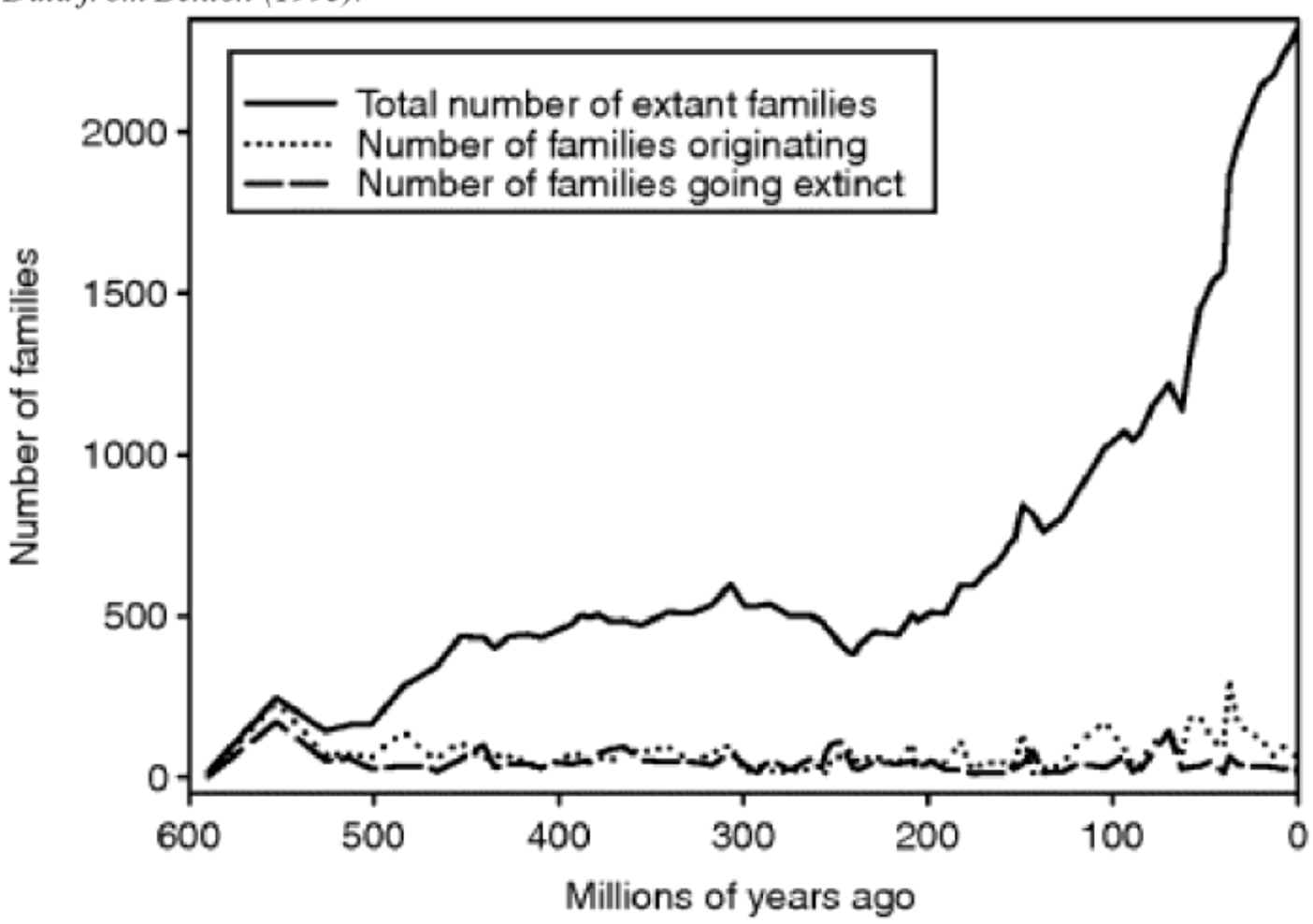
Chapter 8

Conservation Genetics

The Need for Conservation

Biodiversity quite simply refers to all of the different life forms on our planet, and includes species diversity and genetic diversity. We know from the fossil record that species diversity has been steadily increasing over the past 600 million years, despite the fact that as many as 99% of species that have ever lived are now extinct (Figure 8.1). Around 96% of all extinctions have occurred at a fairly constant rate, creating what is known as the background extinction rate. This has been estimated from the fossil record as an average of 25% of all living species going extinct every million years (Raup, 1994). The remaining 4% or so of all extinctions occurred during five separate mass extinctions, which are identified from the fossil record as periods in which an estimated 75% or more of all living species went extinct. The most recent mass extinction occurred in the late Cretaceous (65 million years ago) when approximately 85% of all species, including the dinosaurs, were wiped out.

Figure 8.1 Evidence from the fossil record tells us that the total number of living families has steadily increased over the past 600 million years. Numbers of originations and extinctions have fluctuated but in most time intervals the former outnumbers the latter. *Data from Benton (1993).*



Many biologists predict that we are now entering a sixth mass extinction (Leakey and Lewin, 1995). Over the past 400 years or so, several hundred species are known to have disappeared. Although this might sound like a lot, these recent extinctions actually represent a very small percentage of described taxa and therefore do not suggest anything close to a mass extinction (Table 8.1). Instead, it is the predicted rates of extinctions over the next century that are the main cause for concern. The best estimates of these are provided by the International Union for Conservation of Nature and Natural Resources (IUCN), which regularly compiles 'Red lists' on the numbers of species that are known to be at risk. Several categories are used (e.g. critically endangered, endangered, vulnerable), and these are based on a number of parameters including current population size, number of mature adults, generation time, recent reductions or fluctuations in population size, and population fragmentation (see http://www.redlist.org/ for more details).

Table 8.1 The numbers of species extinctions that have been recorded over the past 400 years (after Primack, 1998). Note that the true numbers are undoubtedly higher than this because numerous undescribed species will also have gone extinct, for example a large number of plant and invertebrate species was probably wiped out during the destruction of tropical rainforests over the past few decades

Taxonomic group	Number of extinctions	Percentage of taxonomic group
Mammals	85	2.1
Birds	113	1.3
Reptiles	21	0.3
Amphibians	2	0.05
Fish	23	0.1
Invertebrates	98	0.01
Flowering plants	384	0.2

The Red list that was compiled by the IUCN in 2010 reported that 21% of all described mammal species, 12% of all described bird species and 29% of all described amphibian species are threatened (http://www.iucnredlist.org/documents/summarystatistics/2010_1RL_Stats_Table_1.pdf). We know little about the total proportion of threatened species in other taxonomic groups simply because we lack the relevant information for most species. For example, 32% of fishes that have been evaluated are classified as threatened, but because only around 5% (4446 species out of an estimated 31 300 species) of all fish species have been assessed, this value gives us limited insight into the status of fishes as a whole. Similarly, 26% of evaluated insects have been placed in the threatened category, but <0.1% of insect species have so far been investigated. Few data are available for most groups of plants with the exception of gymnosperms, in which 89% of species have been evaluated, and we know that 35% of these are threatened. Clearly these data are far from complete, but if it turns out that similar proportions of *all* described species in the various taxonomic groups are threatened, then the fate of very many species hangs in the balance (Table 8.2). It is for this reason that many people believe that we are currently on the brink of a sixth mass extinction.

Table 8.2 Numbers and proportions of threatened species according to the IUCN 2010 Red List. Note that for most taxonomic groups only a very small proportion of species has been evaluated

Taxonomic group	Number of described species	Number of evaluated species	Number of threatened species as % evaluated	Number of threatened species as % described
Vertebrates				
Mammals	5 490	5 490	21%	21%
Birds	9 998	9 998	12%	12%
Reptiles	9 084	1 672	5%	28%
Amphibians	6 433	6 284	29%	30%
Fishes	31 300	4 446	5%	32%
Subtotal	62 305	27 890	10%	22%
Invertebrates				
Insects	1 000 000	2 886	0.1%	26%
Molluscs	85 000	2 305	10%	45%
Crustaceans	47 000	1 735	1%	35%
Corals	2 175	856	11%	27%
Arachnids	102 248	32	0.02%	56%
Velvet worms	165	11	5%	82%
Horseshoe crabs	4	4	0%	0%
Others	68 658	52	0.03%	46%
Subtotal	1 305 250	7 881	0%	34%
Plants				
Mosses	16 236	93	0%	86%
Ferns and allies	12 000	211	1%	66%
Gymnosperms	1 021	909	32%	35%
Flowering plants	281 821	10 916	3%	73%
Green algae	4 053	2	0%	0%
Red algae	6 081	58	0.1%	16%
Subtotal	321 212	12 189	3%	70%
Fungi and protists				
Lichens	17 000	2	0.01%	100%
Mushrooms	31 496	1	0.003%	100%
Brown algae	3 067	15	0.2%	40%
Subtotal	51 563	18	0%	50%
TOTAL	1 740 330	47 978	1%	36%

It is also likely that extinction rates will be accelerated by climate change (McLaughlin *et al*., 2002), which includes global warming, changes in precipitation patterns and a higher frequency of extreme weather events. Some of this biodiversity loss will arise because in a rapidly changing environment, species have to either adapt or disperse in order to survive. Over the short term, adaptation may not be possible, and we know from Chapter 4 that there is tremendous variation in the ability of species to disperse. Even those which can disperse may face limitations such as the summit trap phenomenon, which occurs when species that live on mountains move to higher altitudes as conditions warm up, and then have no escape route away from the mountain (see Pertoldi *et al*., 2007).

So why exactly are so many species threatened with extinction? In most cases, the answer to this is anthropogenic activity. Farming, logging, mining, damming and building have destroyed the habitats of countless species around the world. Many endemic species have suffered from human-mediated introductions of alien species, both deliberate and accidental. Hunting, fishing and trading have led to the overexploitation of many species, while countless others have suffered from industrial or agricultural pollution. Although these processes are diverse, a common outcome is a reduction in the sizes of wild populations. When this occurs, species begin to suffer from reduced genetic diversity and inbreeding, and this is where conservation genetics comes into play. In this chapter we will look at some of the most important aspects of conservation genetics by first examining how genetic data can be used to classify distinct species and management units in order to catalogue biodiversity and identify the most appropriate targets for conservation. In subsequent sections we shall build on some of the theory that was presented in earlier chapters by revisiting genetic diversity, inbreeding, population sizes and relatedness, but this time paying particular attention to how they can be applied to some of the issues surrounding conservation biology. We will also build on Chapter 5 by looking at how genomics and adaptive genes are at the heart of some of the more recent advances in conservation genetics.

Taxonomy

Taxonomy is the science that enables us to quantify biodiversity, although its applications extend much further than this because without it our understanding of ecology and evolution would be greatly reduced: put simply, species are the fundamental units of biology. Taxonomy has therefore remained an important area of biological research since Linnaeus developed his extensive classification system in the eighteenth century. Over the years, organisms have been classified on the basis of a wide range of morphological, behavioural and genetic characters. In this section we will limit ourselves to a discussion on the importance of taxonomy to conservation biology, paying particular attention to the contributions that have come from molecular data.

Species Concepts

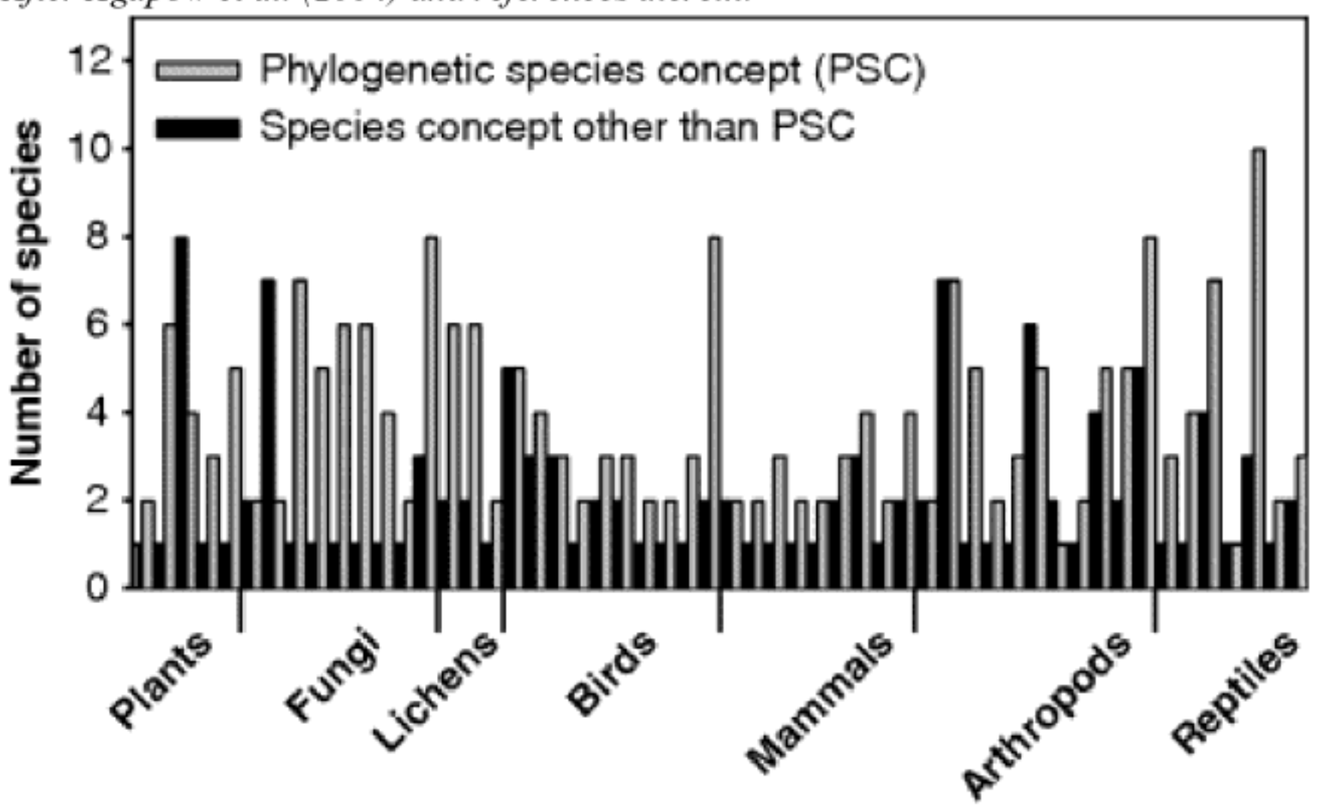
Conservation strategies are often directed at individual species, or at habitats that have been identified as species-rich, and they therefore tend to assume that most individuals have been correctly assigned to a particular species. But is this necessarily the case? Although generally supportive of conservation initiatives, most biologists would argue that the identity of species is far from straightforward. Historically, researchers have often relied on the biological species concept (BSC), which defines species as ' . . . groups of actually or potentially interbreeding natural populations, which are reproductively isolated from other such groups' (Mayr, 1942). Although conceptually straightforward, the BSC does have several shortcomings, for example a literal interpretation does not allow for hybridization, and few can agree on how this dilemma should be solved. In addition, the BSC cannot accommodate species that reproduce asexually or by self-fertilization.

More than 20 different species concepts can now be found in the literature (Hey *et al*., 2003). One alternative to the BSC is the phylogenetic species concept (PSC). This defines species as groups of individuals that share at least one uniquely derived characteristic, and is often interpreted to mean that a species is the smallest identifiable monophyletic group of organisms within which there is a parental pattern of ancestry and descent (Cracraft, 1983). The PSC circumvents to some extent the problem of asexual reproduction, but it has been criticized for dividing organisms on the basis of characteristics that may have little biological relevance, and also for creating an overwhelmingly large number of species. Furthermore, two groups that are identified as separate species under the PSC may retain the potential to reproduce with one another. If reproduction between these two groups did occur, they would no longer be monophyletic and would therefore have to be reclassified as a single species.

The PSC tends to identify a greater number of species than the BSC. One review of 89 studies concluded that the PSC identified 48.7% more species than the BSC (Agapow *et al*., 2004; see Figure 8.2). If the PSC was to replace the BSC as the most widely accepted species concept, the number of endangered species will increase while the geographical range of many will decrease. This in turn would lead to a wide-scale re-evaluation of numerous conservation programmes; for example, the location of high-profile biological hotspots, in which large numbers of endemic species can be found, may change depending on which concept is used to determine the number of species in a given region (Peterson and Navarro-Siguenza, 1999). Many biologists therefore advocate a less dramatic approach in which multiple species concepts are retained, provided it is clear which concept is being employed at any given time; some situations will lend themselves to the BSC, others to the PSC, while still others (for example those involving unicellular or parasitic taxa) may lend themselves to another approach altogether (de Meeus *et al*., 2003). This tactic has the advantage of being well balanced, but suffers from the uncertainties that surround variable taxonomic criteria.

Figure 8.2 Some examples of how the number of species in different taxonomic groups varies, depending on whether or not the phylogenetic species concept (PSC) is used for classification purposes.

After Agapow et al. (2004) and references therein.

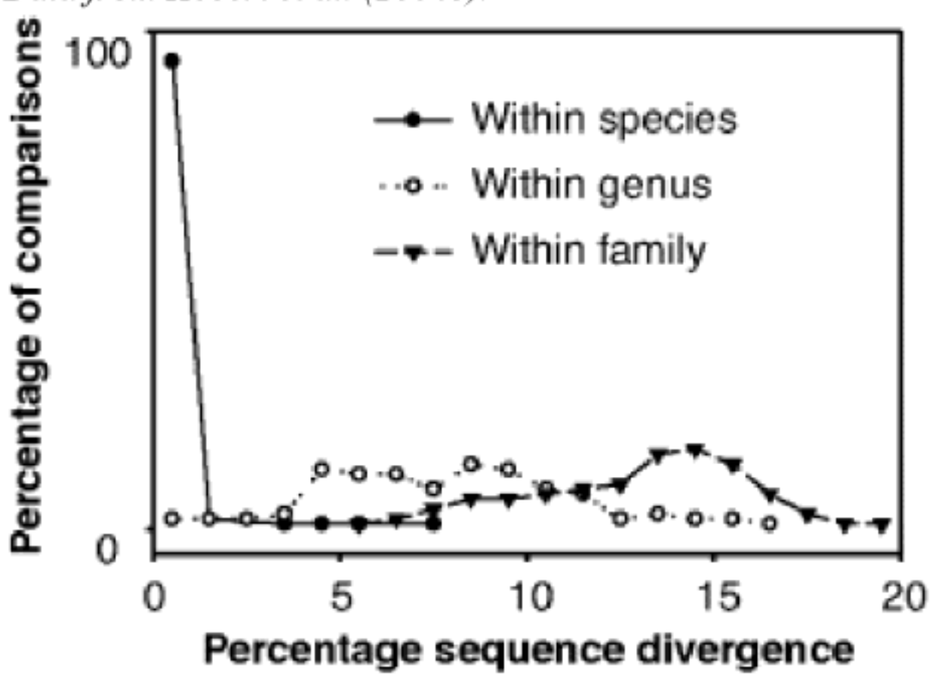


DNA barcoding

A more recently established approach to taxonomy seeks to identify species solely on the basis of a **DNA barcode** (or **genetic barcode**), which consists of one or a few DNA sequences. Barcoding is founded on the premise that variation in the barcoding gene is lower within species than between species. As a result, species can be identified if their sequence matches (or is similar to) other conspecific sequences; conversely, new species can be identified if their sequence is sufficiently different from the comparison sequences. The most widely used DNA barcode is a region of the cytochrome oxidase I (COI) gene in mitochondrial DNA (Hebert *et al*., 2003; Savolainen *et al*., 2005). One of the earliest applications of DNA barcoding was based on a comparison of 260 bird species, in which COI was found to be species-specific, and was also an average of 18 times more variable *between* species (7.05–7.93%) than *within* species (0.27–0.43%) (Figure 8.3; Hebert *et al*., 2004b). This is one of the findings that led to an international collaboration known as the Consortium for the Barcoding of Life, which is promoting the eventual acquisition of DNA barcodes for all living species.

Figure 8.3 The extent to which the mitochondrial cytochrome oxidase I gene varies among 260 species of North American birds. Comparisons are based on levels of sequence divergence within and among species, genera and families.

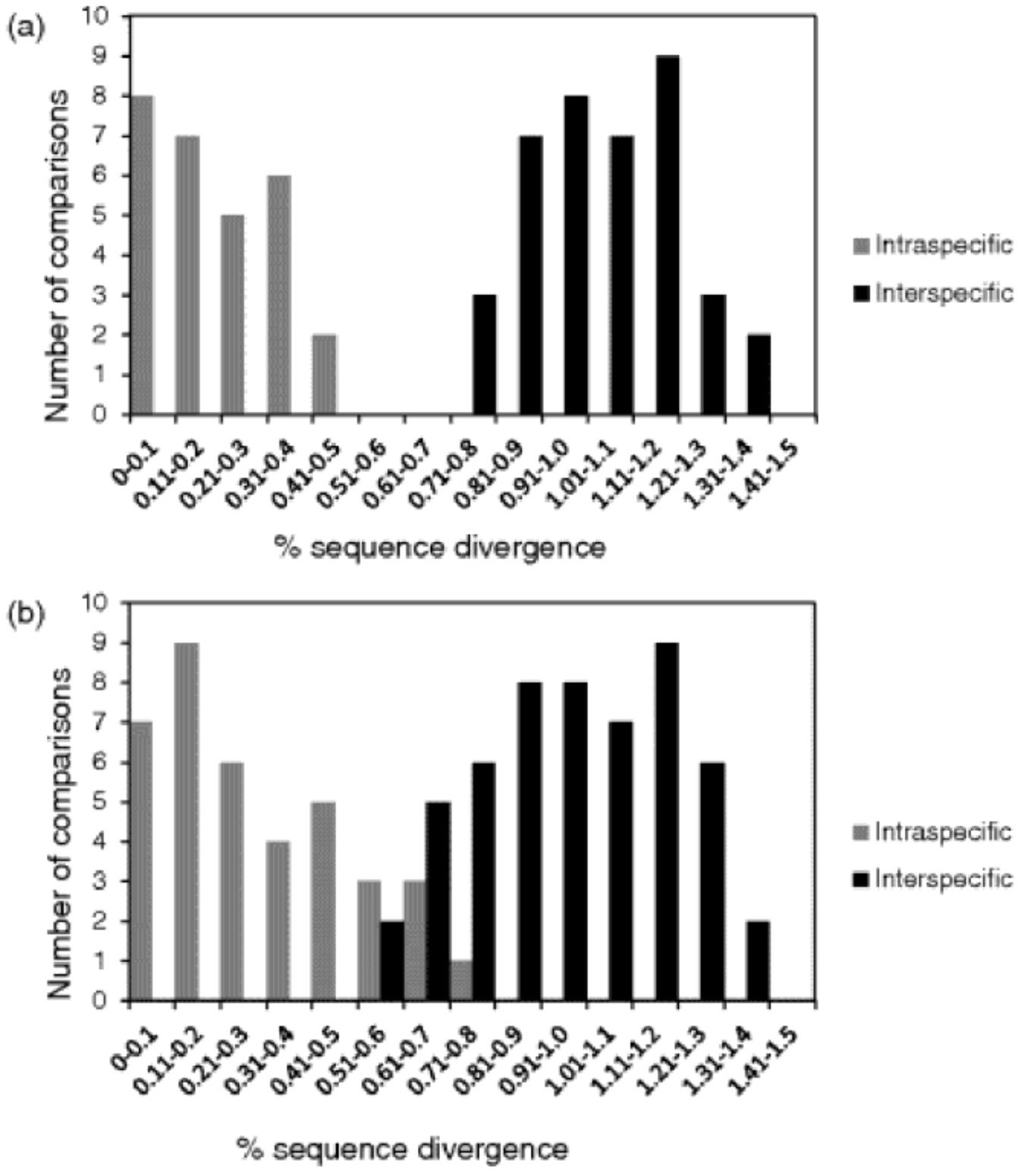
Data from Hebert et al. (2004b).



Although increasingly widely used, DNA barcoding is not without controversy. Critics have pointed out that the range of intraspecific sequence divergence can be difficult to predict. Although Hebert *et al*. (2004b) found that avian intraspecific divergence was consistently <0.44% and therefore lower than interspecific divergence, a study by Johnson and Cicero (2004) found that interspecific sequence divergences ranged from 0% to 8.2% in 39 comparisons of avian sister species. DNA barcoding is most straightforward when there is a barcoding gap between intra- and interspecific comparisons (Figure 8.4). Another problem can arise when sequences are not species-specific, and we know from Chapter 6 that both hybridization and incomplete lineage sorting mean that this will not always be the case. Because hybridization occurs between species within all major taxonomic groups, and an estimated one-quarter of all animal species have yet to reach the stage of reciprocal monophyly (Funk and Omland, 2003), DNA sequences will

sometimes transcend the boundaries of putative species. Lou and Golding (2010) used Bayesian analysis to show that DNA barcoding can work well even in the absence of a barcoding gap, except when the degree of incomplete lineage sorting is extreme.

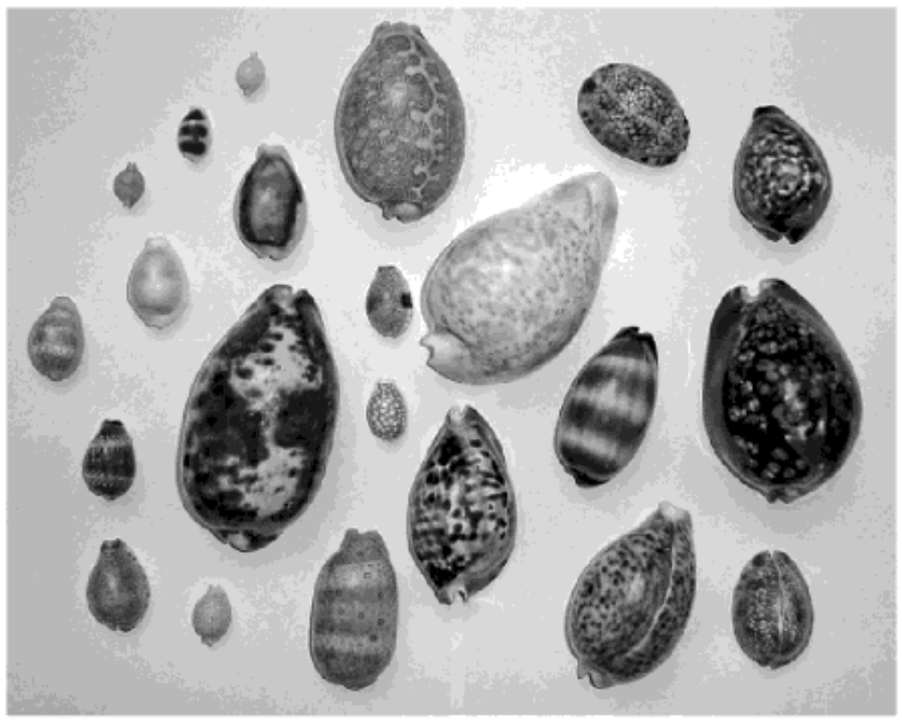
Figure 8.4 Two hypothetical scenarios describe the relationship between intra- and interspecific sequence divergence within a congeneric group of species. (a) intra- and interspecific sequence divergence does not overlap, resulting in a barcoding gap; (b) intra- and interspecific sequence divergence overlaps, and there is no barcoding gap. The latter scenario makes it more difficult to identify species on the basis of sequence similarity.



Meyer and Paulay (2005) found that DNA barcoding works best on groups for which taxonomy has already been reasonably well studied. They compared barcodes from a diverse group of cypraeid marine gastropods (cowries; Figure 8.5) and found that barcoding performs poorly in incompletely sampled groups. Many intra- and interspecific comparisons did not generate a barcoding gap, leading to error rates in species identification of ~17%. The authors concluded that barcoding could be a useful tool for differentiating between taxonomically well-understood clades from which a substantial proportion of species (and of lineages within species) have been sampled, but may not be able to differentiate between closely related species in groups that have been taxonomically understudied.

Figure 8.5 Shells from a range of cowrie species. DNA barcoding can discriminate well between taxonomically studied groups, but performs less well on closely related species from taxonomically understudied groups

(Meyer and Paulay, 2005). Photo attributed to Bricktop.



Other researchers have highlighted potential issues surrounding the sample sizes on which DNA barcodes are typically based. Matz and Neilson (2005) suggested a sample size of 12 individuals per species, although the DNA barcoding database typically includes only 5–10 sequences per species (Hajibabaei *et al*., 2007). Zhang *et al*. (2010) studied a data set from skipper butterflies of the *Astraptes fulgerator* complex in which cryptic species had previously been identified, partially on the basis of DNA barcoding (Hebert *et al*., 2004a). They determined that in order to discover 80% of the haplotypes in this data set, a sample size of between 10 and 216 would be necessary from each species (a variable number because of different evolutionary histories and levels of variability within each species). They conclude that it is unrealistic to expect that a single, universal sample size can be applied to all barcoding studies, and recommend that the evolutionary history of each study species be taken into account.

An additional challenge for DNA barcoding comes from the fact that cytochrome oxidase is not an appropriate barcode for all taxonomic groups. In plants, for example, it is not sufficiently variable to differentiate among species (recall from Chapter 2 that mtDNA is relatively invariant in plants). As an alternative barcode, the Canadian Barcode of Life Plant Working Group suggested that a combination of two chloroplast regions – *rbcL* and *matK* – be used as barcodes in plants, although they found that only about 75% of species could be discriminated on that basis (CBOL Plant Working Group, 2009). Another region that has been investigated extensively for plants is the internal transcribed spacer (ITS) of the nuclear ribosomal DNA gene, although in some species this is too variable to provide reliable discrimination, and in some cases more than one variant of the sequence is found within individuals (it is part of a repetitive gene; see Chapter 1). Spooner (2009) compared the performance of potential chloroplast and ITS barcodes in a well-studied but complicated plant group, *Solanum* sect. *Petota*, which includes wild and cultivated potatoes. The evolutionary history of potatoes has been complicated by interspecific hybridization, introgression, allopolyploidy, a mixture of sexual and asexual reproduction, and possible recent species divergence (Spooner and Salas, 2006). Against this backdrop, no useful DNA barcode has yet been identified: ITS has too much variation within species and the chloroplast regions were insufficiently variable. Table 8.3 summarizes some of the DNA regions that have been proposed as barcodes in a range of taxonomic groups (see also Box 8.1).

Table 8.3 Some examples of gene regions that have been identified as potentially suitable barcodes in various taxa

Taxonomic group	Gene region	Reference
Animals	Cytochrome oxidase I (COI) mitochondrial region	Herbert *et al*. (2003)
Plants	Two chloroplast regions, *rbcL* and *matK*	CBOL Plant Working Group (2009)
Plants	Second internal transcribed spacer (ITS2) of nuclear ribosomal DNA	Chen *et al*. (2010)
Arbuscular mycorrhizal fungi	Nuclear rDNA (1500 bp fragment spanning small subunit, ITS region, and large subunit)	Stockinger *et al*. (2010)
Red algae	Cytochrome oxidase I (COI) mitochondrial region	Robba *et al*. (2006)
Microbial eukaryotes	Small subunit nuclear rDNA	Huber *et al*. (2007)
Bamboo corals	Mitochondrial regions: mismatch repair gene homologue (msh1) and intergenic region (igr4) between cyt b and NADH6	van der Ham *et al*. (2009)

Box 8.1 DNA barcoding and gene flow

The precision with which DNA sequences can delineate species will depend in part on the frequency with which DNA introgression occurs between species. Petit and Excoffier (2009) argue that because high rates of *intra*specific gene flow can prevent or minimize

*inter*specific introgression, gene regions which experience high rates of intraspecific gene flow should be more suitable for species delineation than regions which experience low rates of intraspecific gene flow. The rationale for this is based on the fact that most species have dynamic ranges, which can allow for repeated meetings between closely related species between which reproductive barriers are incomplete. Introgression will often follow, but if introgression involves alleles that experience high intraspecific gene flow then drift will be minimized and the introgressed alleles less likely to reach appreciable frequencies. Conversely, if intraspecific gene flow is relatively low, introgression of alleles should be more apparent.

Petit and Excoffier (2009) tested this idea by comparing rates of introgression in markers with different modes of inheritance (biparental versus uniparental) in species with male-biased dispersal and species with female-biased dispersal. Recall from Chapters 2 and 7 that in species with female-biased dispersal, maternally inherited markers (e.g. mtDNA) should experience more intraspecific gene flow than biparentally (nrDNA) or paternally (Y chromosome) inherited markers; conversely, in species with male-biased dispersal, Y chromosome markers should experience more intraspecific gene flow than either maternally or biparentally inherited markers. If Petit and Excoffier (2009) were correct in their hypothesis, this should mean that nuclear markers would experience more introgression than mtDNA markers in species with female-biased dispersal, whereas mtDNA should experience more introgression than nuclear markers in species with male-biased dispersal. Their results overwhelmingly supported this prediction: 16 species with female-biased dispersal had a higher introgression rate in nrDNA compared to mtDNA, whereas 21 species with male-biased dispersal had a higher introgression rate in mtDNA compared to nrDNA (Figure 8.6). They conclude that when using markers to delineate species, the precision should be highest when based on genetic regions that are experiencing relatively high degrees of intraspecific gene flow.

Despite the various challenges associated with DNA barcoding, there are many supporters of this approach to cataloguing the earth's biodiversity. Although barcoding is not perfect, Packer *et al.* (2009) argue that the same can be said for taxonomic delineations that are made on the basis of morphological characteristics; limitations of morphology are particularly evident in (but not limited to) cryptic species, cryptic life stages (e.g. beetle larvae), and microbial species that require (but will not always be amenable to) culturing prior to identification. Similarly, Dexter *et al.* (2010) quantified the success rate of ecologists identifying trees on the basis of morphology – a challenging task because most trees encountered in the field must be identified using vegetative characters, but most species descriptions rely on fruit and flower characters – against known DNA sequences. They found that 6.8–7.6% of all individuals were erroneously identified on the basis of morphological characters, although they pointed out that DNA methods were also error-prone. Their conclusion was that a combination of morphological and molecular data would provide the most accurate species identification.

Figure 8.6 (a) African elephant (Loxodonta africana), which exhibits male-biased dispersal and in which mitochondrial introgression exceeds either nuclear or Y chromosome introgression (Roca *et al.*, 2005; photo attributed to Trevor Ohlssen).

(a)

(b)

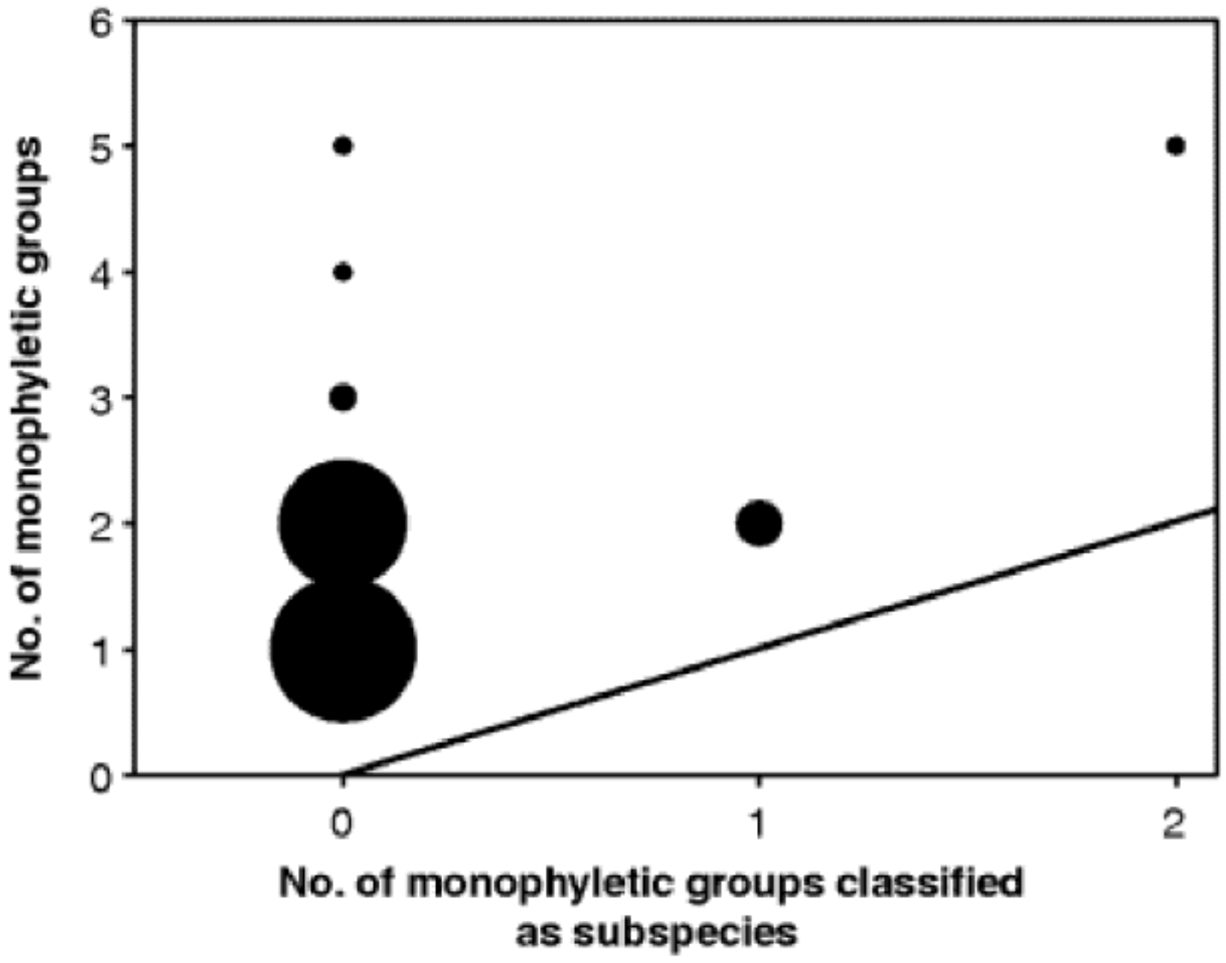
So far, we have been discussing the pros and cons of using a single region of DNA to delineate species (DNA barcoding). One important area in conservation genetics that we have not yet discussed is whether a barcoding region of ITS or mtDNA is biologically informative. This is part of the bigger question in conservation genetics which surrounds the application and relevance of neutral and non-neutral markers, and is one that we will return to repeatedly throughout this chapter. We will start this topic with a discussion of conservation units.

Subspecies

Possibly even more confusing than the species concept is the demarcation of subspecies. Although advocated by Linnaeus, the classification of subspecies was seldom used until the mid-twentieth century. The adoption of subspecies around this time was particularly widespread in birds. Reclassification was usually based on morphological characteristics, and as a result the current classification of bird subspecies does not agree with the distribution of monophyletic mitochondrial lineages. A review of the literature has shown that bird species contain on average approximately two monophyletic mtDNA lineages, but are subdivided into an average of 5.5 subspecies (Zink, 2004; Figure 8.7). The cactus wren (*Campylorhynchus brunneicapillus*), for example, has only two evolutionarily distinct mitochondrial lineages but six named subspecies.

Figure 8.7 The number of monophyletic mitochondrial lineages per species, compared to the number of these lineages that currently match subspecies classifications. The size of each circle is proportional to the number of comparisons in each category. The diagonal line indicates where the circles would be located if the monophyletic mitochondrial lineages in each species were in complete agreement with designated subspecies. Because all circles are above this diagonal line, all species contain monophyletic groups that are not classified as subspecies.

After Zink (2004) and references therein.



Discrepancies such as these may mean that conservation efforts are directed at genetically indistinct subspecies while distinct lineages receive less attention, and this has led Zink (2004) to call for the re-classification of subspecies. This is a somewhat controversial demand, since there are a number of reasons why the morphology and genetics of recently diverged species may not agree, one of these being incomplete lineage sorting. Furthermore, as we learned in Chapter 4, quantitative trait variation may exceed the genetic differences that are revealed by neutral molecular markers. Subspecific status should therefore be revoked with caution, because morphological differences, however slight, may reflect local adaptation even if neutral molecular markers show no differentiation.

Understanding the delineation of subspecies is highly relevant to conservation policy; for example, one-third of the bird taxa on the US endangered species list are subspecies (Fallon, 2007). It is therefore critical that if we are to protect all evolutionarily significant lineages, appropriate criteria are used to identify subspecies. However, there are many instances in which taxa that appear to be distinct subspecies, or even species, on the basis of morphological characteristics show no differentiation at neutral genetic markers (Funk and Omland, 2003). Swamp sparrows (*Melospiza georgiana*) have provided an excellent system in which to compare divergence on the basis of morphology, neutral genetic divergence and adaptive traits. The coastal plain swamp sparrows (*M. g. nigrescens*) are morphologically distinct from the subspecies that live in inland freshwater marshes (*M. g. georgiana*, *M. g. ericrypta*), and yet show no genetic differentiation on the basis of neutral molecular markers (see references in Ballentine and Greenberg, 2010). However, when nestlings from both coastal and inland subspecies were raised in a common animal care environment, it was clear that there were heritable differences in bill size and plumage coloration. The authors of this study concluded that the neutral genetic markers (allozymes, mtDNA and microsatellites) that had previously shown no differentiation between the subspecies were not suitable for identifying evolutionarily important taxonomic subdivisions (Ballentine and Greenberg, 2010).

Conservation Units

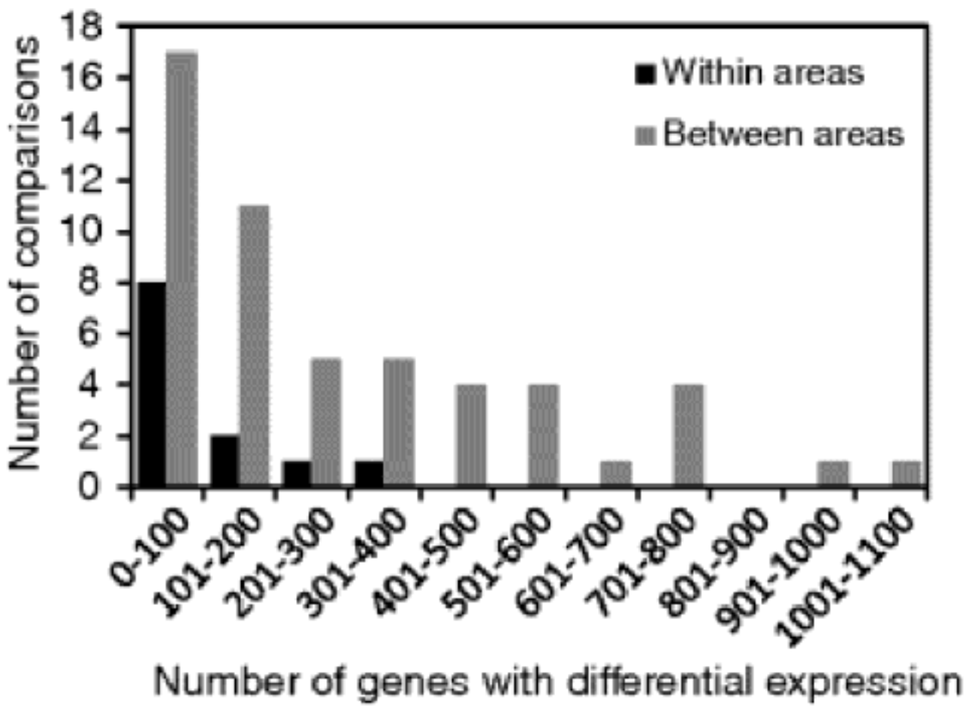
In an attempt to circumvent some of the problems that may be associated with taxonomic classification, conservation biologists sometimes concentrate on management units (MU) and evolutionarily significant units (ESU). An MU is 'any population that exchanges so few migrants with others as to be genetically distinct from them' (Avise, 2000), and is analogous to the stocks that are identified in fisheries. Distinct MUs are often identified on the basis of significant differences in allele frequencies at multiple neutral loci. An ESU consists of one or more populations that have been reproductively isolated for a considerable period of time, during which they have been following separate evolutionary pathways. Examples of this may include lineages that diverged in separate refugia during glacial periods (see Chapter 6). ESUs have often been characterized by reciprocal monophyly in mtDNA and significant allele frequency differences at neutral nuclear loci (Moritz, 1994).

As with higher taxonomic divisions, there is a growing trend in conservation genetics to base the identification of conservation units on patterns of adaptive variation. This, however, does not mean that estimates of genetic diversity based on neutral markers are uninformative: studies have shown that bird species with low levels of neutral genetic diversity are more likely to go extinct (Evans and Sheldon, 2008). In addition, neutral markers can provide unbiased (i.e. not influenced by natural selection) estimates of genetic drift and time since reproductive isolation (Luikart *et al*., 2003). However, when considering the adaptive potential of different conservation units within their particular habitats, non-neutral genetic markers can provide more informative estimates of adaptive evolutionary divergence (Gebremedhin *et al*., 2009).

A combination of neutral genetic data and data from the transcriptome (Chapter 5) was used to characterize conservation units of threatened Atlantic salmon, *Salmo salar*, around the Bay of Fundy in Canada. Genetic differentiation among populations from four different geographical regions around the bay was inferred from neutral microsatellite loci, and gene expression was quantified using a cDNA salmonid microarray. Microsatellite data showed differentiation among all four geographical areas; similarly, the transcriptomic variation showed that a greater number of genes were being differentially expressed between each of the four areas compared to between different sites within each of the four areas (Figure 8.8). In general, patterns of variation in gene expression were consistent with the levels of genetic differentiation that were based on microsatellite loci, although the two measures of genetic differentiation were not strongly correlated. The authors of this study therefore suggest that these data collectively demonstrate that different geographical regions each harbour unique salmon lineages, and ideally all of these should be conserved (Tymchuk *et al.*, 2010).

Figure 8.8 The numbers of differentially expressed genes was generally higher between geographical areas than within geographical areas in Atlantic salmon around the Bay of Fundy, and this supports the conclusion based on neutral markers that each geographical area harbours one or more unique genetic lineages

(Tymchuk et al., 2010).



Hybrids

The preservation of distinct MUs and ESUs is generally seen as desirable because each unit contributes to a species' genetic diversity. Conservation of hybrids, on the other hand, is a much more controversial issue. The U.S. Endangered Species Act (ESA), for example, originally proposed that hybrids would not be protected. This clause has since been revoked, although a proposed replacement policy on 'inter-crosses' (avoiding the sometimes pejorative term 'hybrids') has yet to be officially integrated into the ESA. However, hybridization in plants and animals can have both positive and negative influences on biodiversity. On the one hand, hybridization involves the creation of novel genomes, and can therefore be an important evolutionary process that has led to many new plant and animal species throughout history (Mallet, 2007). On the other hand, hybridization brings with it the potential for significant introgression of genomes, which can be particularly problematic if the genomes from a common species 'swamp' a rare species, thereby eroding the threatened species' genome, compromising local adaptation and ultimately hastening the road to extinction.

The risk of genome swamping means that hybridization is usually considered an undesirable event in conservation genetics; however, in many species it may be difficult to detect. For example, picture-winged *Drosophila* (Figure 8.9) on the Hawaiian Archipelago hybridize with other species, a process that is likely facilitated by the rapid divergence and speciation of the Hawaiian *Drosophila*: within the genus, species that are genetically more similar are more likely to produce viable and fertile offspring (Coyne and Orr, 1997), and hence should be more likely to hybridize. However, it is unclear whether hybridization in Hawaiian picture-winged *Drosophila* is natural or a result of anthropogenic activities. Introduced *Drosophila* species do not appear to hybridize with Hawaiian *Drosophila*, but habitat alterations and introduced predators are altering the distribution and abundance of species which may facilitate future hybridization events (Price and Muir, 2008). In other species, particularly birds and mammals, hybridization may be easier to detect. Today there are around 500 000 plains bison in North America, and only about 4% of these (20 000 animals) are in conservation herds. Furthermore, hybridization between bison and cattle has been extensive in the past, and only one of the conservation herds with no known cattle ancestry has an N_e of >1000. One of the targets of proposed conservation management strategies is therefore to minimize cattle ancestry in additional herds (Hedrick, 2009). Other examples of 'problem' hybrids in conservation biology are given in Table 8.4.

Figure 8.9 Drosophila heteroneura (left) and D. silvestris (right) are two native Hawaiian pictured-winged Drosophila that are known to hybridize in nature. The hybrid males (middle) and females survive and successfully breed but there is evidence that backcross males have reduced mating success that may limit the integration of these species.

Photo provided by Don Price, and reproduced with permission.

Table 8.4 Some examples of hybrids that are threatening rare or declining species. Note that most of the examples involve introduced species

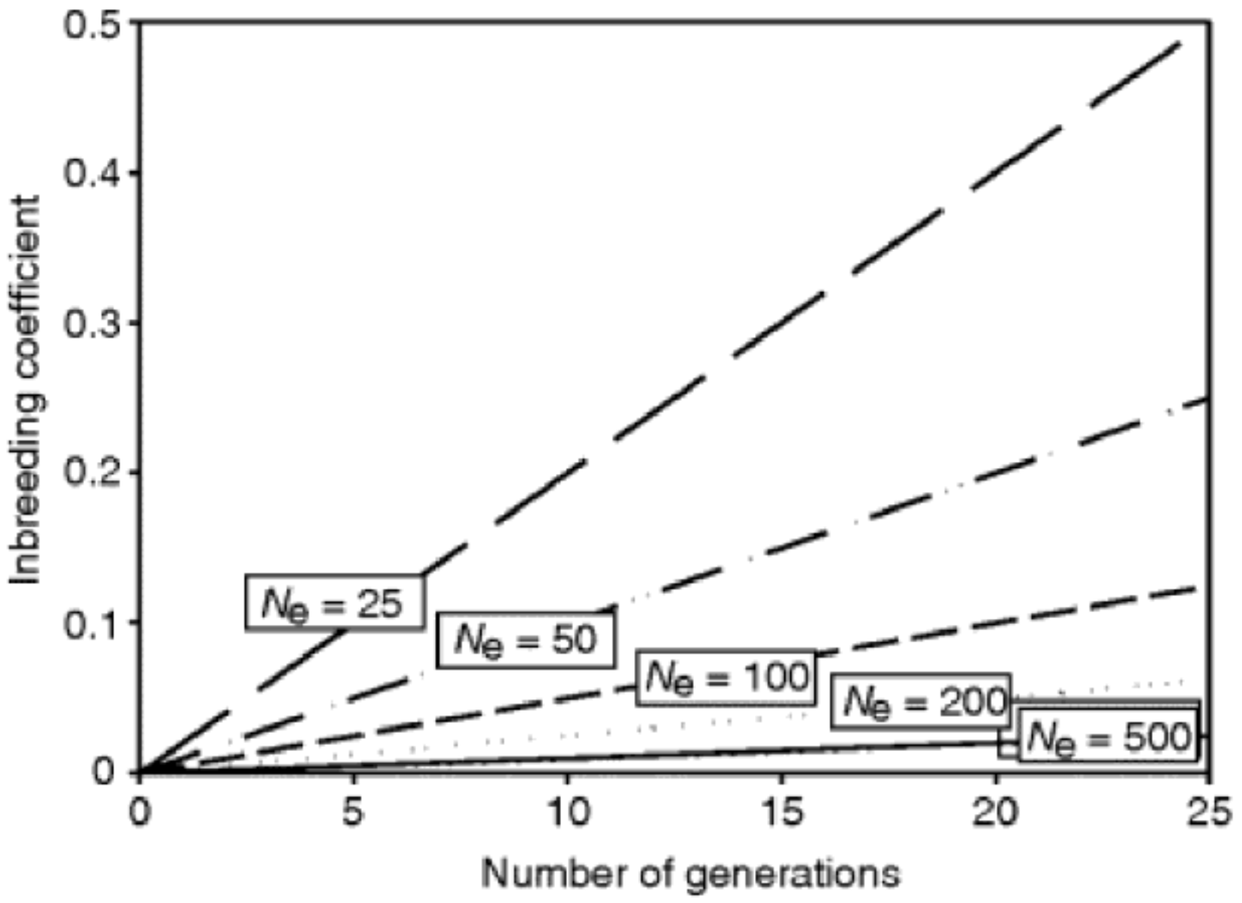
Rare or declining parental species	Common parental species	Reason for (accelerated) hybridization	Reference
Spotted owl (*Strix occidentalis*)	Barred owls (*Strix varia*)	Barred owls have expanded geographical range	Haig *et al.* (2004)
New Zealand Grey Duck (*Anas superciliosa*)*	Mallard duck (*Anas platyrhynchos*)	Mallard ducks introduced to New Zealand	Rhymer *et al.* (1994)
Hawaiian duck (*Anas wyvilliana*)	Mallard duck (*Anas platyrhynchos*)	Mallard ducks introduced to Hawaii	Fowler *et al.* (2009)
Australian dingo (*Canis lupus dingo*)	Domestic dog (*Canis lupus familiaris*)	Human settlers brought domestic dogs to Australia	Elledge *et al.* (2008)
Bull trout (*Salvelinus confluentus*)	Brook trout (*Salvelinus fontinalis*)	Brook trout an introduced species in bull trout's range	DeHaan *et al.* (2010)
Yellowstone cutthroat trout (*Oncorhynchus clarkii bouvieri*)	Rainbow trout (*Oncorhynchus mykiss*)	Rainbow trout an introduced species in Yellowstone cutthroat trout's range	Gunnell *et al.* (2008)
Butternut tree (*Juglans cinerea*)	Japanese walnut (*Juglans ailantifolia*)	Japanese walnut is introduced species	Ross-Davies *et al.* (2008)
Red-legged (*Alectoris rufa*) and rock (*A. graeca*) partridge	Chukar partridges (*A. chukar*)	Massive release (for hunting) of captive reared hybrids of native (red-legged and rock) and non-native (chukar) partridges	Barilani *et al.* (2007)
Caspian locust tree (*Gleditsia caspic*)	Honey locust tree (*Gleditsia triacanthos*)	Honey locust tree is non-native, planted as ornamental shade tree	Schnabel and Krutovskii (2004)

*Now effectively extinct – only hybrids remain.

Although molecular data have made valuable contributions to studies of taxonomy, and have sustained ongoing debate about taxonomic criteria, they do raise (or in many cases reinforce) a number of questions. For example, all individuals are genetically unique, but just how much genetic dissimilarity should we tolerate within a single MU? How much genetic divergence is required before ESUs are designated distinct species? How can molecular taxonomy accommodate incomplete lineage sorting and hybridization? Our inability to answer these questions to everyone's satisfaction does not mean that identifying the most appropriate units for conservation is an impossible task, although we need to remain aware of the limitations and assumptions that surround many taxonomic decisions. For the rest of this chapter we will, for the most part, be talking about species and populations as unambiguous entities, but we must keep in mind the possibility that species and population boundaries will be redrawn at some point in the future.

in which H_t and H_0 represent heterozygosity at generation t and generation zero, respectively, and F is the inbreeding coefficient (Frankham *et al*., 2002). We were introduced to the first part of this equation in Chapter 3 (equation) as a way to estimate the rate at which heterozygosity will be lost from a population. By expanding this equation to include the inbreeding coefficient, we can see how drift, which is influenced by population size, will simultaneously reduce genetic diversity and promote inbreeding.

Figure 8.10 The increase over time in the inbreeding coefficients (F) of five populations of different sizes, all of which were completely outbred at time zero ($F = 0$), and all of which are closed to immigrants. The rate at which inbreeding levels increase within a population is inversely proportional to its effective size.



Inbreeding threatens the survival of small populations when it leads to a reduction in fitness, a phenomenon that is known as **inbreeding depression**. There are two ways in which this can occur. The first of these is known as **dominance**, so-called because the favourable alleles at a locus are usually dominant, and the deleterious alleles have been maintained within the populations because they are recessive. The increased homozygosity that results from inbreeding means that deleterious alleles are more likely to occur as homozygotes, and when this happens their effects cannot be masked by the dominant favourable allele and inbreeding depression will result. The second phenomenon that can lead to inbreeding depression is known as overdominance, or heterozygote advantage, which means that individuals that are heterozygous at a particular locus have a higher fitness than individuals that are homozygous for either allele. In Chapter 3 we were introduced to sickle-cell anaemia, a classic example of overdominance in which heterozygotes benefit from a high resistance to malaria.

Although inbreeding depression has been a central theme in conservation biology for decades, and although general mechanisms for it are widely accepted, we still know little about its underlying molecular basis, for example which genes are involved, how many genes are involved, what gene pathways are involved, and so on. However, some recent genomic studies have started to give us insight into these molecular level questions (Paige, 2010). The first whole-genome study on the relationship between inbreeding and gene expression was done by Kristensen *et al*. (2005) on *Drosophila melanogaster*. They compared gene expression in inbred and outbred lines of *D. melanogaster*, and determined that inbreeding changes transcription levels for a number of genes. The genes that showed differential expression in inbred lines were disproportionately involved in metabolism and stress responses, for example heat-shock protein genes (which are involved in stress response) were up-regulated more (i.e. expressed in greater amounts) in inbred flies. This suggests that inbreeding acts like an environmental stress factor, and the metabolic costs of this stressor leave less energy for reproduction (hence a reduction in fitness associated with inbreeding). This effect was even more pronounced in a follow-up study when flies were placed in a more stressful (high temperature) environment, which had the effect of increasing the differential expression of heat-shock protein and metabolism genes in inbred versus outbred flies (Kristensen *et al*., 2006). This suggests that inbred organisms will be particularly challenged in stressful environments, a conclusion that is consistent with other studies such as one which found that inbreeding depression is on average 6.9 times higher for mammals in the wild compared to mammals that are kept in captivity (Crnokrak and Roff, 1999).

Demontis *et al*. (2009) extended the study of gene expression in inbred *Drosophila* by looking at 40 SNPs in coding regions of genes that were previously identified as being differentially expressed in inbred and outbred lines. They compared fast inbred lines (which took one generation to reach a predefined level of inbreeding) with slow inbred lines (which took 19 generations to reach the same level of inbreeding) to test the hypothesis that slow inbreeding causes less inbreeding depression than fast inbreeding. It has been suggested that this difference is the result of more efficient **purging** (selection against – and hence elimination of – deleterious, homozygous alleles; see Box 8.3) and/or or more efficient selection for heterozygotes in populations that take longer to become inbred. Demontis

the risks associated with this outweigh the potential benefits, because it is extremely difficult to predict the efficacy of purging (Leberg and Firmin, 2008).

Heterozygosity Fitness Correlations

The examples described in the previous section, which used whole-genome data to study the effects of inbreeding, were conducted on *Drosophila melanogaster* which is a model species for which a great deal of genetic information is now available. Although transcriptome characterization of non-model organisms is possible (e.g. Vera *et al*., 2008), recall from Chapter 5 that genomics studies on non-model organisms are still very expensive to conduct, and remain few and far between. However, there are other ways to obtain information about inbreeding and inbreeding depression from molecular genetic data, and one of these is by looking at heterozygosity fitness correlations (HFCs).

HFCs are based on two principles: first, multilocus heterozygosity values can be used as a measure of inbreeding, and second, inbreeding depression leads to a reduction in fitness. If we combine these two principles, we reach the conclusion that inbreeding depression should be characterized by a correlation between low heterozygosity and reduced fitness. This is most commonly tested for by comparing observed heterozygosity values to one or more individual fitness components such as rate or % of seed germination; growth rate; time to reproduction; the number of flowers, fruits, or seeds; sperm quality or volume; or longevity.

Although caution should be used when interpreting results that are based on a limited number of loci, or on a limited number of individuals (Chapman *et al*., 2009), a correlation between heterozygosity and fitness is widely accepted as evidence of inbreeding depression (reviewed in Szulkin *et al*., 2010). See Table 8.5 for some examples of HFCs. Such correlations may be strengthened by studies that are based on multiple species. Fitzpatrick and Evans (2009) reported a link between sperm quality and heterozygosity (based on microsatellite loci) across 20 mammal species, a correlation that was driven by the low heterozygosity and poor sperm quality in the endangered species. However, others have argued that because inbreeding leads to a genome-wide reduction in heterozygosity, HFCs are unlikely to be caused by inbreeding depression because they are based on only a small number of neutral markers, which are unlikely to represent genome-wide changes in homozygosity.

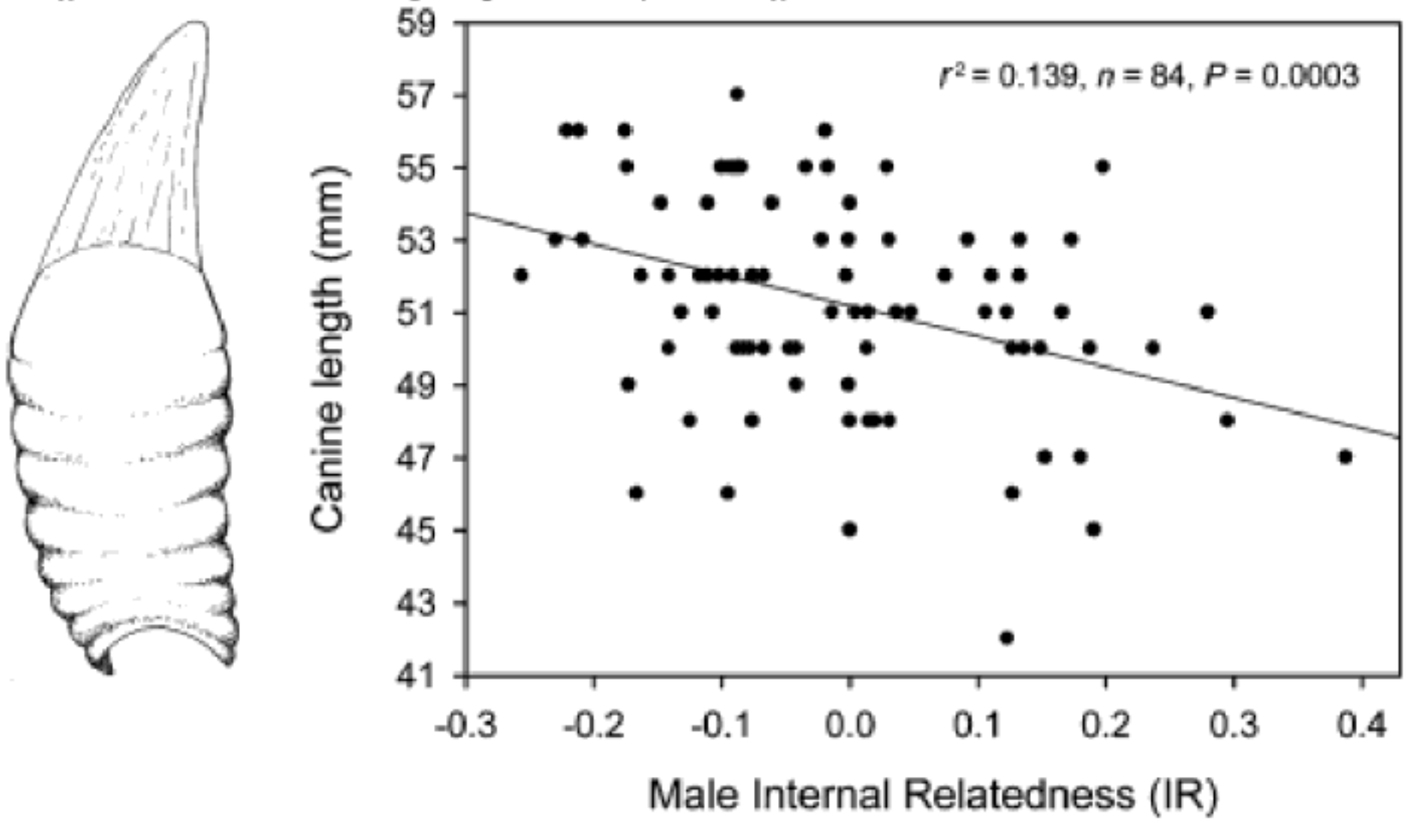
Table 8.5 Species in which a positive correlation has been found between heterozygosity and fitness (HFCs)

Species	Characteristic	Reference
Iberian red deer (*Cervus elaphus hispanicus*)	Smaller antler size in individuals with low heterozygosity	Perez-Gonzalez *et al*. (2010)
Alpine marmots (*Marmota marmota*)	Greater survival of juveniles with higher heterozygosity	Cohas *et al*. (2009)
Great tits (*Parus* major)	Individuals with earlier egg-laying date and larger clutch size had higher heterozygosity	Tomiuk *et al*. (2007)
Seychelles warblers (*Acrocephalus sechellensis*)	Maternal heterozygosity correlated with offspring survival	Brouwer *et al*. (2005)
Pocket gopher (*Thomomys* bottae)	Metabolic cost of burrowing is higher in individuals with low heterozygosity	Hildner and Soulé (2004)
Butterfly blue (*Scabiosa columbaria*; perennial plant)	Populations were less able to compete with Bromus grass when heterozygosity was low	Pluess and Stocklin (2004)
Common mussel (*Mytilus edulis*)	Improved immune response in highly heterozygous individuals	Carissan-Lloyd *et al*. (2004)

Another possible explanation for HFCs is overdominance or, more likely, associative overdominance (an increase in fitness of heterozygotes at a neutral locus that that is linked to a locus that is under selection). This was illustrated by two complementary studies on Antarctic fur seals (*Arctocephalus gazella*). In the first study, Hoffman *et al*. (2010b) identified a correlation between heterozygosity at nine microsatellite loci and the sizes of upper canine teeth that had been collected from adult male seals that died of presumably natural causes. Because tooth size is a good predictor of body size in this species, this provided evidence of a link between heterozygosity and body size (Figure 8.12). The authors could not pinpoint the mechanism behind this trend, but suggested that because the observed pattern appeared largely attributable to a small subset of loci, associative overdominance – and not inbreeding depression – was the most likely proximate mechanism. In a follow-up study (Hoffman *et al*., 2010a), the researchers increased their data set to an impressive 76 microsatellite loci, and found that inbred individuals were largely absent, and the HFC was lost. If the original HFC was a result of inbreeding depression then this pattern should have been retained across a larger set of loci, particularly as most of the loci had been located at unique locations around the dog genome. Because the HFC was lost, the authors had further grounds to support their earlier conclusion that it was associative overdominance, and not inbreeding depression, that had generated their earlier finding of an HFC.

Figure 8.12 Relationship between multilocus heterozygosity, expressed using the measure internal relatedness, and canine length for 84 adult male Antarctic fur seals that died of natural causes at Bird Island, South Georgia

(Hoffman et al., 2010a). Figure provided by Joe Hoffman.



Self-Fertilization

So far we have been looking at inbreeding depression in species that reproduce solely by outcrossing. We will now turn our attention to **self-fertilization** (or **selfing**), which involves the fusion of gametes that have been produced by the same individual and is therefore the most extreme form of inbreeding. Around 40% of all flowering plant species are capable of self-fertilization. We might expect selfing plants to exhibit high levels of inbreeding depression, but in actual fact they are often less prone to inbreeding depression than outcrossing species. This may be because they are more adept at purging deleterious alleles, although as with obligately outcrossing species, purging seems to be more effective in some populations than in others.

In the eelgrass (*Zostera marina*), for example, selfing plants produce seeds more frequently and in larger numbers than outcrossing plants (Rhode and Duffy, 2004). In the wild daffodil *Narcissus longispathus*, on the other hand, inbreeding depression can be pronounced (Figure 8.13). This is a herb that is endemic to a few mountain ranges in south-eastern Spain, and which can reproduce by either self-fertilization or outcrossing. In one study, heterozygosity was found to be much higher in parental plants than in seedlings, a discrepancy that is taken as evidence for strong selection against inbred offspring (Barrett *et al.*, 2004). This is therefore an example of self-fertilization leading to inbreeding depression in the form of high seedling mortality. Despite these obvious drawbacks, the authors of this study suggest that self-fertilization is maintained in this species because it allows prolific reproduction during the founding of new populations, even if mates are unavailable.

Figure 8.13 Narcissus longispathus (Amaryllidaceae), a rare self-compatible trumpet daffodil restricted to a few mountain ranges in SE Spain.

Photograph by Spencer C. H. Barrett.

Many hermaphrodite animals are also capable of both outcrossing and self-fertilization, including a number of tapeworm, snail and ascidian species. The parasitic tapeworm *Schistocephalus solidus* has a complex life cycle with a copepod (freshwater zooplankton) as its first intermediate host, the three-spined stickleback (*Gasterosteus aculeatus*) as its second intermediate host, and one of several fish-eating bird species as its final host. Researchers who were interested in whether or not selfing led to inbreeding depression in this parasite used microsatellite data to compare the genotypes of adults and offspring in order to establish whether juveniles were the product of self-fertilization (one parent) or outcrossing (two parents). They then discovered that outcrossed parasites produced a significantly more intense infection than selfed parasites, and as a result they were more likely to progress in their life cycle to the point where they could reach their final host. Despite an advantage to outcrossing, this species nevertheless maintains an ability to self-fertilize, presumably for reproductive assurance because there is no guarantee that a tapeworm will be able to find a partner with which to outcross (Christen and Milinski, 2003).

Inbreeding Avoidance

A final testimony to the hazards associated with inbreeding are the lengths to which individuals will often go in order to avoid it. In Chapter 7 we were introduced to two important mechanisms of inbreeding avoidance. The first of these was sex-biased dispersal. If one sex is philopatric and the other disperses before reproducing, then the breeding males and females within a population or breeding group should not be related to each other. That is not to say that inbreeding avoidance is the only reason why sex-biased dispersal occurs, since other factors such as competition for territories or for mates may also come into play, but for some species it is undoubtedly a driving force. In black grouse (*Tetrao tetrix*), for example, females seem unable to discriminate among relatives for the purposes of mating, although copulations between close relatives are rare. Because females do not discriminate among mates on the basis of relatedness, inbreeding should be more prevalent in flocks that contain a greater number of relatives. By comparing the relatedness of male and female grouse in their parental flock to the relatedness of males and females in a non-parental flock, Lebigre *et al*. (2010) demonstrated that female-biased dispersal was reducing inbreeding within populations.

Even when neither males nor females tend to disperse from their family groups, incestuous matings can often be avoided. A study that was published in 1995 reviewed data from a number of species that live in family groups, and found that 18 out of 19 avian species, and 17 out of 20 mammalian species showed a strong tendency to avoid mating with relatives (Emlen, 1995). This leads us to the second mechanism of inbreeding avoidance that we were introduced to in Chapter 7, and that is mate choice. If mates are chosen at least partially on the basis of inbreeding avoidance then species must have a basis for recognition. Some species will use phenotypic characters, for example the call of the American toad (*Bufo americanus*) is more similar in closely related individuals, and therefore can be used as a cue to avoid inbreeding (Waldman *et al.*, 1992). Other species use olfactory cues, which may help them to identify genetically dissimilar mates, for example sand lizards prefer the odour of individuals that have distantly related MHC alleles (Olsson *et al.*, 2003).

Of course, inbreeding avoidance is impossible in very small populations, but remember that inbreeding does not necessarily lead to inbreeding depression. A question that often appears in conservation genetics is how large a population must be if it is to avoid inbreeding depression. The work of animal breeders suggests that populations with an effective size of 50 or more should usually be able to avoid inbreeding depression and retain reproductive fitness (Franklin, 1980). However, this estimate refers to the short-term avoidance of inbreeding depression, and other studies suggest that an effective size of between 500 and 1000 is necessary if populations are to maintain their long-term evolutionary potential (Franklin and Frankham, 1998). Note also that this is the *effective* population size, and if we accept that the average N_e/N_c ratio is around 0.14 (Chapter 3), the minimum population census size necessary for long-term survival will be closer to 4000. Somewhat alarmingly, many species have a total N_c that is <500 (Table 8.6) and, although they are not all necessarily doomed to extinction, they are undoubtedly at a greater risk than large populations because of their relatively low levels of genetic diversity and their often high levels of inbreeding.

Table 8.6 Some examples of endangered or critically endangered species that had an $N_c < 500$ at their most recent assessment. Source: IUCN and BirdLife International

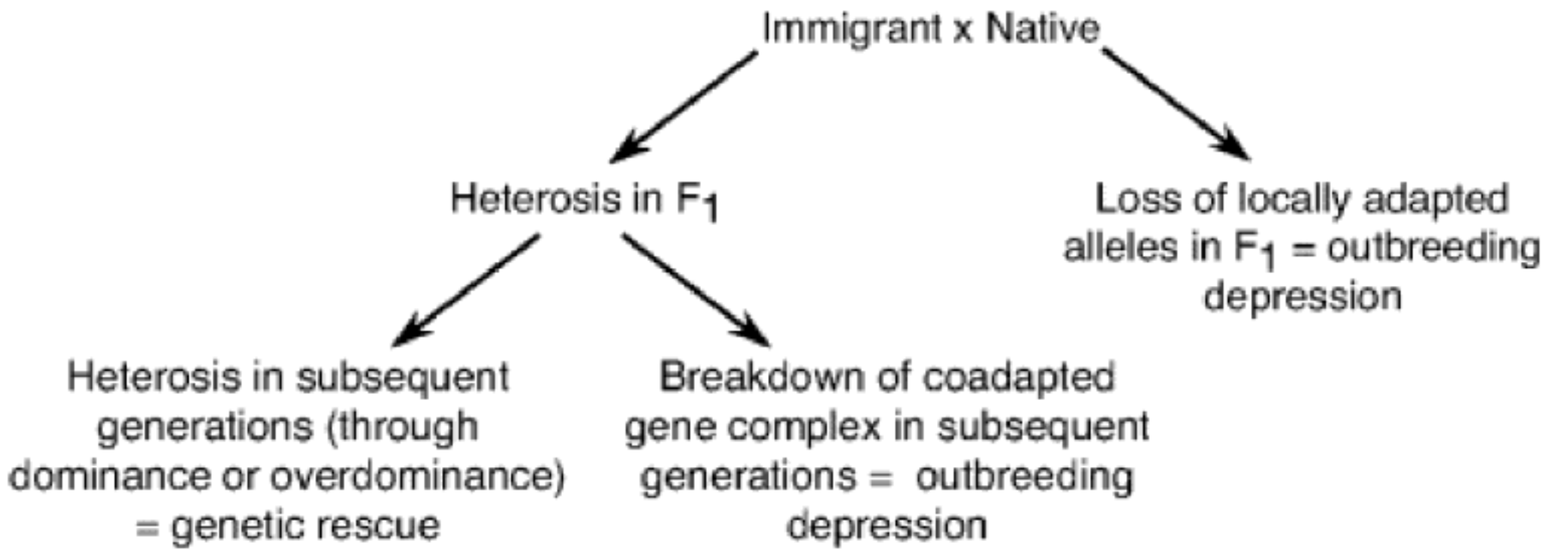
Species	**Geographic range**	N_c
Baishanzu fir (*Abies beshanzuensis*)	Baishanzu Mountain, China	5
Greenflower Indian mallow (*Abutilon sandwicense*)	Oahu, Hawaii	200–300
Bastard quiver tree (*Aloe pillansii*)	Namibia, South Africa	<200
Visayan wrinkled hornbill (*Aceros waldeni*)	Western Visayas, Philippines	120–160
Blue-eyed ground-dove (*Columbina cyanopis*)	Brazil	<250
Anegada ground iguana (*Cyclura pinguis*)	Virgin Islands	<200
Aruba Island rattlesnake (*Crotalus durissus unicolor*)	Caribbean	350
Javan rhinoceros (*Rhinoceros sondaicus*)	Java, Vietnam	<100
Ethiopian wolf (*Canis simensis*)	Ethiopia	400

Outbreeding Depression

Inbreeding depression is not the only possible deleterious outcome of mating between individuals with suboptimal levels of relatedness; another potential threat comes from **outbreeding depression**. This occurs when genetically dissimilar individuals interbreed and the fitness values of their offspring are lower than those of either parent. This can occur following matings between individuals from two different species or subspecies, or even individuals from two different lineages within a single subspecies. Two genetic factors can lead to outbreeding depression. The first is the loss of locally adapted genotypes. If individuals from two populations that are each adapted to their natal environments hybridize, their offspring will contain a mixture of alleles that may not be well suited to either environment. When this occurs, outbreeding depression will be evident in the first generation of offspring.

The second genetic cause of outbreeding depression is the loss of positive epistatic interactions. **Epistasis** refers to the interaction of genes from multiple loci, and their collective influence on particular traits. The group of loci involved in an epistatic interaction is known as a **co-adapted gene complex**, and if this complex is broken up through recombination then outbreeding depression may result. Because the set of chromosomes from each parental lineage will remain intact in their offspring (the F_1 generation), outbreeding depression may not be immediately evident, and in fact fitness may temporarily increase because the F_1 generation will have high heterozygosity values. By the F_2 generation, however, recombination will have disrupted the adaptive gene combinations, causing a sudden reduction in fitness (Figure 8.14). This process was evident in the tide pool copepod *Tigriopus californicus* when broods representing both pure populations and inter-population hybrids were raised under different conditions of temperature and salinity. As is typical following the disruption of co-adapted gene complexes, adverse affects were not evident in the F_1 generation of inter-population hybrids, but the fitness of the F_2 generation was substantially reduced (Edmands and Deimler, 2004).

Figure 8.14 Some outcomes that may result from matings between immigrant and native individuals. Note that genetic rescue assumes that the native population was experiencing some level of inbreeding depression prior to the mating between natives and immigrants.



Although theoretically well established, the extent to which outbreeding depression threatens the survival of wild populations remains a matter of debate; some researchers maintain that its importance is overstated, while others believe that it is a widespread phenomenon that would be detected more often if appropriate studies were conducted (see Edmands, 2007, for further discussion). It is also likely that the literature on outbreeding depression will grow as an increasing number of formerly separated species and populations come into contact with one another as a result of species introductions into novel geographical areas, and alterations in species' distributions as a result of climate change. Empirical examples of outbreeding depression in natural populations include hybrids from two different populations of pink salmon (*Oncorhynchus gorbuscha*) that are separated by around 1000 km, and which had decreased survival in the F_2 generation relative to either parental population; this is consistent with an epistatic model of outbreeding depression (Gilk *et al.*, 2004). In another example, the male courtship song in offspring that were generated by inter-population crosses of the fruit fly *Drosophila montana* had a frequency different to that found in either parental population, which led to reduced mating success and lowered fitness, once again suggesting outbreeding depression (Aspi, 2000).

Another example of outbreeding depression comes from wild populations of the herbaceous scarlet gilia (*Ipomopsis aggregata*), in which matings between parents separated by only 100 m produced offspring with a reduced lifetime fitness that was caused by outbreeding depression, although not surprisingly the decline in fitness depended in part on environmental heterogeneity and the associated selection regimes (Waser *et al.*, 2000). Effects at an even finer spatial scale were found in Nelson's larkspur (*Delphinium nelsonii*) when flowers were hand pollinated using pollen from between 1 m and 30 m away. Progeny from intermediate crossing distances (3 m and 10 m) grew approximately twice as large as the progeny that resulted from crossings between either nearby or more distant plants. This was presumably because pollen from intermediate distances did not adversely affect the fitness of offspring, whereas pollen from plants that were 1 m or 30 m away led to inbreeding depression and outbreeding depression, respectively (Waser and Price, 1994).

Translocations

Once we have identified which populations are most at risk from low genetic diversity, management strategies can be drawn up that will help to increase their chances of long-term survival. One of the most effective ways of slowing the decline of small, genetically depauperate populations is through the introduction of immigrants.

Genetic Rescue

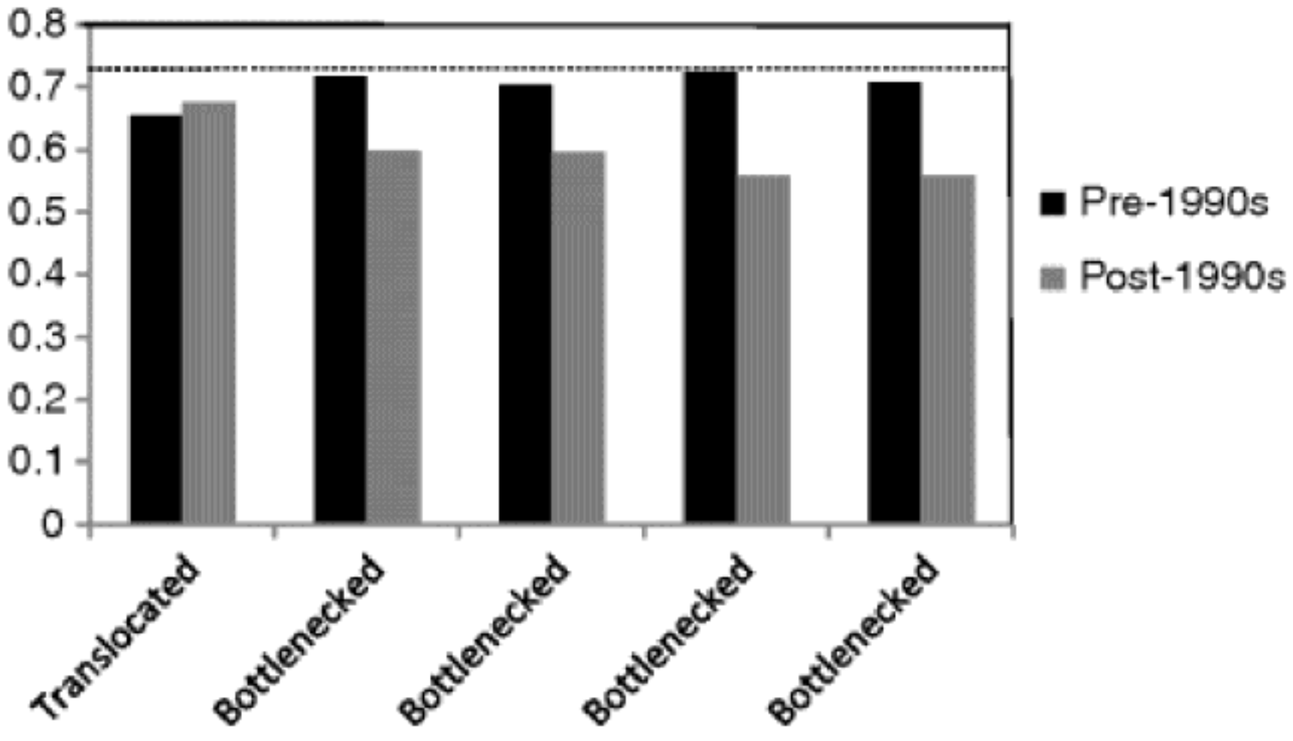
When migrants are translocated from one population to another, they will often introduce new alleles into the recipient population. If this results in a reduction of inbreeding depression, it is known as **genetic rescue** (Thrall *et al.*, 1998). Genetic rescue will increase the growth rate of a population over multiple generations from the time when the novel genes were introduced. This is usually attributed to **heterosis**, which is elevated fitness in the offspring of genetically divergent individuals (sometimes known as **hybrid vigour**). Recall that inbreeding depression can be attributable to either dominance or overdominance. As we might expect from a process that effectively reverses inbreeding depression, heterosis can result from either the production of relatively fit heterozygous individuals or, more likely, the masking of deleterious alleles.

There are several success stories in which genetic restoration has dramatically improved the fitness of populations, possibly saving them from extinction. One of the best known examples of this occurred in the last remaining population of the Florida panther (*Felis concolor coryi*) (Figure 8.15). In recent decades the effective size of this population has been around 25, and it is therefore not surprising that genetic variation, as revealed by microsatellite loci, is much lower than that found in populations of any other North

Finally, it is important to note that the recovery of a population does not depend solely on genetic rescue. Greater prairie chickens (*Tympanuchus cupido pinnatus*) in south-eastern Illinois experienced declines in both population size and genetic diversity in the latter half of the twentieth century, which led to translocations into the Illinois population from larger, genetically diverse populations. This resulted in higher genetic diversity in the genetically rescued population, which includes many descendants of the translocated birds. From the point of view of conservation genetics this may be considered a success story (Figure 8.16). However, demographic data show that the potential recovery of prairie chickens in Illinois remains uncertain: although fitness is increasing, the population is not growing in size. The authors of this study conclude that if the factors that caused the original decline are not addressed (most notably habitat loss), prairie chickens may not survive in Illinois even though they are showing signs of genetic rescue (Bouzat *et al.*, 2006).

Figure 8.16 Changes in genetic diversity over time, measured as H_e, for prairie chicken populations. 'Translocated' refers to the Illinois population that received translocated birds, whereas 'bottlenecked' refers to four other populations that experienced bottlenecks but did not receive any translocations. The pre-1990s samples for the translocated population were collected between 1971 and 1991, whereas those for the bottlenecked populations were collected in the 1950s (which may explain the lower starting point in the translocated population). The dashed line indicates the current average H_e value for populations that did not experience bottlenecks in the twentieth century. Note that out of populations for which data from two time points are available, only the genetically rescued population has experienced an increase in genetic diversity. The trend is essentially the same for allelic richness and haplotype diversity.

Data from Bouzat et al. (2006).



Source Populations

Although translocations are often successful, care must be taken when moving individuals across large geographic distances. If the extant source population at one site shows substantial genetic differences from the extinct or endangered population at the destination site, then there is a greater risk of outbreeding depression or maladaptiveness. One approach to minimizing these risks is to use museum specimens to reconstruct evolutionary relationships. Biologists used this methods to screen several potential donor populations in China and Russia before identifying an appropriate source of Oriental white storks (*Ciconia boyciana*) for re-establishing the Japanese population, which went extinct in 1986 (Murata *et al.*, 2004). The lack of extant Japanese storks made genetic comparisons somewhat challenging, but researchers circumvented this problem by cutting small pieces of skin from 17 Japanese storks that had been stuffed and mounted on display in Toyooka City, Japan, and two nearby villages. They compared mtDNA haplotypes from these samples to haplotypes from Chinese and Russian storks that had previously been used in captive breeding programmes. The maximum divergence between Japanese, Chinese and Russian storks was 2.6%, which is much lower than the levels of intraspecific control region sequence divergence that have been found in some other bird species; furthermore, one haplotype was found in both a Japanese and a Chinese stork, suggesting a relatively recent historic connection between the Japanese and continental populations. Finally, a maximum likelihood phylogenetic tree showed no distinction between the evolutionary lineages in Japan, China and Russia. The authors of this study therefore concluded that translocation of storks from the continent to Japan would be appropriate, at least on the basis of genetic compatibility.

Another example of using museum specimens to determine the suitability of source populations comes from a study of north-eastern beach tiger beetles (*Cicindela dorsalis dorsalis*), a federally listed threatened subspecies that includes a population in Massachusetts, USA, that has a haplotype which is currently found nowhere else. DNA analysis from museum specimens determined that this same haplotype was historically distributed across the north-eastern seaboard, and its currently restricted distribution in Massachusetts is most likely a result of recent habitat fragmentation and genetic drift; therefore, re-introductions from adjacent areas into Massachusetts seem appropriate (Goldstein and DeSalle, 2003). The stochastic effects that drift and habitat fragmentation will have on the distribution of haplotypes are more likely to be seen in populations that are considered to be at risk, and because these are the populations for which

(b)

Ecological restoration projects must also factor in the possibility of outbreeding depression, a threat that has been realised in some projects that aim to restore biodiversity in intensively managed farmland. Such projects often use seed mixtures of wildflowers that are produced by commercial suppliers, and which may have originated many miles from the site of restoration. The potential consequences of using seeds from distant sources were investigated in a study of three arable weed species: common corn-cockle (*Agrostemma githago*), red poppy (*Papaver rhoeas*) and white campion (*Silene alba*) (Keller *et al.*, 2000). Swiss plants were crossed with plants that originated in England, Germany and Hungary, and the fitness of the hybrids was compared with that of the parental plants. Outbreeding depression was indicated by reduced biomass in the F_2 generation of all red poppy crosses, and in the F_1 generation that was generated by a cross between Swiss and German corn-cockles. Seed mass decreased in the F_2 generation of the white campion crosses, and survival was reduced in the F_1 and F_2 progeny of red poppies. Results such as these suggest that whenever possible, habitats should be restored using seeds of relatively local origin. This should minimize the likelihood of both outbreeding depression and genetic swamping.

Captive Breeding

If a species is unable to survive in the wild then the only way that it can be saved from extinction is through captive breeding. There are a number of species that would now be extinct were it not for captive breeding, including the California condor (*Gymnogyps californianus*), Père David's deer (*Elaphurus davidianus*), Arabian oryx (*Oryx leucoryx*), the black-footed ferret (*Mustella nigripes*), the Franklin tree (*Franklinia alatamaha*) and the Potosi pupfish (*Cyprinodon alvarezi*). In many more cases, captive breeding programmes have been initiated for species that are rapidly dwindling in the wild, in order to maintain as large a gene pool as possible and, in some cases, to provide a source of plants and animals for translocation programmes. Space, money, expertise and other resources that are needed for this costly endeavour are limited, and decisions about which species will be captively bred are often motivated by their appeal to humans, with mammals, birds and flowering plants generally receiving much more attention than invertebrates or lower plants.

Maximizing Genetic Diversity

Logistical constraints often mean that captive populations are dispersed among multiple zoos, aquaria and wildlife parks. Each institution will normally house only a handful of individuals from a particular species, and this could have serious repercussions for long-term genetic diversity. Successful captive breeding therefore often requires co-operation between institutions to create what is effectively human-mediated gene flow between very small populations. This has been facilitated by a number of enterprises including the Species Survival Plan Programs (SSP) of the American Zoo and Aquarium Association, which were started in 1981 as co-operative conservation programmes for selected species in zoos and aquaria in North America. This programme currently oversees the captive breeding of more than 115 different species, most of which involve many different institutes. These are often programmes to conserve 'flagship species', which are well-known animals that tend to arouse strong feelings in the public and a widespread desire to preserve and protect populations both in captivity and in the wild; these include the giant panda, California condor, and lowland gorilla.

The type of co-operation exemplified by the SSPs is needed if captive breeding programmes are to achieve a commonly stated goal of maintaining 90% of genetic diversity for a period of 100 years, while increasing the inbreeding levels by no more than 10%. The effective population size needed to maintain genetic diversity for this length of time will depend to some extent on the generation time of the species in question. Assuming that the population size remains constant, and note that we are talking about N_e and not N_c, the necessary effective population size has been derived from the rate at which heterozygosity is expected to decline within populations of different sizes following genetic drift (Frankham *et al.*, 2002; see also Chapter 3), and is approximately equal to:

(b)

In a similar vein, the Frozen Ark is a last ditch attempt to preserve some of the genetic diversity of animals. This project, which is a collaboration between the London Natural History Museum, the Zoological Society of London, and Nottingham University, aims to collect, store and preserve DNA and tissue from as many endangered species as possible; the goal is to collect the DNA of from each of the >16 000 animal species that are currently on the IUCN Red Data List, plus viable cells (somatic cells, eggs, embryos and sperm) from as many of these species as possible. A recent initiative within the Frozen Ark was to formalize a global group of experts to develop guidelines for the cryopreservation of genetic material from coral species, which may be used in future restoration programmes, as discussed in the earlier section on restoration genetics. Priority is given to species with a high likelihood of extinction in the near future, with the first members of the ark including the yellow seahorse (*Hippocampus kuda*), Scimitar horned oryx (*Oryx dammah*), Socorro dove (*Zenaida graysoni*) and the Seychelles Fregate beetle (*Polposipus herculeanus*). If the DNA is stored appropriately it could remain intact for tens of thousands of years, possibly longer. It is too soon to know exactly what this genetic information will be used for in the future, although one possibility is that science fiction-type cloning will allow scientists to resurrect formerly extinct species.

Other genetic diversity banks have more specific short-term applications. At the Wildlife Breeding Research Centre in South Africa, for example, vets and biologists have established a sperm bank for lions, and have an ongoing programme in which they travel around artificially inseminating females so as to reduce inbreeding in small, isolated populations. The social structure of this species means that if a strange male is introduced to a pride then the members of that pride will chase him away or even kill him, which would do nothing to ameliorate inbreeding. Artificial insemination therefore bypasses some of the behavioural deterrents against the genetic enhancement of lion populations. Cryopreservation of sperm and oocytes may facilitate captive breeding of other taxonomic groups (e.g. Ledda *et al.*, 2001; Browne *et al.*, 1982), although detailed methods generally have to be worked out separately for each species and the technology is currently available for only a small proportion of animals.

Overview

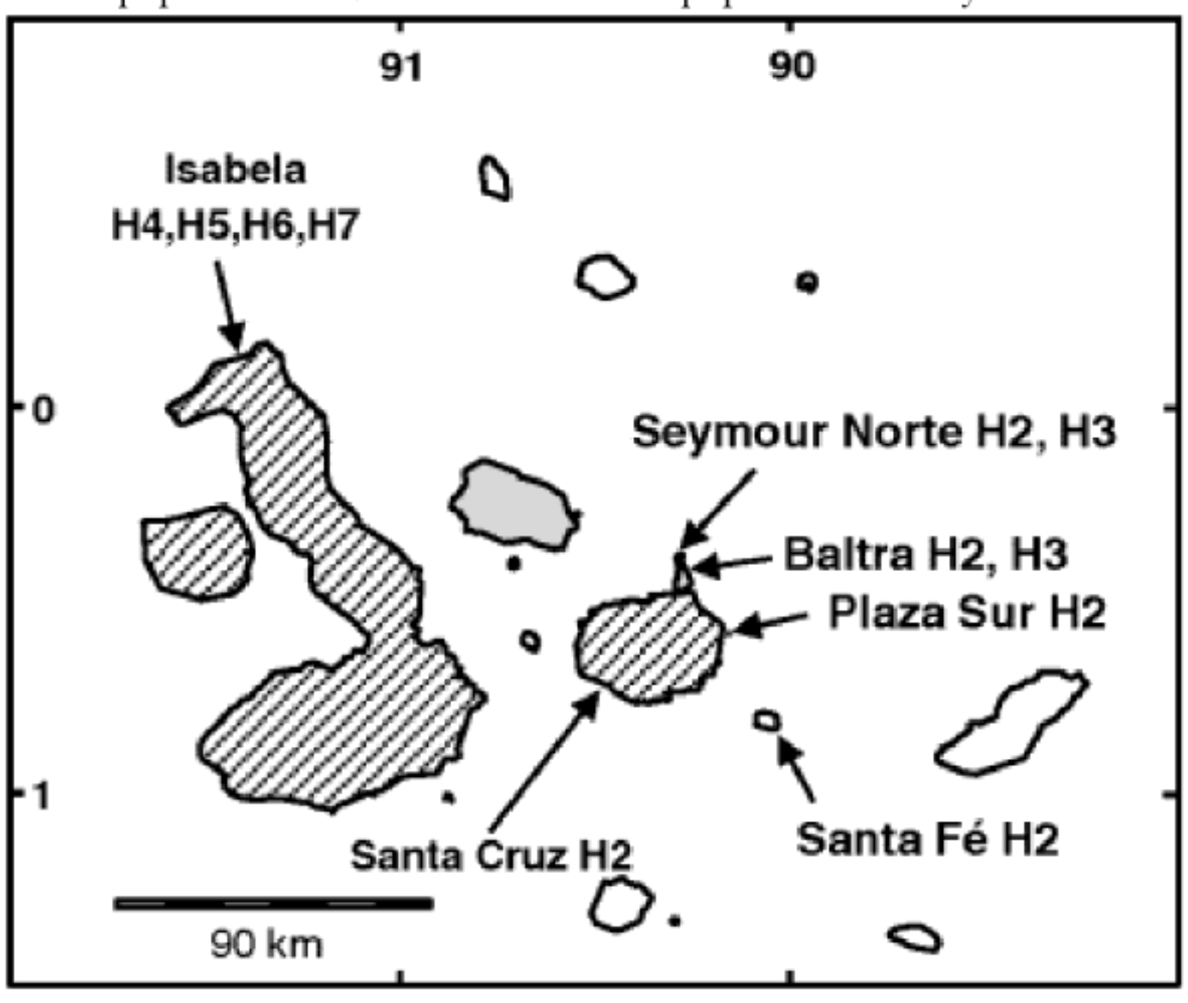
Conservation biology is very much an uphill battle. Human populations and their consumption of resources continue to grow and more and more habitat is lost every day. The future does not look good for a growing number of species, although on a brighter note we would likely have lost even more species by now if we had no conservation programmes. Molecular genetics can help us to make informed decisions about the management of both wild and captive populations, and for this reason conservation genetics remains one of the most important applications of molecular ecology.

Chapter Summary

- Although in most taxonomic groups only a small proportion of species have been assessed, the numbers of threatened species have led many people to believe that we are on the brink of a sixth mass extinction.
- Conservation strategies tend to assume that species can be accurately classified, although none of the >20 species concepts currently in the literature are universally accepted. DNA barcoding is the most recent approach to species identification. Conservation may also be based on management units and evolutionary significant units, whereas the protection of hybrids is more controversial.
- Threatened populations are usually small and therefore lose genetic diversity at a relatively rapid rate following genetic drift. Because drift is more important than selection at determining the fate of alleles in small populations, deleterious alleles are more likely to reach fixation and increase the genetic load.
- Small populations are susceptible to inbreeding, and if this leads to a reduction in fitness as a result of either dominance or overdominance, then the population will experience inbreeding depression.

8.6. In the 1940s, land iguanas (*Conolophus subcristatus*) were extirpated from Isla Baltra on the Galápagos Archipelago. Historical records show that some iguanas were moved from Isla Baltra to nearby Isla Seymour Norte in the 1930s, an island that had previously lacked land iguanas. As part of a proposed translocation programme, biologists compared mitochondrial haplotypes from current populations to Isla Baltra specimens that had been collected in the 1940s to determine whether it would be appropriate to restock Isla Baltra using iguanas from Isla Seymour Norte (Hofkin *et al.*, 2003). The six haplotypes that they found were distributed across the archipelago as shown in Figure 8.20.

Figure 8.20 The distribution of land iguana haplotypes(H2-H7) in past and current populations in the Galápagos Archipelago. White islands have always lacked land iguana populations, grey islands represent extirpated populations, and hatched islands represent extant populations. Note that the current population on Seymour is a translocated, artificial population.



On the basis of this haplotype distribution, what would be the most conservative approach to follow when translocating iguanas from Isla Seymour Norte to Isla Baltra?

Further Reading

Books

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Online Activities

The exercises provided to support this chapter are meant to integrate a number of concepts that have been covered throughout the text book. In one exercise, several sequences of unknown origin have been provided, and these will be used to determine if they are derived from CITES or IUCN red listed species. A second exercise provided data sets that allow students to determine the most appropriate source population that can be used to augment a declining population. Online activities can be accessed at www.wiley.com/go/freeland_molecular2e.