic data, P averaged close to 25%, with considerable variation across species (Nevo 1978). However, with the advent of high resolution molecular genetic techniques, P tends to go towards 100% with only a few exceptional species and loci, so this measure of genetic diversity provides little quantitative discrimination with modern data sets.

Another simple measure is N, the 'number of alleles'. With N, it is possible to provide a more quantitative assessment of genetic variation than with P. For example, suppose a locus has two alleles, each with frequency of 0.5; whereas a second locus has 10 alleles, each with frequency of 0.1. Both are polymorphic loci by the 95% criterion, so no discrimination between them is made using P. However, the second locus would be measured as containing more genetic diversity using N. Thus, N provides a greater degree of discrimination of levels of polymorphism than a simple categorical criterion. However, N still has its limitations. For example, consider one locus with two alleles, each with frequency of 0.5, and a second locus with two alleles, one with a frequency of 0.95 and the other with a frequency of 0.05. Once again, both loci are polymorphic, but in this case N also provides no discrimination between them with respect to genetic diversity.

A measure that is sensitive to the frequency of alleles is 'heterozygosity', or H. Heterozygosity can be measured in two different fashions. The first is observed heterozygosity; that is, the probability that an individual will be heterozygous at a locus. Obviously, this measure of genetic diversity is applicable only to diploid genetic elements and is sensitive not only to allele frequencies, but to genotype frequencies as well (and hence, all the factors that determine the relationship of allele to genotype frequencies). This sensitivity to genotype frequencies creates difficulties in interpreting H as a measure of genetic diversity. For example, consider a population of 100% self-mating plants. The observed heterozygosity is expected to be close to zero for all loci, regardless of how many alleles they have or the frequencies of those alleles. To avoid this problem, the more common use of heterozygosity as a measure of genetic diversity is expected heterozygosity; namely, the probability that two copies of a locus drawn at random from the gene pool have different allelic states. Note, by defining expected heterozygosity in terms of randomly drawn pairs of genes, expected heterozygosity can even be applied to haploid genetic elements, such as mitochondrial DNA. Mathematically, the observed heterozygosity for a locus is:

$$H = 1 - \sum_{i=1}^{N} p_i^2 , \qquad (1)$$

where p_i is the frequency of allele i. H can also be averaged over loci to create a multilocus measure of genetic diversity. As can be seen from equation (1), H depends both upon N and upon the frequencies of alleles, so more information than just allele number is incorporated into this measure. In the hypothetical example of the previous paragraph, the first locus has a heterozygosity of 0.5, whereas the second locus has $H = 0.095$. Hence, the first locus has greater genetic diversity by this criterion even though $N = 2$ for both loci.

With modern molecular data sets, it is often possible to measure H at the level of individual nucleotides as well as for a locus as a whole. Going back to the level of a locus (or any defined DNA region), restriction site and DNA sequence data sets also allow the estimation of the 'average number of nucleotide differences', or

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K, between two randomly drawn genes. This measure is a quantitative refinement over the usual expected heterozygosity. To see its utility, consider the following hypothetical example. Suppose that two loci both have two alleles, each with frequency of 0.5. However, suppose the two alleles at the first locus differ at only one nucleotide site, whereas at the second locus, the two alleles differ at ten nucleotide sites. For both loci, $H = 0.5$, but for the first locus, $K = 0.5(1) = 0.5$, and for the second locus, $K = 0.5(10) = 5$.

Another locus-level measure possible with restriction site or sequence data is the 'number of segregating sites', or S. S is simply the number of restriction sites or nucleotides that are polymorphic in the population in the DNA region under study. Note that alleles (or haplotypes) at the locus are defined by their joint genetic state at all segregating sites considered simultaneously. For example, suppose a DNA region is polymorphic for three different restriction sites, so $S = 3$. However, there could be as few as two alleles (e.g. $+++$ and $---$) or as many as eight ($+++, ++-, +-+, -++, +--, -+-, --+, ---$). Hence, S is measuring a different aspect of genetic diversity than N.

A final way of summarizing the genetic diversity at a locus is to construct an 'allele or haplotype tree' that reflects the evolutionary relationships among the alleles. Algorithms for estimating such trees and placing confidence limits on them, even when the complication of recombination exists, have already been developed (Templeton *et al.* 1992). Over the past decade, a considerable body of theory has been developed that indicates that the topology of haplotype trees and the frequencies of haplotypes are strongly interrelated (see Ewens (1990) and Hudson (1990) for reviews of this theory). Moreover, a sufficient number of haplotype trees are now available to test these theoretical predictions, and the predictions so far have been found to be valid (Crandall & Templeton 1993). Hence, haplotype trees contain a considerable amount of information about genetic diversity. Haplotype trees open up the possibility of assessing genetic diversity in a qualitatively new fashion. For example, in assessments of species diversity, it has been recognized that using only the number or frequencies of species may not be the best strategy – one must also consider the evolutionary uniqueness of a species (Crozier 1992; Faith 1992; May 1990; Vane-Wright *et al.* 1991). The ability to estimate haplotype trees now allows the possibility of incorporating phylogenetic considerations at the level of genetic diversity as well. An example of such an application will be given in the next section.

3. A RECONSIDERATION OF THE 50/500 RULE

The concern for preserving genetic diversity within a species or population is explicitly reflected in the '50/500 rule' of conservation biology (Franklin 1980). Both numbers in this rule are motivated by a concern for the preservation and maintenance of genetic diversity. The 50 refers to the population size needed as a founding source to prevent serious inbreeding depression (Franklin 1980) and to ensure that high levels of genetic diversity are present in the founders (Primack 1993); the 500 refers to the population size required for long-term maintenance of additive genetic variation as a balance between loss due to genetic drift versus creation due to mutation (Franklin 1980; Primack 1993). The utility of the 50 rule with respect to inbreeding will not be discussed here as the issue of inbreeding is discussed elsewhere (Templeton 1994). The current discussion will be limited to the utility of this rule with respect to preserving and maintaining genetic diversity.

First, consider the recommendation of 50 founders. The '50' in this number is not the actual number of animals or plants in the founder population, but rather is the inbreeding effective size of the founding population relative to the ancestral population from which the founders were derived (Simberloff 1988). It is well known that inbreeding effective sizes are more closely related to the number of ancestors than to the size of the current generation (Crow & Denniston 1988); hence, in the absence of information about the number of ancestors, it is impossible to implement this rule. Nevertheless, a few generalities can be made. If the ancestral population were formerly large in size but is now declining (a common situation in conservation programs) and the ancestral population had much gene flow over its geographical distribution, then even a handful of founders could easily satisfy the '50' rule and carry over substantial amounts of genetic variability from the ancestral population (Nei *et al.* 1975; Templeton 1980).

An empirical example of small founder populations bringing over much genetic variation is provided by the captive population of Speke's gazelle (*Gazella spekei*). By examining the details of response of the captive herd to pedigree inbreeding, it was concluded that the ancestral population structure was outcrossing with a large inbreeding effective size (Templeton 1987). The original captive herd of these species was founded from only one male and three females (Templeton & Read 1983, 1984), but this would be more than sufficient to satisfy a founding inbreeding effective number greater than 50 given the inference about ancestral population structure. Under this ancestral structure, a significant portion of the population's genetic variation (as measured by P or H) is present as individual heterozygosity such that just a handful of individuals can result in trivial reductions of these genetic variation indices (Nei *et al.* 1975). That this was indeed the case for the captive herd of Speke's gazelle was empirically shown by genetic surveys performed several years after the formation of the herd. These genetic surveys revealed that the captive herd had high levels of genetic variation as measured by P or H: indeed, this captive herd had greater levels of genetic variation than most natural populations of mammals, particularly large mammals (Templeton *et al.* 1987). As this example shows, the '50' in the 50/500 rule is a strange number that often bears little or no relationship to the actual number of founders and when interpreted as 'the

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number of founders' is actively misleading with respect to predicting the carry over of genetic variation from the ancestral population into the founder population.

However, other measures of genetic variation, such as N, allele number, are much more sensitive to founder size; hence, the 50 rule might have validity if this measure of genetic variation is deemed more appropriate. To examine this issue, it is important to understand why N is more sensitive to founder size than P or H. For many loci, there are a few common alleles and a large number of rare alleles. Because rare alleles by definition are found in very few individuals, whenever a sample of only a small number of individuals is taken from a population, many rare alleles will not be included in the sample. However, because such alleles have low frequencies, they have only a minor effect on measures of genetic diversity that weight alleles by their frequency (such as H) or that use categorical discriminations based upon the more common alleles (such as P). For the measures of molecular diversity, neither K nor S are as sensitive to founding size as N, although K is slightly more sensitive to population size bottlenecks than S, but S declines more rapidly than K when there is prolonged small population size (Tajima 1989, 1992). Using coalescent theory, Crandall & Templeton (1993) have shown that the number of alleles reaches a plateau rapidly with increasing sample size, such that little additional gain in N is expected after sampling 50 to 100 individuals unless one is willing to increase the sample size by orders of magnitude. This result strengthens the rationale for the 50 rule, but only if N is the appropriate measure of genetic diversity and no other.

A phylogenetic perspective can be used to evaluate the biological significance of N as a measure of genetic diversity. As expected from neutral theory and as demonstrated by Crandall & Templeton (1993), rare alleles are primarily clustered on the tips of haplotype trees and represent one-step mutational derivatives from the more common haplotypes that are primarily interior nodes of the haplotype tree. Hence, the loss of even a substantial number of rare alleles is akin to a trimming of the twigs of a tree that leaves the basic structure of the tree intact. In particular, the deepest evolutionary lineages of haplotypes are rarely effected, and it is these lineages that contain almost all of the genetic diversity as measured by K or S (and also P or H). Moreover, new rare alleles can quickly be generated by the mutational process from the same handful of common alleles that were the ancestors of the original set of rare alleles. Hence, when phylogenetic weighting is considered, the extraordinary sampling efforts that must be exerted to preserve rare alleles cannot be justified.

One objection to the above conclusion might be that even though rare, some of these alleles might be selectively important. When the neutrality assumption is not true, there are two general classes of selection models that need to be considered. The first class is selection that maintains polymorphism, such as balancing selection. Takahata (1990), Takahata & Nei (1990), and Takahata *et al.* (1992) have shown that balancing selection actually accentuates the tendency of genetic diversity as measured by K or S (and also P or H) to be concentrated in old, common haplotype lineages found in the interior of the tree. The second class of selection models involves directional selection either against or for a new mutation. Golding (1987, 1992) has shown that mutations with deleterious fitness effects do not persist long and are therefore found primarily at the tips of haplotype trees. Directional selection in favour of a new haplotype is expected to swiftly increase its frequency, thereby removing it from the rare allele class. Therefore, directional selection in favour of a new mutant haplotype is a problem only in the extremely unlikely event that a population is sampled shortly after the mutation occurred but before selection has increased its frequency. The other situation of concern is that with environmental change, some rare haplotype that is neutral or deleterious today may become beneficial in the future. There is, of course, no way of predicting this possible outcome. However, because these rare alleles tend to be one-step derivatives of a common allele, they have a high probability of arising repeatedly, as has apparently occurred for sickle-cell aenemia in Africa (which is a one nucleotide change from the common haemoglobin A allele) (Lapoumeroulie *et al.* 1992). Other than these situations, the presence of selection further erodes the rationale for expending much effort on the preservation of rare alleles. In light of these phylogenetic considerations, the 50 rule is not important when attempting to carry over most of the evolutionary and selective significant genetic variation from a larger, panmictic ancestral population for the simple reason that an inbreeding effective size greater than 50 will almost always occur regardless of the census founding size.

All of the above arguments were predicated upon the assumption that the ancestral population was close to panmixia and had large amounts of gene flow over its geographical distribution. However, many natural populations show geographic subdivision such that a large component of their genetic variation is found as differences among local populations. Under these conditions, the amount of genetic diversity found in a founder population depends critically upon how the founders were sampled geographically (Templeton 1980, 1991*b*, 1993). For example, molecular genetic surveys of collared lizard (*Crotaphytus collaris*) populations in the Ozarks of central North America revealed that most of the genetic diversity exists as fixed differences among local populations (Templeton 1993; Templeton *et al.* 1990). Hence, a founder population of two lizards collected from different local populations would display more genetic variation for all diversity measures with the possible exception of N than a sample of 50 lizards from a single local population. When dealing with subdivided populations, the geography of sampling is more important than the numbers sampled with respect to preserving genetic variation (Templeton 1993; Templeton *et al.* 1990). Thus, the 50 rule is not merely irrelevant when

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dealing with subdivided populations, it is actively misleading.

The 500 in the 50/500 rule is also an effective size, but in this case it is the variance effective size (Simberloff 1988) that should balance the loss of additive genetic variance for a phenotypic character versus the creation of new additive variance through mutation. The number of 500 was derived by combining a theoretical model in which there is no dominance or epistatic variance with a single experiment on the rate of production of new variation for bristle characters in some lines of *Drosophila melanogaster* (Franklin 1980). The theoretical assumptions were made on the basis of mathematical convenience and are inconsistent with a growing body of experimental evidence that dominance and epistatic variance is significant for a large number of quantitative traits (Bryant 1989; Bryant & Meffert 1990, 1991, 1992; Carson & Wisotzkey 1989; Mitchell-Olds 1991; Templeton 1989). Moreover, additive variance is not directly related to any of the other measures of genetic diversity discussed previously. The additive variance depends not only upon genetic variation at the molecular level, but also depends upon the genotype-phenotype relationship and the genetic architecture (number of loci, distribution of phenotypic affects over loci, etc.) of the specific phenotype under consideration. Indeed, with founder and bottleneck effects (common situations in conservation biology), there can even be an inverse relationship between molecular genetic variation and additive genetic variation. For example, if a population is polymorphic at a locus with major phenotypic effect that also interacts with a large number of epistatic modifiers, natural selection is expected to adjust the various allele frequencies at all loci such that there is little or no additive variance despite the presence of molecular variation. However, fixation of an allele at the major locus would immediately cause the epistatic interactions of the modifier loci to become responsive to selection (i.e. additive), as first pointed out by Templeton (1980). Note that a loss of genetic variation at the molecular level is actually associated with an increase of additive genetic variance for a phenotype; a prediction that has been both theoretically elaborated (Bryant *et al.* 1986; Goodnight 1988; Willis & Orr 1993) and empirically verified (Bryant 1989; Bryant & Meffert 1990, 1991, 1992; Carson and Wisotzkey 1989; Mitchell-Olds 1991). Consequently, the theoretical model used to justify the 500 rule is without generality. Moreover, the rate of production of new additive variance also depends critically upon the specific genetic architecture of the trait under consideration. As a consequence, the figures used by Franklin (1980) cannot be generalized to other strains of *Drosophila melanogaster*, much less to other species. Moreover, they cannot even be generalized to other non-bristle traits within the same strains of *Drosophila melanogaster*. It is disturbing that a single experiment on bristle number in one strain of one species of fruit fly is being used as a general guide for conservation management policy.

One might argue that the 50/500 rule is still useful when there is an absence of other information: an all too common situation in conservation biology. When Franklin (1980) first proposed the 50/500 rule, he made explicit the theoretical assumptions (with the unfortunate exception of failing to tell the readers that 50 was an inbreeding effective size but 500 was a variance effective size), and he acknowledged 'the meager information' that underlined the 500 rule. In the context of the time, the 50/500 rule was a reasonable working hypothesis, although the results summarized here indicate that this hypothesis can now be rejected both theoretically and empirically. Accordingly, the 50/500 rule should not be used because it is frequently misleading for accomplishing the goal of maintaining genetic diversity. Unfortunately, it is commonplace to treat the 50/500 proposition as a 'rule' rather than as an hypothesis. This attitude reduces the priority that agencies give to doing the research needed for designing optimal programmes for the particular species or communities being managed. It is ironic that some conservation biologists and managers, who ought to appreciate biodiversity, attempt in their management decisions to treat each species as if it were completely interchangeable with all other species: thereby denying the importance of the very biodiversity they are attempting to preserve. Instead of invoking 'universal' rules, conservation biologist ought to recognize the role of biodiversity in management policies. Not all species are the same, and we need more research and a willingness to try novel approaches rather than naively apply a 'rule' that has no demonstrable generality.

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