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The problems with hybrids: setting conservation guidelines

Fred W. Allendorf, Robb F. Leary, Paul Spruell and John K. Wenburg

Rates of hybridization and introgression are increasing dramatically worldwide because of translocations of organisms and habitat modifications by humans. Hybridization has contributed to the extinction of many species through direct and indirect means. However, recent studies have found that natural hybridization has played an important role in the evolution of many plant and animal taxa. Determining whether hybridization is natural or anthropogenic is crucial for conservation, but is often difficult to achieve. Controversy has surrounded the setting of appropriate conservation policies to deal with hybridization and introgression. Any policy that deals with hybrids must be flexible and must recognize that nearly every situation involving hybridization is different enough that general rules are not likely to be effective. We provide a categorization of hybridization to help guide management decisions.

HYBRIDIZATION (see Glossary) provides an exceptionally tough set of problems for conservation biologists. The issues are complex and controversial, beginning with the seemingly simple task of defining hybridization[1]. Detection of hybridization can also be difficult, although it is becoming easier through the development of various molecular techniques over the last two decades. In spite of improved molecular data that can be collected with relative ease, interpreting the evolutionary significance of hybridization and determining the role of hybrid populations in developing conservation plans is more difficult than is usually appreciated. According to Stone[2]: 'It is an understatement to say that hybridization is a complex business!'.

The harmful effects of hybridization, with or without INTROGRESSION, have led to the extinction of many populations and species in many plant and animal taxa[3]. Hybridization is especially problematic for rare species that come into contact with other species that are more abundant. Rhymer and Simberloff[3] have concluded that the severity of this problem has been underestimated by conservation biologists. The increasing pace of the three interacting human activities that contribute most to increased rates of hybridization (introductions of plants and animals, fragmentation, and habitat modification) suggests that this problem will become even more serious[3].

Hybridization has long been recognized as playing an important role in the evolution of plants[4]. However, recent studies have found that hybridization has also

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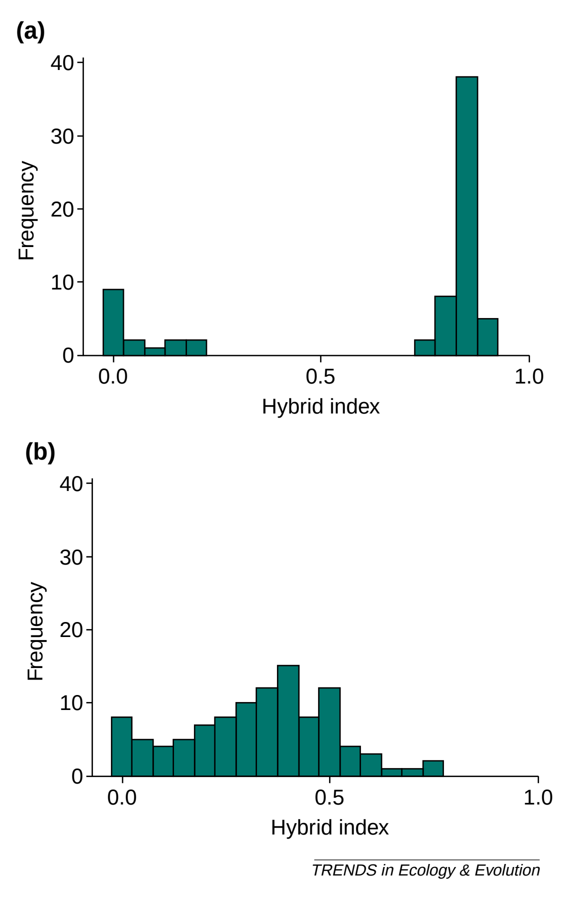

Fig. 1. Hybrid index scores for two population samples of sympatric coastal cutthroat trout *Oncorhynchus clarki clarki* and rainbow trout *O. mykiss*. The hybrid index indicates the relative probability that the multiple locus genotype of an individual arose by random mating within each of the parental taxa. The index ranges from 0 to 1, with the two extremes occurring with high probability in coastal cutthroat trout (0) and rainbow trout (1). Most individuals are similar to one of the parental taxa in a bimodal hybrid zone (a), whereas all individuals are hybrids after many generations of backcrossing in a unimodal[13] hybrid zone (b). The hybrid swarm shown in (b) contains approximately 25% admixture from rainbow trout and 75% from coastal cutthroat trout. Reproduced, with permission, from Ref. 10.

played an important role in the evolution of animals[5–8]. Early conservation policies generally did not allow protection of hybrids. However, recent recognition of the historical role of hybridization as an evolutionary process has caused a re-evaluation of these policies. Our purpose is to provide guidelines that deal with problems associated with increasing anthropogenic hybridization and, at the same time, consider the important evolutionary role of natural hybridization.

The perspective here comes from our research on salmon and trout and our experiences providing advice to various agencies on appropriate management actions[9]. Natural hybridization is more common in fish than in other vertebrates, and non-native fish species have been introduced extensively worldwide[10,11]. In addition, interspecific hybrids among genetically divergent salmonid fish are often fertile, and hybridization frequently results in creation of HYBRID SWARMS[12] (Fig. 1). Therefore, hybridization has been a particularly common and difficult problem for the conservation of fish. Nevertheless, the problems and suggestions that we discuss here can be applied to most plant and animal taxa. We restrict our examples to animals, because of the common occurrence of asexual reproduction in plants that raises additional issues beyond the scope of this article.

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Detection and analysis of hybridization

The detection of hybrid individuals relied upon morphological characteristics until the mid-1960s. However, not all morphological variation has a genetic basis, and the amount of morphological variation within and among populations is often greater than is actually recognized[10]. The detection of hybrids using morphological characters generally assumes that hybrid individuals will be phenotypically intermediate to parental individuals[11]. This is often not the case, because hybrids sometimes express a mosaic of parental phenotypes[10]. Furthermore, individuals from hybrid swarms that contain most of their genes from one of the parental taxa are often morphologically indistinguishable from that parental taxon[14]. Morphological characters do not allow one to determine whether an individual is a first generation hybrid (F1), a backcross, or a later generation hybrid. These distinctions are crucial, because if a population has not become a hybrid swarm and still contains a reasonable number of parental individuals, it could potentially be recovered by removal of hybrids or by a captive-breeding program.

The use of molecular genetic markers greatly simplifies identification and description of hybridized populations (Box 1). This procedure began with development of protein electrophoresis (allozymes) in the mid-1960s (Ref. 15). Recent advances in molecular techniques, especially the development of PCR, have greatly increased the number of loci that can be used to detect hybridization. In addition, these techniques are more applicable to small populations that are threatened with extinction, because sampling can be noninvasive[16].

Hybrid policies

An early series of interpretations of the US Endangered Species Act (ESA)[17] concluded that hybrids should not receive protection under the ESA because protection of hybrids would not help recover a listed species and could jeopardize continued existence of that species. However, this 'Hybrid Policy' was withdrawn in December 1990 because 'New scientific information concerning genetic introgression has convinced us that the rigid standards set out in those previous opinions should be revisited'[18].

A proposed policy on 'intercrosses' was published in 1996 (Ref. 18). The proposed policy used the term 'INTERCROSS' rather than 'hybrids', because of negative connotations associated with that term. This Intercross Policy was scheduled to be finalized one year later, but has still not been approved; it now

Box 1. Interpretation of genetic data

Genetic data must be interpreted at both the individual and population level to understand the history of hybridization in populations[a]. Hybrid individuals can be first-generation (F1) hybrids, second-generation hybrids (F2s), backcrosses to one of the parental taxa, or later-generation hybrids. Parental types and F1 hybrids can be reliably identified if many loci are examined. However, it is very difficult to distinguish between F2s, backcrosses and later-generation hybrids, even if many loci are examined[b]. New statistical approaches for assigning individuals to their population of origin based on many highly polymorphic loci could be especially valuable for identifying hybrids[c].

Describing hybrid populations by allele frequencies can be misleading. Hybrid populations are best described by the distribution of hybrid genotypes. For example, consider a sample of 100 individuals from a hybrid population examined at ten loci. In one population, ten F1 hybrids and 90 parental individuals are detected. A proportion of an admixture of 5% in this population over all ten loci would be estimated. The proportion of admixture would also be 5% in a population in which each individual carried a single non-native allele at any one of the ten loci. However, the pattern of hybridization of these two populations is markedly different. Hybridization is perhaps recent or rare in the first case, whereas the second population appears to be a hybrid swarm (Fig. 1b). Estimation of the proportion of admixture is therefore a useful measure only in hybrid swarms.

The distribution of GAMETIC DISEQUILIBRIA (D; see Box Glossary) between pairs of loci is helpful to describe the distribution of hybrid genotypes and to estimate the 'age' of hybridized populations[d]. Recently hybridized populations will have high D because they will contain parental types and many F1 hybrids. By contrast, genotypes will be randomly associated among loci in hybrid swarms that have existed for many generations. This will occur rather quickly for unlinked loci, because D will decay by one-half each generation. However, nonrandom association of alleles at different loci might persist for many generations at pairs of loci that are closely linked.

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Box Glossary

Gametic disequilbria: genotypic associations between loci that result in a nonrandom distribution of gametic types. For two loci, the disequilibrium coefficient is often used (Eqn I):

$$D = P_{AB} - p_A p_B \quad \text{[I]}$$

where P_{AB} is the observed frequency of the AB gamete and p_A and p_B are the allele frequencies at loci A and B. D is expected to decay each generation in a random mating population at a rate of $D' = D(1 - r)$, where r is the recombination rate between the two loci.

appears that the proposed policy is likely to be withdrawn. Thus, no official policy provides guidelines for dealing with hybrids under the ESA. The absence of a final policy probably results from the difficulty in writing a hybrid policy that would be flexible enough to apply to all situations, but that would still provide helpful recommendations.

Rigid adherence to guidelines for dealing with hybrids can be problematic. For example, the US National Marine Fisheries Service (NMFS) recently published a status review for coastal cutthroat trout *Oncorhynchus clarki clarki* under the ESA (Ref. 19). They examined eight diagnostic allozyme loci between coastal cutthroat trout and rainbow trout *O. mykiss*. Allele frequencies were estimated to describe the pattern of genetic relatedness among populations after individuals that had at least one rainbow trout allele at four or more of those loci were excluded. As NMFS pointed out, many of the individuals included in the data were undoubtedly hybrids, even though they passed the test of having rainbow trout alleles at less than four of the eight loci. For example, most offspring from backcross matings between F1 hybrids and coastal cutthroat trout would not meet the NMFS definition of hybrids, and therefore would have been included in the data set; however, approximately 25% of the genes of such backcross progeny would have come from rainbow trout.

The guideline used by NMFS does not adequately account for the pattern and extent of hybridization among populations. Eliminating only individuals meeting their criterion from hybrid swarm populations could cause a misinterpretation of the genetic relationships among populations. Some samples could contain a few F1 hybrids, in which case, inclusion of the population after removal of those individuals would be appropriate. However, populations that appear to be hybrid swarms with an extremely high proportion of apparent hybrids (up to 82% in the NMFS study) should be excluded from analysis of genetic relationships, because all individuals in such samples are likely to be hybrids. Failure to exclude such populations can distort patterns of relatedness among populations and lead to inappropriate designations of 'distinct population segments' under the ESA. That is, genetic similarity between some populations might be a result of introgression from rainbow trout, rather than because of common ancestry.

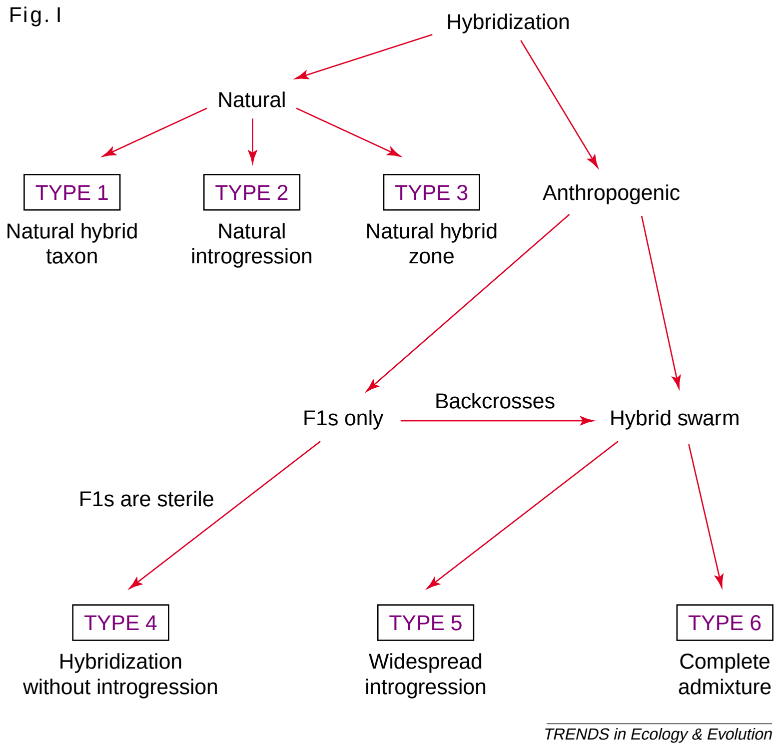
Categorization of hybridization

Here, we identify six types of hybridization and discuss what conservation measures should be taken for each (Box 2). Although the situations described here refer primarily to interspecific hybridization, the principles are general and also apply to intraspecific hybridization (Box 3).

Box 2. Categorization of hybridization

Figure I provides a framework with which to categorize hybridization. Each type should be viewed as a general descriptive classification that is used to facilitate discussion rather than as a series of strict, all encompassing divisions. Types 1–3 represent hybridization events that are a natural part of the evolutionary legacy of taxa; these taxa should be eligible for protection. Types 4–6 divide anthropogenic hybridization into three categories that have different consequences from a conservation perspective.

Fig. I



TRENDS in Ecology & Evolution

Type 1: natural hybrid taxon

Virgin River roundtail chub *Gila seminuda* are listed as endangered under the Endangered Species Act of the USA (ESA). It is a hybrid taxon that appears to have originated from hybridization between *G. elegans* and *G. robusta* in the Pleistocene long before human influence in the Colorado River system[a].

Type 2: natural introgression

Moorean land snails *Partula tainiata* and *P. suturalis* occur sympatrically on the island of Moorea in French Polynesia. In spite of being markedly different both phenotypically and ecologically, estimates of genetic distance based on molecular markers between some sympatric populations of these species are lower than is typical for conspecific comparisons for these taxa[b]. Clarke *et al.*[b] concluded that this apparent paradox was best explained by ‘molecular leakage, the convergence of neutral and mutually advantageous genes in two species through occasional hybridization’.

Type 3: natural hybrid zone

Red- and yellow-shafted northern flickers *Colaptes auratus* hybridize in the Great Plains of North America[c]. Their narrow hybrid zone extends from Canada through Texas (USA) and has been remarkably stable historically. The reproductive success of hybrids is equal to that of the parental types, and there is no assortative mating within the hybrid zone. Nevertheless, the parental types are thought to be maintained by sexual selection and by natural selection associated with environmental differences between eastern and western North America.

Type 4: hybridization without introgression

Bull trout *Salvelinus confluentus* are currently listed as threatened under the ESA. Hybridization with introduced brook trout *S. fontinalis* has been documented throughout much of their range. However, there have been few reports of hybrids beyond the first generation (F1) (Ref. d). Thus, the major detrimental effect of hybridization in this case is wasted reproductive effort rather than genetic mixing. Removal of the non-native species and F1 hybrids is likely to be beneficial, and restoration of degraded habitat could help decrease hybridization.

Type 5: widespread introgression

Westslope cutthroat trout *Oncorhynchus clarki lewisi* have suffered from widespread hybridization with introduced rainbow trout[e] *O. mykiss*. However, many pure westslope populations remain, especially in isolated headwater areas throughout the range of the subspecies. Hybridized populations are of little conservation value (although they could have other values), and efforts should focus on maintaining and expanding the remaining pure populations.

Type 6: complete admixture

New Zealand grey duck *Anas superciliosa* have been severely affected by hybridization with introduced mallard ducks *A. platyrhynchos*[f]. Few, if any, pure populations remain and there does not appear to be any selection against the hybrids. Here, conservation of hybrids should be considered, because it is the only available option if we are to avoid the complete loss of the hybridized species.

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Box 3. Should we be concerned about intraspecific hybridization?

It is sometimes argued that intraspecific hybridization should not be a concern because populations of the same species generally share alleles so that new genetic types that might cause outbreeding depression will not be created by introgression[a,b]. Moreover, it is argued that the introduction of new genetic variation into a population will generally be beneficial and provide genetic variation, so that natural selection can increase the fitness of populations[a].

Hybridization between genetically similar populations in the same species can have an important effect on adaptive divergence among populations. For example, consider two populations that have only slightly different allele frequencies at a locus with two alleles. Assume population A has frequencies of 0.7 and 0.3 of alleles *A* and *a*, and population B has frequencies of 0.3 and 0.7 for these same two alleles. This amount of divergence is common among intraspecific populations ($F_{st} = 0.16$; see Box Glossary). The genotypic distributions of these two populations at this locus will be very similar so that hybridization will only cause a minor shift in the frequencies of different genotypes in these populations. Thus, one could argue that hybridization between these two populations would have little effect.

This argument fails to take into account multiple locus genotypes. Two populations with this amount of genetic divergence (i.e. $F_{st} = 0.16$) will have similar differences in genotypic distributions at thousands of polymorphic loci throughout the genome. For example, assume that a particular phenotype requires the presence of the dominant allele (*A*; that is, genotype *AA* or *Aa)* at a series of loci in two isolated populations in Hardy–Weinberg proportions with a mean F_{st} of 0.16 (with allele frequency divergence as described in the first paragraph and assuming linkage equilibrium). The frequency of this phenotype in populations A and B becomes more different the more loci there are that contribute to this trait (Table I):

Table I. Frequency of an adaptive phenotype determined by different number of loci in two populations with moderate genetic divergence[a]

Number of loci	Frequency *AA* + *Aa*	
	Population A	Population B
1	0.91	0.51
5	0.62	0.03
10	0.39	<0.01

[a] $F_{st} = 0.16$

The genotype at many loci is likely to be important for complex adaptive differences between populations[c]. These local adaptations might be lost through intraspecific hybridization, and therefore actions that increase the amount of genetic exchange among locally adapted populations could be harmful. For example, the release from hatcheries or the escape from aquaculture facilities of salmon could harm wild populations through intraspecific hybridization and the loss of local adaptations[d–f].

The loss of such adaptations could be difficult to detect because local adaptation of native populations might only be essential during periodic episodes of extreme environmental conditions (e.g. winter storms, drought, or fire)[g]. For example, Rieman and Clayton[h] have suggested that the complex life histories of bull trout (mixed migratory behaviors, etc.) are adaptations to periodic disturbances, such as fire.

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Box Glossary

Fixation index (F_{st}): the proportional reduction in heterozygosity due to population subdivision. F_{st} is often used as a measure of the amount of genetic divergence among populations (Eqn I):

$$F_{st} = 1 - \frac{H_s}{H_t} \tag{I}$$

where H_s is the mean expected heterozygosity within local populations, and H_t is the expected total heterozygosity using the mean allele frequencies of populations.

Dowling and Secor[5] presented compelling evidence that genetic mixing has played an important role in evolution of some taxa. We classify hybrid taxa that have arisen by natural genetic admixture as resulting from type 1 hybridization. Species resulting from such historical hybridization events should be eligible for protection, just like any other species.

Many hybrid taxa of vertebrates are unisexual[5]. For example, unisexual hybrids between the northern redbelly dace *Phoxinus eos* and the finescale dace

Phoxinus neogaeus occur across the northern USA (Ref. 20). Reproduction of such unisexual species is generally asexual or semisexual, and they are often regarded as evolutionary dead ends[20]. However, it appears that some tetraploid bisexual taxa had their origins in a unisexual hybrid (e.g. all salmonid fish[21]). Recent evidence suggests that all vertebrates went through an ancient polyploid event that might have involved hybridization[22].

We classify introgression that is natural but does not lead to a creation of a new taxon as type 2 hybridization. For example, hybridization between phenotypically distinct species of land snails *Partula tainiata* and *P. suturalis* inhabiting islands of French Polynesia has resulted in species from one island resembling each other genetically more than they resemble conspecifics from other islands[23]. This same mechanism could explain discordance in apparent phylogenetic relationships between rainbow trout and several subspecies of cutthroat trout based upon allozymes versus mitochondrial DNA (mtDNA) and morphology[24].

The capture of mtDNA is another example of type 2 hybridization. For example, brook trout *Salvelinus fontinalis* from Lake Alain, Québec (Canada), have mtDNA haplotypes that are indicative of Arctic char *S. alpinus*. However, all nuclear genes that have been examined appear to be characteristic of brook trout[25]. Similarly, coyote *Canis latrans* mtDNA has been found in some wolf *C. lupus* populations from eastern North America[26,27].

Populations resulting from type 2 hybridization contain alleles from other taxa, but ongoing hybridization is not increasing the frequency of those alleles. Such introgression is part of the evolutionary process and should not preclude protection of taxa that result from type 2 hybridization.

We classify HYBRID ZONES as type 3 hybridization. Recent molecular analysis of plants and animals has revealed that hybrid zones occur widely in many taxa[1]. Barton and Hewitt[28] reviewed 170 reported hybrid zones and concluded that hybrids were selected against in most hybrid zones that had been studied. Nevertheless, some hybrid zones appear to be stable and persist over long periods of time by a balance between dispersal of parental types and selection against hybrids[1].

The remaining three types of hybridization involve situations in which human activities have caused hybridization. Situations in which primarily F1s have been detected are termed type 4 hybridization. In this case, hybridization is not a threat through genetic mixing, but wasted reproductive effort could pose a demographic risk. For example, bull trout *Salvelinus confluentus* (Box 2) in a Montana stream were almost replaced by introduced exotic brook trout within a few years after initiation of hybridization[29]. In a similar situation, females of the European mink *Mustela lutreola* hybridize with males from the introduced North American mink *M. vison*. Embryos are aborted so that hybrid individuals are not detected, but wastage of eggs through hybridization has accelerated decline of the European species[30]. The presence of primarily F1 hybrids should not jeopardize protection of populations affected by type 4 hybridization. However, care should be taken to determine conditions that favor the native species to protect and improve its status and reduce the wasted reproductive effort of hybridization.

The existence of hybrid swarms (Fig. 1b) makes conservation and recovery of threatened taxa much more difficult (types 5 and 6 hybridization). In some situations, hybridization might have begun only recently or might be geographically restricted so that parental populations still exist (type 5 hybridization), but if conservation actions are not taken, all populations could become hybrid swarms (type 6 hybridization).

Once hybridization has begun, it is difficult to stop, especially if hybrids are fertile and mate both among themselves and with parental individuals. After a few generations, this process will result in a hybrid swarm in which essentially all individuals are of hybrid origin. Hybrid swarms can form even if there is selection against hybrids because all the progeny of hybrid individuals will be hybrids. As successive generations of hybridization accrue in a population, the proportion of individuals of hybrid origin increases progressively, whereas the proportion of parental individuals decreases progressively. Eventually, selective differences between parental individuals and hybrids become irrelevant, because parental individuals no longer exist in the population.

In some species, such as the New Zealand grey duck *Anas superciliosa*, nearly all populations have become genetic admixtures[31] (type 6 hybridization). Similarly, a domestic horse *Equus caballus* mare is one of 13 founding individuals of the Przewalski's horse *E. przewalskii* captive-breeding program[32]. In spite of this hybridization, Przewalski's horses represent a distinctive gene pool that is the object of continuing conservation efforts[32]. Although any remaining nonintrogressed populations in the case of type 6 hybridization should be given highest priority, remaining hybrids might be protected in the hope that they will fill the ecological role of the native taxon.

We appreciate how difficult it can be to distinguish between natural and anthropogenic hybridization. Nevertheless, this distinction is of primary importance. The alternative is to either not allow protection of natural hybrids or to protect anthropogenic hybrids that could contribute to extinction of parental species and waste limited resources available for conservation. We consider two case studies as exemplars of the application of our classification scheme to difficult situations (Boxes 4 and 5).

Box 4. The red wolf

The red wolf *Canis rufus* was originally found throughout the southern USA (Ref. a). Habitat disruption and reduction of red wolf numbers allowed coyotes *C. latrans* to invade the range of the red wolf, and hybridization between red wolf and coyotes led to the loss of almost all the red wolf populations through genetic mixing. By the 1960s, pure populations of red wolves were found only in Texas and Louisiana, and the red wolf was listed as endangered under the US Endangered Species Act (ESA) in 1967.

Molecular genetic analysis of red wolves led to the suggestion in 1991 that the red wolf is a hybrid taxon resulting from hybridization between the gray wolf *C. lupus* and coyotes[b]. This conclusion has been hotly debated[c–h]. Much of the debate now centers on whether the hybridization is historical (thousands of years ago) or recent (hundreds of years ago)[f]. Under the former, the red wolf is an ancient component of its ecosystem that has nearly disappeared because of anthropogenic hybridization, and should be protected[g]. The other position holds that the red wolf is a creation of recent hybridization that does not warrant protection.

Nowak and Federoff[g] reject the hybrid origin of red wolves because of evidence that the red wolf is not intermediate between gray wolves and coyotes, as would be expected with type 1 hybridization. However, the red wolf could have resulted from type 2 hybridization in which some coyote genetic material became introgressed into red wolf. If it could be definitively shown that red wolves are solely the result of recent hybridization between gray wolves and coyotes owing to anthropogenic factors, it would constitute type 5 hybridization. We would recommend against protection of the red wolf in this case because the parental taxa are extant and widespread.

Reich *et al.*[f] argue for 'recent' hybridization, but conclude that this hybridization could have originated as long as 12 800 years ago. We believe that it might not be possible to ever know with certainty the evolutionary history of the red wolf. The age of the hybridization could potentially be estimated by an examination of gametic disequilibrium between pairs of diagnostic loci that are known to be linked in the gray wolf and coyote (Box 1). An absence of gametic disequilibrium would suggest an ancient hybridization. However, any gametic disequilibrium detected could either be the result of a recent hybrid origin of the red wolf or recent anthropogenic hybridization in those red wolf samples that are available.

We support the conclusion that the red wolf is an 'evolutionary entity' worthy of protection[e]. Although anthropogenic factors have increased the occurrence of hybridization over the past 200 years or so, red wolves appear to constitute a type 1 or type 2 hybrid taxon that warrants protection. The cost of mistakenly concluding that the hybridization is anthropogenic is too great. Given that the genetic evidence might not be able to provide a clear answer, we should protect the red wolf as a component of the evolutionary legacy of canids.

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Management questions

There are several controversial questions that often arise when developing conservation plans in situations where hybridization is a concern.

Is there an acceptable proportion of admixture?

The creation of hybrid swarms between native and introduced species is widespread among salmonid fish in the western USA. For example, most local populations of native westslope cutthroat trout are now hybrid swarms with rainbow trout[24]. We have been asked many times what PROPORTION OF ADMIXTURE (>0%, 5%, or 25%) must be present before a population should no longer be considered 'westslope cutthroat trout'.

An argument could be made that any admixture should preclude a population from being protected in the case of type 5 or type 6 hybridization. We believe otherwise. The amount of admixture that precludes protection will vary with each situation. Setting some arbitrary limit of admixture below which a population will be considered 'pure' is problematic. First, estimating the proportion of admixture precisely is difficult because of a limited number of diagnostic markers. In addition, it is often hard to distinguish between a small proportion of admixture (e.g. <5%) and natural polymorphisms that might exist in some populations. Finally, setting an arbitrary threshold could give way to further erosion of the genetic integrity of the parental taxon by constantly lowering the definition of 'pure'. If 5% is acceptable, why not 6% or 10%? We believe that clearly stating the basis for the chosen specific management actions is more important than is the exact proportion of admixture deemed 'acceptable'.

Several factors need to be considered when assessing the potential value of a hybridized population. One factor is how many PURE POPULATIONS of the taxon remain. The smaller the number of pure populations, the greater the conservation and restoration value of any hybridized populations. In addition, the greater the phenotypic (behavior, morphology, etc.) differentiation between the hybridized population and remaining pure populations, the greater the conservation value of the hybridized population. Another factor to consider is

Box 5. Pallid and shovelnose sturgeon

Pallid sturgeon *Scaphirhynchus albus* were initially described as a morphological variant of the shovelnose sturgeon *S. platorynchus* in 1905 (Ref. a), but they were recognized in 1954 as a separate species based on morphology[b]. Pallid sturgeon are distinguished from shovelnose sturgeon by several morphological features, including their larger size[c]. Shovelnose sturgeon were common throughout most major rivers of the Mississippi River drainage basin, but their abundance has declined[d]. Pallid sturgeon are restricted to large rivers in the Missouri and lower Mississippi River drainage basins and were listed as endangered under the US Endangered Species Act (ESA) in 1990 (Ref. c).

An allozyme study found that pallid and shovelnose sturgeon were indistinguishable at 34 monomorphic and three polymorphic loci[e]. A recent study found significant differences in allele frequencies at five microsatellite loci between sympatric samples of pallid and shovelnose sturgeon from three localities[f]. This provides evidence for some reproductive isolation or assortative mating between these species, but the amount of divergence (mean $F_{st} = 0.16$) between the three pairs of sympatric samples is well within the range that is usually found between intraspecific populations. In addition, pallid sturgeon from the Atchafalaya River in the southern Mississippi River drainage basin were more similar to sympatric shovelnose sturgeon samples than they were to two samples of conspecifics from the Missouri River in Montana.

A study of mitochondrial DNA (mtDNA) from 29 pallid and 37 shovelnose sturgeon found a total of 15 haplotypes with an amount of sequence divergence that is similar to that which is usually found within species (maximum 2%)[c]. Four of the five haplotypes found in pallid sturgeon were also present in shovelnose sturgeon. Significant differences in haplotype frequencies were found between sympatric samples of pallid and shovelnose sturgeon in both the Missouri and Atchafalaya Rivers. However, as was also found using microsatellites, pallid sturgeon from the Atchafalaya River were more genetically similar to sympatric shovelnose sturgeon than they were to Missouri River pallid sturgeon. In addition, evidence for substantial hybridization between pallid and shovelnose sturgeon has been found using morphology, microsatellites and mtDNA in the Atchafalaya River samples[c,f].

The recognition of the pallid sturgeon under the ESA is based on the conclusion that the similarity between pallid and shovelnose sturgeon is due to recent anthropogenic hybridization (type 4 or 5) that is probably the result of habitat degradation[g]. However, the similar allele and haplotype frequencies between these fish suggest that either they never evolved complete reproductive isolation or the lower Mississippi River is a natural hybrid zone between these taxa (type 3). The small amount of mtDNA sequence divergence between haplotypes provides strong evidence that these species have not been distinct lineages long enough to accumulate the amount of sequence divergence generally observed between species. Regardless of whether the hybridization between these taxa is anthropogenic or natural, the sturgeon from the lower Mississippi River appear to be a hybrid swarm. That is, based on the available samples from the Atchafalaya River, there are no pure pallid sturgeons in the lower Mississippi River.

We believe that the pallid sturgeon represents an important evolutionary component of sturgeon in the Mississippi River that is worthy of protection. Nevertheless, the available genetic evidence suggests that pallid and shovelnose sturgeon are not isolated evolutionary lineages. The conservation policy for these taxa should consider these fish as a complex of populations that naturally exchange genes, rather than as two isolated evolutionary lineages. The current listing of pallid sturgeon as a separate species would not allow protection of pallid sturgeon from the southern Mississippi River, because they are clearly hybrids. However, pallid sturgeon in the Atchafalaya River should be protected because the hybridization between pallid and shovelnose sturgeon appears to be natural.

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whether the continued existence of hybridized populations poses a threat to remaining pure populations. The greater the perceived threat, the lower the value of the hybridized population.

Can parental individuals be 'rescued' from hybrid populations?

Management agencies often want to know whether it is possible to select nonhybridized individuals from hybrid populations to be used in founding new populations or for use in captive breeding. This approach can work in the case of type 4 hybridization in which populations consist primarily of parental individuals and F1 hybrids, as long as a sufficiently large number of diagnostic loci are examined to ascertain fully that only parental individuals are used for recovery[33]. However, this approach is not suitable for hybrid swarms (type 5 and type 6 hybridization) because virtually all individuals in the population are hybrids, although on the basis of molecular analysis they might appear to be parental individuals.

Managers sometimes note that a certain proportion of the fish in a hybrid swarm only have alleles that are characteristic of the native taxon at the diagnostic loci analyzed, and they mistakenly interpret these to be pure individuals. This

Glossary

Admixture: the production of new genetic combinations in hybrid populations through recombination.
Diagnostic locus: a locus that is fixed or nearly fixed for different alleles in two hybridizing populations.
Extrinsic outbreeding depression: outbreeding depression that results from reduced adaptation to environmental conditions.
Genetic mixing: the loss of a formerly distinct population through hybridization.
Hybridization: interbreeding of individuals from genetically distinct populations, regardless of the taxonomic status of the populations[a].
Hybrid swarm: a population of individuals that all are hybrids by varying numbers of generations of backcrossing with parental types and mating among hybrids.
Hybrid taxon: an independently evolving, historically stable population or group of populations possessing a unique combination of heritable characteristics derived from two or more discrete parental taxa.
Hybrid zone: an area of contact between two genetically distinct populations where hybridization occurs[b].
Intercross: all crosses between individuals of different ‘species’ as defined under the ESA (i.e. taxonomic species, subspecies and distinct population segments of vertebrates).
Intrinsic outbreeding depression: outbreeding depression that results from genetic incompatibility between the hybridizing taxa (e.g. chromosomal rearrangements that disrupt pairing during meiosis).
Introgression: gene flow between populations whose individuals hybridize.
Outbreeding depression: a reduction in fitness in hybrid individuals relative to the parental types.
Proportion of admixture: the proportion of alleles in a hybrid swarm that comes from each of the hybridizing taxa.
Pure population: a population in which there has been no hybridization and therefore contains only individuals from the parental population.

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interpretation is not correct because, in a hybrid swarm, the genes of the parental taxa are randomly distributed among the individuals in the population. Consider a randomly mating hybrid swarm that contains a 10% admixture from an introduced species. All individuals in this population will be hybrids and contain approximately 10% of their alleles from the non-native taxon. However, 81% of all individuals will appear to be native parental individuals at any individual DIAGNOSTIC LOCUS ($0.9 \times 0.9 = 0.81$), and 66% will appear ‘pure’ at two loci ($0.81 \times 0.81 = 0.66$).

When is intentional hybridization desirable?

Another difficult question is under what circumstances should purposeful hybridization be used as a tool in conservation? Some populations of listed taxa are small or have gone through a recent bottleneck, and therefore they contain little genetic variation. In some cases, it might be advisable to increase genetic variation in these populations through intentional hybridization. For example, a headwater population of topminnow *Poeciliopsis monacha* that had lost all detectable heterozygosity because of a population bottleneck caused by drought was being outcompeted by a sympatric asexual hybrid taxon from the same genus[34]. Experimental replacement of 30 females with 30 females from a downstream population that had high heterozygosity restored the original heterozygosity and the competitive ability of the sexual population.

In extreme cases, some taxa might only be recovered through the use of intentional hybridization. However, the very characteristics of the local populations that make them unusual or exceptionally valuable could be lost through this purposeful introgression. In addition, such introductions could cause the loss of local adaptations and lower the mean fitness of the target population[35]. The most well-known example of this dilemma is the decision to bring in pumas *Puma concolor stanleyana* from Texas (USA) to reduce the apparent effects of inbreeding depression in Florida panthers *P. c. coryi*[17,36].

Hybridization is least likely to result in OUTBREEDING DEPRESSION when there is little genetic divergence between the populations. INTRINSIC OUTBREEDING DEPRESSION is probably not a major concern in most circumstances of intraspecific hybridization. However, in some circumstances genetic exchange between intraspecific populations could result in EXTRINSIC OUTBREEDING DEPRESSION through loss of important local adaptations that are crucial for viability of local populations[37] (Box 3). This is more probable as the amount of genetic divergence between populations increases at molecular markers. Thus, populations that are genetically similar at molecular markers and are similar for a wide range of adaptive traits are the best candidates for intentional hybridization.

We recommend that intentional hybridization should be used only after careful consideration of potential harm. Intentional hybridization would be appropriate when the population has lost substantial genetic variation through genetic drift and the detrimental effects of inbreeding depression are apparent (e.g. reduced viability or an increased proportion of obviously deformed or asymmetric individuals). Populations from as similar an environment as possible (that is, the greatest ecological exchangeability[38]) should be used as the donor population. In these situations, even a small amount of introgression might sufficiently counteract the effects of reduced genetic variation and inbreeding depression without disrupting local adaptations[39].

Conclusions

Hybridization is a natural part of evolution. Taxa that have arisen through natural hybridization should be eligible for protection. Nevertheless, increased anthropogenic hybridization is causing extinction of many taxa (species, subspecies and locally adapted populations) by both replacement and genetic mixing. Policies should be designed to reduce anthropogenic hybridization. Hybrid taxa resulting from anthropogenic causes should be protected only in exceptional circumstances. They could warrant protection when hybrids contain the only remaining genetic information from a taxon that has otherwise been lost by genetic mixing or when the circumstances of their origin is unclear.

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