GENOMICS OF HYBRIDIZATION

Hybridization and endangered species protection in the molecular era

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Abstract

After decades of discussion, there is little consensus on the extent to which hybrids between endangered and nonendangered species should be protected by US law. As increasingly larger, genome-scale data sets are developed, we can identify individuals and populations with even trace levels of genetic admixture, making the ‘hybrid problem’ all the more difficult. We developed a decision-tree framework for evaluating hybrid protection, including both the processes that produced hybrids (human-mediated or natural) and the ecological impact of hybrids on natural ecosystems. We then evaluated our decision tree for four case studies drawn from our own work and briefly discuss several other cases from the literature. Throughout, we highlight the management outcomes that our approach provides and the nuances of hybridization as a conservation problem.

Keywords: admixture, California tiger salamander, conservation genomics, Endangered Species Act, intercross policy, wolf

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Hybrid or admixed individuals and populations constitute one of the most challenging problems for protection and management under the US Endangered Species Act (ESA). From the perspective of endangered species management, the essential questions concern whether hybrids of a protected species (which, under the ESA, include species, subspecies and vertebrate distinct population segments) are legally protected. Answering this question has proved to be one of the thorniest challenges presented by the ESA, for two related reasons. First, the identification of hybrids, including the extent of genomic contributions from parental species, has been a persistent empirical challenge, especially for natural populations with unknown pedigrees. Second, even when hybrids can be identified and ancestry quantified, deciding which individuals and populations should be protected under specific historical, environmental and biological circumstances remains contentious and unresolved. Over the last few years, genomics has provided the means to solve the first problem at the finest levels of resolution. However, this increased genetic clarity has actually made the second problem even more challenging, and highlights the need for a better articulated hybrid policy, as well as flexibility in our approach to species protection. A clear message from the debate on hybrid protection is that a one-size-fits-all approach to hybrid management is neither a realistic nor a desirable goal, given the multiple ways in which hybrid and pure populations coexist in nature. The hybrid problem is a nuanced one and requires appropriately nuanced solutions (Allendorf et al. 2001).

Legal policy for hybrids in the United States

Current US policy is unresolved with respect to the protection of hybrids, but the consensus view is that hybrid individuals involving endangered species are not protected under the ESA. An attempt at a clear ‘intercross’ or hybrid policy was proposed in 1996 (US Fish and Wildlife Service 1996; Haig & Allendorf 2006). This
policy had two essential components. First, individuals that more closely resembled the endangered taxon than an intermediate between the two parental forms would receive protection. Specifically, the policy proposed that a hybrid between listed taxon A and unlisted taxon B would receive protection if that individual was more similar to A than to an A/B intermediate. Under an additive model of genetic admixture, this would imply that individuals that were >75% A would receive protection (an intermediate is presumably 50%, and the proposed rule stated that the individual in question be more similar to a 100% A than to a 50% A). Second, if genetic rescue (Carlson et al. 2014) via admixture is the only way to prevent extinction, then hybrids would receive protection, and admixture may be used as a conservation tool (Hedrick 1995).

The 1996 proposed intercross policy was published in the Federal Register (the official publication outlet of the US government) 20 years ago, and has not been accepted as policy or rejected outright by the US Fish and Wildlife Service (USFWS) or the National Marine Fisheries Service (NMFS). One of the major roadblocks for acceptance of the rule has been setting a quantitative admixture threshold (in this case, 75% or more of the endangered taxon) for protection status. Another key concern is whether all genes or phenotypes should count equally when tallying the genetic composition of an individual, as the proposed rule suggested. As recent research has demonstrated, some hybrid alleles confer considerable selective advantages (or disadvantages, e.g. Rieseberg et al. 1999; Hedrick 2013), whereas many other allelic variants differentiating closely related species are functionally interchangeable. For example, it is now clear that ancient hybridization between Neanderthals and modern humans enhanced immunity and adaptation in specific environments, but also contributed genetic variants which may be deleterious in a modern context (e.g. Dannemann et al. 2016; Deschamps et al. 2016).

To help resolve the conflict over when, if ever, hybrids should receive protection, one emerging idea is to focus on the concept of ‘ecological authenticity’ rather than the overall fraction of native alleles. Specifically, if hybrids are ecologically interchangeable with pure natives, then they are serving the same ecological role and maintaining at least some of the genomic content of the endangered taxon, and they should receive protection (Fitzpatrick et al. 2010). Although never explicitly stated, this approach presumes that the target ecological condition is that of the endangered taxon before any deleterious effects associated with extreme inbreeding depression may have reduced overall fitness. In keeping with this idea, genetic rescue (e.g. Hedrick 1995; Westermeyer et al. 1998; Madsen et al. 1999; Vila et al. 2002; Adams et al. 2011) is sometimes considered a viable option for endangered species management, particularly for taxa that suffer from inbreeding depression and reduced viability. Carlson et al. (2014) defined genetic rescue as the ‘increase in the absolute fitness of numerically small populations exhibiting inbreeding depression resulting from the influx of genetic variation from immigrants’; the key idea is that by bringing in new genetic variation from conspecific or heterospecific populations, an endangered species that suffers from severely depleted genetic variation can be genetically restored.

What to preserve, and the ‘problem’ of too much genetic data

A decade ago, genetic inferences for natural populations were often based on a handful of markers and decisions on the genetic purity of an endangered population or lineage were frequently straightforward. However, as genomewide data have become accessible for virtually any taxon, such classifications are more challenging. Again, consider human populations, one of the best understood vertebrates with respect to interspecific admixture. Current estimates based on whole-genome analyses of archaic hominids indicate that up to 4% of the genome of modern non-African humans is derived from Neanderthal admixture, and up to 6% from ‘Denisovan’ interbreeding and admixture (e.g. Meyer et al. 2012; Wall et al. 2013). If modern humans were endangered (clearly, not the case), would this imply that all such admixed individuals should not be protected? Here, the case is reasonably clear that ancient hybridization and the presence of archaic human alleles is simply a part of modern Homo sapiens’ history. The movement of genetic variation across species and populations is a natural phenomenon that happens routinely as a part of the evolutionary process. Presumably, many protected species include a combination of allelic variation that was unchanged from a common ancestor, unique variation that evolved within the lineage, and
alleles that derive from admixture. The critical point for conservation is that such historic admixture is part of the evolutionary history of the species or population and does not reflect a response to anthropogenic actions such as habitat degradation or the introduction of non-native species.

A decision tree for hybrid protection using genomewide data

Ultimately, genomewide data can provide endangered species managers with an estimate of the fraction of the genome that is derived from another species or population, and identify the specific genes or chromosomal segments that have been introgressed. These data can also be used to recover the history of recent selection on a chromosomal region and, by inference, the potential importance of a non-native genomic region on the fitness of individuals in an admixed population. Although the definition of what constitutes a 'species' and how it should relate to conservation actions remains a difficult topic to fully resolve (Fitzpatrick et al. 2015), genomic data at least provide a full empirical context to address the problem.

Given the increasing ability to apply genomewide data to admixed, declining populations and species, we suggest that a relatively simple decision tree may be a valuable guide for resource managers tasked with deciding whether to protect or remove hybrids. Obviously each case will have its own unique attributes, and landscape analyses and ecological and natural history observations are also critical components of the determination of whether to protect or control hybrid populations. However, it should be possible to establish a relatively simple set of decisions, as we do here. The proposed intercross policy of 1996 was one such attempt with a decision rule providing protection of individuals which are 75% or more native endangered taxon. Quantitative guidelines such as this are appealing in their simplicity, although they ignore the ecological authenticity of admixed individuals and the historical processes which resulted in admixture. Ecological and evolutionary processes are important, and should be incorporated into a coherent hybrid policy. With these points in mind, we propose the following decision tree for protection of hybrid populations as endangered species.

Hybrid status assessment using genomewide data: a decision-tree approach

Rule 1. Is admixture a natural occurrence between two native species or does it derive from recent anthropogenic influences?

- If natural, then protect.
- If not, then go to Rule 2.

Natural hybridization is a part of the evolutionary process, and should not be cause for an individual, population or species to forfeit protection. In the case of broad hybrid zones, for example between a protected and nonprotected pair of subspecies, arbitrary cut-offs designating where in the zone protection ends may be needed. We tentatively suggest >50% genomic composition of the endangered species as a reasonable limit for protection given this value is readily detected by even low-resolution molecular techniques and in many cases will include a substantial fraction of the actual hybrid zone (e.g. vonHoldt et al. 2011).

Rule 2. Are hybrids ecological surrogates for the endangered species that provide similar or identical ecosystem functionality and result in similar community interactions?

- If yes, then protect hybrids and go to Rule 3.
- If no, then go to Rule 3.

Obviously determining ecological equivalency is challenging. For some species, experiments may be possible (see California tiger salamander, below). For others, patterns of inferred selection on genomic regions may suggest strong selection, as may observational studies of admixed populations in the wild. Our main point is that hybrids that clearly function differently than natives should be management targets.

Rule 3. Will restoration of the native habitat for the endangered species select for native alleles and decrease the fraction of genomic contributions from the non-endangered species?

- If yes and yes to 2, then continue to protect and undertake restoration.
- If yes and no to 2 undertake restoration and temporarily protect with the expectation that the frequency of native alleles in the hybrid zone will increase.
- If no and no to 2, then do not protect, pursue active elimination of individuals with non-native ancestry, and reduce the movement of non-native individuals into the endangered species range.

This scheme presumes that restoration can favour the increase of native alleles in the admixture zones which requires the existence of a selection differential between native and non-native alleles.
If parental alleles have similar fitness in the admixture zone or are nearly neutral in effect, then selection cannot enhance the fraction of native or endangered species alleles. Further, in small populations, the efficacy of selection may be reduced and even alleles with different selection coefficients may act as if they were neutral. Specific population modelling can be useful in this case to determine the demographic conditions for restoration (Marsden et al. 2016).

This decision tree is preliminary. Our intent is to provide a starting point for discussion that will lead to a more sophisticated and nuanced approach for hybrid management. After two decades of stalled official policy, our hope is to reinvigorate the debate and help bring about a reasonable hybrid policy that makes ecological sense and supports the protection of endangered species and evolutionary lineages. Essentially, if hybrids lead to ecological catastrophes, then managers should not protect them, and eliminating them may be preferable to keeping them on the landscape, even if they are the sole representatives of an endangered species genome. In such cases, we argue that extinction by hybridization has already occurred, and recovering the original endangered species may be impossible. Alternatively, if hybrids represent a loss of genetic isolation that existed historically, but if no important adaptive variation had accumulated during the isolation from the introgressing taxon, then protecting admixed populations and individuals will have no discernible ecological consequences. In this case, the ecological and evolutionary essence of the endangered species still exists and should be protected regardless of admixture level. Finally, if habitat restoration can be undertaken that might select for increasingly native populations in an admixture zone, that restoration should be pursued before making final decisions on protecting or eliminating hybrids.

**Case studies**

Below, we present a brief synopsis of four case studies and how our decision-tree approach leads to concrete and distinct management recommendations. For each, we provide a summary of the relevant genomic and ecological data, recommendations based on those data, and areas where additional data would help refine these recommendations. This collection of case studies is admittedly limited in taxonomic scope, and reflects systems that the authors are actively investigating, rather than a synopsis of many possible examples. However, they also cover a diverse range of situations and outcomes.

**Mexican wolf**

*Background.* The Mexican wolf (*Canis lupus baileyi*) is the most distinct and endangered lineage of grey wolf in North America and likely derived from an ancient migration of grey wolves into North America (Fan *et al.* 2016; Hendricks *et al.* 2016). The Mexican wolf has a distribution throughout much of the arid lands of Northern Mexico and the American Southwest, and has been considered to range as far north as central Arizona and New Mexico in the United States (Hendricks *et al.* 2016). The subspecies declined in the 1900s due to public and private predator control efforts as well as habitat loss and was extinct in the wild by the 1970s (Hedrick *et al.* 1998). However, a small number of Mexican wolves (<10 founders) were rescued from the wild prior to extinction and actively bred in three captive programmes beginning in 1974. Since 1997, these captive bred wolves have been used as a source for re-introduction to a single site straddling the Arizona–New Mexico border in the southern United States. However, despite continuous supplementation, the re-introduction has not resulted in a self-sustaining population due primarily to removal of wolves having conflicts with livestock, and low native prey base (Wayne & Hedrick 2011). It is important to recognize that wolf introductions can result in self-sustaining populations, as seen with grey wolves introduced to Yellowstone National Park and central Idaho in 1995. About 75 wolves were introduced in total to both these areas, and the combined population now numbers close to 1500 wolves that are continuing to expand and recolonize much of the American west (Wayne & Hedrick 2011).

A prime limitation preventing the re-introduced Mexican wolf population from maintaining numbers and increasing has been limitations on re-introduction sites imposed by the narrow delineation of the historical geographic range, which under the US Endangered Species Act limits the area to which individuals can be re-introduced (Hendricks *et al.* 2016). However, analysis of mtDNA from historic specimens demonstrates that the diagnostic Mexican wolf haplotype was distributed over a far greater geographic range, including areas where northern grey wolves were known to exist (Leonard *et al.* 2005). Recent niche projections using these data extend considerably the current designated range of the Mexican wolf, suggesting new sites for re-introduction (Hendricks *et al.* 2016). Further, the dispersal abilities of wolves, and existing admixture among wolf ecotypes (Schweizer *et al.* 2016) and between coyotes (*Canis latrans*) and wolves (vonHeldt *et al.* 2011), suggest that rigid geographic range boundaries are unlikely for population units in wolves. Rather, genetic substructure in wolves reflects habitat differences which define specific
wolf ecotypes/subspecies, including the Mexican wolf (Geffen et al. 2004; vonHoldt et al. 2011; Schweizer et al. 2016). Consequently, transition zones between habitat types likely were historically a mixture of wolf ecotypes. In this case, Mexican wolves appear adapted for arid lands, whereas Northern grey wolves inhabit wetter coniferous forest often at higher altitudes. These habitats intermix in the Southern Rocky Mountains of the United States, and consequently a mosaic hybrid zone likely was a part of the original landscape. This interpretation is supported by the genetic analysis of historic Mexican wolves (Leonard et al. 2005) which found diagnostic Mexican and Northern grey wolf mtDNA haplotypes across a wide area in the Southwestern United States, defining an admixture zone.

**Recommended action.** Genetic analysis of historic specimens suggested Mexican wolves and Northern grey wolves intermixed historically as would be predicted by the dispersal abilities of wolves and the past distribution of habitats. Consequently, according to our decision tree, the admixture zone which developed between Northern grey wolves and Mexican wolves in the Southern Rockies and beyond represents a natural historic process, and therefore, Rule 1 = Yes. Hybrids should be protected. To restore a historic evolutionary process represented by the admixture zone (which allowed natural selection to sort genes most appropriate for transitional habitats), Mexican wolves and grey wolves should be re-introduced or allowed to expand to a wider area with appropriate habitat. Such actions would acknowledge the importance of evolutionary process embedded in our decision tree.

**Red wolf**

**Background.** The red wolf (*Canis rufus*) is an enigmatic taxon that was protected under the Endangered Species Act soon after its final passage in 1974. As its designation as a distinct species suggests, the red wolf was thought to be a species that inhabited the American Southeast and was extirpated by the early 1970s. As red wolves dwindled in number, they hybridized with coyotes over a large area at least since the 1940s (Nowak 1979) and probably earlier (Roy et al. 1996; vonHoldt et al. 2011).

Prior to its absolute extinction, an attempt to capture red wolves from the wild for captive breeding was initiated. A large number of canids from Louisiana and Texas were captured by USFWS biologists and red wolves were phenotypically assessed to identify 12 putative pure red wolf founders. Subsequently, captive breeding of these 12 individuals supplied red wolves for zoos which were part of the captive breeding programme and provided individuals for re-introduction to an isolated peninsula in North Carolina. However, hybridization with native coyotes which had moved into North Carolina threatened the genetic integrity of the re-introduced population (Fredrickson & Hedrick 2006), and ever since, hybrids have been identified, captured, and either euthanized or sterilized and released as infertile placeholders in the admixture zone boundary between peninsular red wolves and mainland coyote (Stoskopf et al. 2005).

Further confusing this situation is genetic evidence suggesting that the red wolf may never have been a distinct species, and instead formed through human-mediated hybridization between coyotes and Northern grey wolves that co-occupied the American Southeast (Wayne & Jenks 1991; Roy et al. 1996; vonHoldt et al. 2011). This hybridization is hypothesized to have occurred as the southeastern US grey wolf population was depleted through predator control efforts and habitat loss when the area was settled and developed for agriculture after the 1700s (Nowak 1979). Under this scenario, as grey wolf numbers declined, hybridization with the increasingly abundant coyote occurred, creating the ‘Red wolf’ phenotype. This is in contrast to other parts of North America where both species coexist today but wolves are abundant, and hybridization with coyotes does not take place (Roy et al. 1994; vonHoldt et al. 2008).

**Recommended action.** These findings suggest two evolutionary scenarios, each of which has its own set of conservation decisions.

The first assumes that the red wolf was a distinct, endangered species (*Canis rufus*) that has had recent, human-influenced admixture with coyotes (Rule 1 = No). As this admixture likely reflects habitat loss and modification as well as predator control efforts causing red wolves to be rare and coyotes abundant, admixed individuals should be protected. This protection depends on the extent to which they are ecologically similar to pure red wolf (Rule 2 = Yes) and whether habitat modifications can be undertaken that select for the unique genetic characteristics of the red wolf (Rule 3 = Yes). We also note that such restoration efforts may prove costly or even impossible in the modified landscapes of the southeastern United States dominated by coyotes, and that is an important research question for managers.

The second scenario is more complex, and presumes that the red wolf was never a distinct lineage or species, but a result of admixture between coyotes and endangered grey wolves as the latter were eliminated through habitat modification and predator control efforts. Under this scenario, the red wolf never existed as a lineage,
and therefore should not be protected differentially from the grey wolf. However, as a population of grey wolf, it still deserves consideration. Assuming that the hybridization was entirely generated through anthropogenic activities (Rule 1 = No), the question of ecological equivalency (Rule 2) becomes key. Unfortunately, we do not know what the historical ecological role of grey wolves in the southeastern United States was, and therefore, deciding on Rules 2 and 3 is difficult. Given that the large game utilized historically by grey wolves is not currently present over much of the American Southeast and that many of the top predator ecosystem functions are now performed by coyotes who adapt well to urban and agricultural landscapes, it appears that without drastic ecosystem restoration the answer to Rule 2 = No. Under this scenario, Rule 3 would ask whether habitat restoration that would select for grey wolf genes is possible (Rule 3 = Yes). If it is, then the ‘red wolf’ should be protected as a recoverable hybrid grey wolf population.

Eastern wolf

Background. The eastern wolf is thought by some to be a unique species (C. lycaon) of smaller-sized wolf that evolved in North America independent of the grey wolf in the Old World (Wilson et al. 2000; Kyle et al. 2006), and that may be related to, or conspecific with, the red wolf. Under this interpretation, the eastern wolf originally shared natural boundaries with, and may have co-occurred with both grey wolves and coyotes. Other researchers believe, similar to the red wolf, that the eastern wolf is a hybrid between grey wolves which inhabited the Great Lakes area and coyotes, forming a hybrid zone with intermediate sized individuals (Koblmüller et al. 2009; vonHoldt et al. 2011). These intermediate sized individuals were mistakenly classified as a distinct species, the eastern wolf.

What is known is that coyotes entered the Great Lakes area after about 1920 into areas where grey and eastern wolf occurred historically and hybridized with both (Kays et al. 2010). If the eastern wolf is a real entity rather than a hybrid construct, recent work suggests that an essentially pure population of eastern wolves exists in Algonquin National Park in Ontario (Rutledge et al. 2010a,b; Benson et al. 2012). As with red wolves, admixture between coyotes and grey/eastern wolves was enabled by habitat changes driven by agriculture, urbanization, and the reduction in wolf numbers due to predator control efforts. Individuals with mixed grey/eastern wolf and coyote ancestry have now invaded the maritime provenances of eastern Canada and the northeastern United States (Kays et al. 2010). These mixes, sometime called coywolves, attain the largest body size of any coyote (but are smaller than grey wolves), form packs and can prey on large ungulates such as deer (Kays et al. 2010). Consequently, the hybrids may be ecologically more similar to grey wolves than coyotes, and currently function as top canid carnivores in disturbed habitats.

Recommended action. As with the red wolf, two scenarios need consideration. If the ‘eastern wolf’ is a recent, human-mediated hybrid between endangered grey wolves and coyotes (Rule 1 = No, Rule 2 = No), then they should be protected as hybrid grey wolf, particularly if the habitat can be enhanced to increase the genomic fraction in the population deriving from protected grey wolves (Rule 3 = Yes). These habitat considerations might include re-introduction or augmentation of large ungulates such as elk and moose that were present historically, and increasing the area of appropriate habitat for grey wolves (which need large areas without humans). In Yellowstone National Park, where habitats are much more intact, grey wolves and coyotes are both abundant and do not interbreed (vonHoldt et al. 2008), and wolves even suppress the abundance of coyotes, suggesting that coexistence should be possible. In fact, genetic evidence suggests that grey wolves may no longer be interbreeding with coyotes in the Great Lakes where their numbers are large (Koblmüller et al. 2009; Merkle et al. 2009; vonHoldt et al. 2011).

In contrast, if the eastern wolf is a distinct North American species of small wolf suffering admixture with coyote due to human actions (Rule 1 = No), then admixed individuals in some habitats appear to be functioning as ecological surrogates and can be protected under Rule 2 (Rule 2 = Yes). Implementing habitat changes that might increase individuals of high eastern wolf ancestry over that representing coyotes and coywolves is difficult to imagine over much of the northeastern United States, given high human population density. Perhaps the habitat of Algonquin National Park, where pure eastern wolves are thought to exist can provide a model in this regard. However, over much of the presumed historic range of the eastern wolf, coyotes are abundant, habitats are disturbed, and it may be that complete ecological restoration will be extremely difficult.

California tiger salamander

Background. The California tiger salamander (Ambystoma californiense, CTS) is an endemic member of the tiger salamander complex that is restricted to the vernal pool/grassland communities of California’s Great Central Valley and inner coast ranges (Shaffer & McKnight...
Since the initial discovery of widespread hybridization with a related species of non-native tiger salamander, the California tiger salamander has become one of the best-studied examples of the ecological consequences of human-mediated admixture in an endangered amphibian (McCartney-Melstad & Shaffer 2015). Based on tissue collections initially dating from the early 1980s, Riley et al. (2003) documented the intentional introduction of non-native barred tiger salamander (*Ambystoma (tigrinum) mavortium*, BTS) to California and its unintended hybridization with threatened CTS. The combination of deep genetic differentiation across the CTS native range, declining populations, and widespread, human-caused hybridization with invasive BTS led to listing CTS as three Distinct Population Segments under the ESA (US Fish and Wildlife Service 2000, 2004), and later under California law. Subsequent genetic research has demonstrated that at least 20%, of the entire range of CTS currently consists of CTS/BTS admixed populations, and that several ‘superinvasive’ chromosomal segments have invaded at least 100 km further than the rest of the hybrid swarm in the Salinas Valley (Fitzpatrick & Shaffer 2007; Fitzpatrick et al. 2009, 2010).

A unique feature of the CTS/BTS hybrid system has been an explicitly experimental approach to quantify the ecological authenticity of hybrids compared to pure CTS. Initial mesocosm work by Ryan et al. (2009) indicated that hybrids are much more effective predators on native amphibians than pure CTS, and subsequent field studies indicated that (i) larval hybrids are better able to withstand pesticides than CTS in pond cage experiments (Ryan et al. 2013), (ii) larval CTS enjoy a selective advantage over hybrids under short pond hydroperiod conditions that mimic natural vernal pools (Johnson et al. 2013), and (iii) locomotor performance of hybrids was generally greater than native CTS, promoting the movement of non-native genes across landscapes (Johnson et al. 2010). Most recently, a replicated mesocosm experiment investigated the effects of different genetic classes of tiger salamander larvae on vernal pool food webs. Compared to intact communities with pure native CTS, Searcy et al. (2016) demonstrated that the complete removal of all tiger salamander larvae had the most profound ecological impacts on vernal pool food webs, followed by hybrid swarm larva. Pools with larvae that were primarily native CTS but contained a small fraction of superinvasive BTS alleles were ecologically indistinguishable from pure CTS (Searcy et al. 2016). Taken together, these experiments point to three conclusions. First, any tiger salamander is better than complete eradication from a community ecology perspective. Second, hybrids are not equivalent to pure natives. And third, habitat restoration back to natural vernal pool hydroperiods may recover native genotypes.

**Recommended action.** For the CTS/BTS system, the admixture is human-mediated (Rule 1 = No). Rule 2 = No for hybrid swarm salamanders, with the caveat that Searcy et al. (2016) demonstrated that community integrity with hybrid salamander larvae is closer to ecological equivalency with CTS than the complete elimination of all larvae, suggesting that elimination of hybrids without replacement with pure CTS is not advisable. However, for superinvasive larvae, Rule 2 = Yes, suggesting that these widespread, rapidly expanding genotypes should be protected. For hybrid swarm larvae, experimental mesocosm evidence suggests that Rule 3 = Yes, and that restoration of artificial cattle ponds to more closely resemble vernal pool hydroperiods may select for more native genotypes (Johnson et al. 2010). To date, this result has only been confirmed in semi-natural mesocosms, but we are following up this promising result with whole-pond hydroperiod manipulative experiments.

**Additional insights from other systems**

Our intent in this commentary is to provide direction and discussion rather than a universally acceptable protocol that can be applied to all hybrid taxa. By reinventing this discussion, we hope that a comprehensive approach, endorsed by regulatory agencies and the conservation biology community, might emerge that can help guide hybrid management. As such, we note two additional points that deserve mention.

First, for high-vagility species, controlling the movements of genes may be difficult or impossible to achieve. Birds, migratory marine and terrestrial mammals, and some wind-pollinated plants may often fall into this category. For example, the northern spotted owl (*Strix occidentalis caurina*) is a species listed under the ESA which has become a flagship species for conservation of old-growth temperate rainforest in the Pacific Northwest of North America. Northern spotted owls hybridize with barred owls (*Strix varia*), a species that in the past few decades has dramatically expanded its US distribution westward and appears to be displacing spotted owls in old-growth forests (Gutierrez et al. 2007). In this case, hybrids could receive protection under Rule 2 if they are ecological surrogates for protected spotted owl in old-growth forest. Further, Rule 3 would suggest that attempts should be made to halt the range expansion of barred owls/hybrids through control measures and habitat preservation and restoration. However, the efficacy of control via euthanizing hybrids is equivocal, and pure spotted owls may be accidently
killed in the process (Livezey 2010; US Fish and Wildlife Service 2012). If the frequency of barred owl genes can be reduced, the admixed areas should stabilize or possibly revert to a dominance of spotted owl genes. The challenge is keeping barred owls, which are strong fliers and highly vagile, from reinvading. High mobility invasive aquatic species present similar challenges if their increase in range is facility by enhanced anthropogenic connections among aquatic habitats. A special case in this regard are farmed species, such as Atlantic salmon, which escape from salmon farms and hybridize with the few remaining wild salmon along the Canadian and US east coast resulting in a reduction in fitness and potential loss of local adaptive gene pools (McGinnity et al. 2003; Hindar et al. 2006). Because the hybrids are derived from anthropogenic activities (Rule 1 = No), and are not ecological surrogates (many cannot even persist long in the wild, so Rule 2 = No and Rule 3 = No), the hybrids should not receive protection and should be actively eliminated.

Second, unlike high mobile species, plants are sedentary, and there is the potential that the invasive source for admixture can more readily be removed or controlled than for many animal species. Even for wind-pollinated plants, the source of the pollen is an individual plant that can in principle be found and eliminated. However, such control may not be practical for many species, especially crop or introduced garden species that humans are dependent on, or are reluctant to control. In such cases, our three rules would argue for eliminating both hybrids and the non-native species, but public opinion or economics might make that impossible. In such cases, appropriately large buffer zones that serve as pollen/seed traps, and where hybrids are removed, may be the best option.

Summary and conclusions

This commentary is not a final prescription for the problem of hybrid management, but rather to add to the debate about protection initiated by others (e.g. Allendorf et al. 2001; Abbott et al. 2013; Stronen & Paquet 2013; Piett et al. 2015; Fitzpatrick et al. 2015). Our goal is to reformulate conservation goals in the age of genomics and in the light of evolutionary and ecological principles. Specifically, we suggest that hybrids often retain conservation value and that the primary factors that need consideration are as follows: (i) whether hybridization is a results of anthropogenic activities; (ii) whether hybrids perform ecosystem functions and services that are similar to those performed by the endangered entity; and (iii) whether restoration of habitat can enable natural selection that favours the native endangered entity and the genes it contained. We demonstrate these principles with four examples. The first three involve endangered canids with large disperse capabilities and the potential for admixture zones that can change the genetic and phenotypic characters of forms across the landscape as well as alter ecosystem function. Our decision-tree approach leads to different conclusions about hybrid protection in these three examples. In the Mexican wolf, historical baseline data implies a large, natural admixture zone between Mexican and northern grey wolves, and therefore that hybrids should be protected as essential contributing elements in an ecologically complex environment. For the red wolf and eastern wolves, the evolutionary origins of the species remain unsettled, and different interpretations lead us to different conclusions. Finally, California tiger salamanders offer an example where ecological experiments have been successfully completed, suggesting that hybrid genotypes are preferable to complete eradication of all salamander larvae and that habitat restoration to more natural pond hydropodid may select for increasingly native salamander populations.

We suggest that arguments for protection of hybrids be as nuanced as the environments they inhabit and the genetic context imposed by history. Our ‘rules’ are only guidelines, and their implementation depends critically on the quality and preponderance of scientific evidence approach used in civil law, rather than any rigid formula.

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Data accessibility
There are no original data presented in this manuscript.