



Discussion

Strategies to address the conservation threats posed by hybridization and genetic introgression



Justin H. Bohling*

Abernathy Fish Technology Center, US Fish and Wildlife Service, Longview, WA, USA

ARTICLE INFO

Article history:

Received 23 February 2016

Received in revised form 23 September 2016

Accepted 5 October 2016

Available online xxxx

ABSTRACT

Hybridization poses a complex problem for biodiversity conservation but there has been little discussion of strategies and guidelines designed to address it from a management perspective. In this article I review management approaches aimed at addressing hybridization while highlighting outstanding needs. Despite real-world efforts to manage systems in which hybridization is a concern, there has been little effort to develop broadly applicable guidelines or best management practices. Management programs would be enhanced by the characterization of the patterns and processes of hybridization in nature and integrating those into planning and policy. Doing so facilitates the development of holistic strategies that balance the importance of hybridization in many biological systems while addressing situations in which human-facilitated gene flow causes concern. The shift from reactive management to emphasizing measures designed to prevent hybridization from becoming a threat has been a positive development. Examples include assessing the genetic characteristics of populations used in translocation programs. Overlooked, however, has been the management of stable hybrid systems in which the goal is not necessarily preventing intermixing but protecting stabilizing mechanisms through policy and land use management. When hybridization emerges as a legitimate threat, activities such as culling, spatial isolation, targeted harvest, and de-introgression can protect threatened genomic units. Further experimentation of these techniques and collaboration among scientists and managers will provide lessons for establishing general guidelines for the conservation community. I hope this review stimulates discussion about approaches useful for addressing hybridization and promotes further development of new techniques and frameworks.

Published by Elsevier Ltd.

1. Introduction

Among the most difficult issues facing conservation biologists are the challenges posed by hybridization and genetic introgression. Early warnings, such as the seminal review of [Rhymer and Simberloff \(1996\)](#), introduced the potential threats posed by hybridization and its implications for biodiversity. Subsequent reviews have produced a variety of additional perspectives, addressing issues such as policy-making to the value of hybrids for conservation to ethical considerations ([Allendorf et al., 2001](#); [Ellstrand et al., 2010](#); [Jackiw et al., 2015](#); [Wayne and Shaffer, 2016](#)). What has lacked, however, is a discussion of actual strategies and management guidelines designed to address hybridization from a natural resource conservation perspective. Thus, there has been a lag in the development of standard practices and tactics that can be shared among the conservation community. This is unfortunate, for anthropogenic forces are increasingly altering historical patterns of gene flow, endangering species and reducing biodiversity ([Crispo et al., 2011](#); [Rhymer and Simberloff, 1996](#); [Seehausen et al., 2008](#)).

The goal of this review is to discuss approaches for addressing hybridization when it emerges as a problem and develop a general framework in which to approach the management of hybrid systems. This includes topics such as measuring risk due to hybridization and integrating principles of genetics and evolutionary biology into monitoring and modeling protocols. Many of these strategies require further examination, however, and I will discuss outstanding needs for managing hybrid systems. Given the complexity of this topic, I also include an overview of relevant terminology. My objective is to provoke discussion about the way hybridization is addressed in conservation circles and facilitate efforts to develop standards geared towards this issue when it becomes a conservation threat.

2. What is hybridization?

The terminology used in connection with hybridization and introgression is diverse, which complicates the issue for managers, policymakers, and other stakeholders (see [Arnold, 1997](#) for a more thorough review). I will define several terms relevant for this review. Focusing on hybridization itself I refer to the definition used by [Harrison \(1990\)](#): the “interbreeding between any 2 populations that

* Corresponding author at: 1440 Abernathy Creek Rd., Longview, WA 98632, USA.
E-mail address: Justin_bohling@fws.gov.

are distinguishable on the basis of ≥ 1 heritable character". This definition, although broad, is useful for several reasons. It is comprehensive enough to cover not only interbreeding between species (interspecific hybridization) but also between diagnosable units within a species, such as subspecies or varieties (intraspecific hybridization). Also, Harrison's definition can be extended from natural hybrid systems to those involving domesticated varieties and intentional intermixing under artificial circumstances. 'Introgression' is the movement of genetic material from one unit (often called 'parental' groups) into the gene pool of another. For this to occur the hybrids must be fertile and then interbreed with one or more of the parental groups, which is referred to as 'backcrossing'. A term often used interchangeably with introgression is 'admixture', which is another term for gene flow. The difference is that admixture refers to the contemporary movement of genes between populations, whereas introgression is the persistence and establishment of those genes in a population. Even if hybridization and admixture end between two genetic groups, the legacy of introgression can still persist. Throughout this review I will use the term 'system' to describe a biological system composed of distinctive genetic groups at any taxonomic level (e.g. species, subspecies, and varieties) that have the potential to interbreed, whether they do or not.

One of the more debated and confusing aspects of hybridization is the distinction between so-called 'natural' and 'artificial' or 'anthropogenic' hybridization (see Allendorf et al., 2001 for a perspective). The issue is finding a definition that protects the evolutionary legacy of hybridization while simultaneously distinguishing systems impacted by human activity. I will use the terms 'natural' and 'artificial' to reflect not the "desirability" of hybridization but instead the circumstances under which it occurs. For systems in which reproductively-compatible biological units (e.g. species and subspecies) naturally co-occur at the same time and place (either across their range or in a contact zone) I will consider these 'natural', regardless of the extent of historical or contemporary hybridization. In other words, species in 'natural' systems historically have had the potential to interbreed in nature, whether they have or not. On the contrary, 'artificial' hybridization is solely the consequence of human activity mixing units that previously had no opportunity to interbreed (i.e. historically allopatric). Examples include movement of organisms across continents or watersheds that were previously inaccessible. Another is the spread of domestic/commercial varieties.

The distinction is significant for management and policy. For example, each has implications for tolerance of introgression: managers may accept some introgression as part of the evolutionary legacy of 'natural' systems, whereas 'artificial' hybridization may be viewed as disrupting evolutionary isolation and demand more extreme management measures. Also since artificial hybridization is a product of human activity, prevention is different compared to natural systems in which the species already coexist. Clearly with the variety of ways hybridization manifests itself many situations will challenge this simple dichotomy, but this distinction is useful for categorizing hybrid systems based on the initial circumstances under which they occur.

3. What is the conservation concern?

In terms of biodiversity conservation there are many scenarios in which hybridization can have negative consequences (see Allendorf et al., 2001; Crispo et al., 2011; Laikre et al., 2010; Rhymer and Simberloff, 1996 for further discussion). While it has been common in the past to treat any hybridization and/or introgression as "undesirable" from a conservation perspective, this view may be overly conservative and ignores our growing understanding of this phenomenon (Allendorf et al., 2001; Jackiw et al., 2015; Searcy et al., 2016; Wayne and Shaffer, 2016). The greatest concern is hybridization causing the extinction of distinct genetic, phenotypic, and/or evolutionary units. The fear is that a distinct genomic unit will disappear due to 'genetic swamping' in which co-adapted gene complexes collapse under

elevated gene flow (Lenormand, 2002). This collapse can either be due to introgression of genetic material from one group into another or outbreeding depression, depending on the extent of hybrid fertility (Crispo et al., 2011). Many terms have been applied to this scenario, such as 'reverse speciation', 'genetic assimilation', 'lineage fusion', 'genetic pollution', and 'genetic extinction'. I will use 'genomic extinction' for extinction via hybridization because it reflects the main concern from a conservation standpoint: the loss of combinations of genes and genotypes that have a unique evolutionary history (Allendorf et al., 2013). Following genomic extinction the result is frequently a 'hybrid swarm' in which all the individuals composing a population are hybrids. Too much swamping can dilute the gene pool to the point where ancestry from one or more of the parental groups may become undetectable, even lost, from the gene pool.

What causes hybridization to emerge as a conservation concern? The distinction between natural and artificial systems is important. With natural systems, disturbances to the environment, population dynamics, and social systems caused by human activity can alter relationships between parental groups (Crispo et al., 2011; Rhymer and Simberloff, 1996; Seehausen et al., 2008). In this case the concern is not necessarily the presence of intermixing, but that hybridization dynamics will shift and cause the genomic extinction of one or multiple parental groups.

Artificial systems can become a concern for a number of reasons: they can result in genomic extinction, outbreeding depression, biotic homogenization and loss of intraspecific variation, and even simply undesired introgression. They can be created by the introduction of non-native species or genotypes, either intentional or accidental, which become established and interbreed with native populations. Another scenario is the intentional, often continual, release of non-native varieties into the range of native populations, frequently for commercial or recreational purposes (Laikre et al., 2010).

4. How can we determine risk?

As with any purported conservation threat, the question is whether hybridization, either natural or artificial in origin, poses a legitimate risk to the viability of a population. There are two problems with measuring risk in hybrid systems. First, hybridization dynamics are stochastic: simply surveying a population at a snapshot in time for the presence of hybrids is not an adequate means to quantify risk. Even under artificial circumstances, observing a few hybrids at one moment in time does not necessarily mean it will grow into a larger problem, especially if the fertility of hybrids is unknown. As an example, retrospective analysis of gray wolves on Vancouver Island, Canada revealed an episode of introgression from domestic dogs: however, contemporary individuals did not display evidence of admixture (Muñoz-Fuentes et al., 2010). Genetic analysis of a few specimens raised fears that hybridization with bobcats (*Lynx rufus*) would threaten endangered lynx (*L. lynx*) in the southern portion of their range (Schwartz et al., 2004); a later continental-wide survey revealed hybridization to be a rare event (Koen et al., 2014). Introgression itself can be temporary: strong selection can purge introgressed alleles from foreign populations (Harbicht et al., 2014). Natural hybrid systems can be quite stable: at low and even moderate levels of interbreeding and introgression parental groups can maintain their evolutionary and genetic character (Mallet, 2008; Poelstra et al., 2014). Thus, as with any threat, it is important to assess whether hybridization is a serious problem that requires human intervention. A few hybrids, especially in natural systems, are not a sign of imminent danger: temporal, spatial, demographic, and ecological data provide context in which to base the monitoring.

The second issue is actually defining extinction risk due to hybridization. The issue is not in the definition of genomic extinction, it is in the implementation: there are no standard criteria to determine when a parental unit is "lost". Studies using population viability analysis (PVA) in the context of hybridization have considered the disappearance of pure

parentals as extinction (Fredrickson and Hedrick, 2006; Wolf et al., 2001). This may be too stringent, though, and rejects alternative philosophies regarding the value of admixed to conservation (Jackiw et al., 2015; Stoskopf et al., 2005; Wayne and Shaffer, 2016). Plus, it can be extremely difficult to define what a “pure” individual is, especially for natural systems with a legacy of introgression. These decisions regarding thresholds for extinction are likely to be case-specific. A decision tree such as those proposed by Wayne and Shaffer (2016) and Stoskopf et al. (2005) provide a guide for setting thresholds for defining extinction based on the nature of the system and the goals of managers.

Making progress in assessing the risks posed by hybridization depends on our ability to monitor and model hybrid systems, especially for natural systems. In terms of monitoring, there are already a plethora of tools available. Many fall under the umbrella of genetic monitoring (Schwartz et al., 2007), which provides a framework for determining the extent of hybridization, trends over time, and responses to management. Application of molecular markers has been critical for managers monitoring the extent of introgression in populations of cutthroat trout (*Oncorhynchus clarki*) (Kovach et al., 2015; Muhlfeld et al., 2014), European wildcats (*Felis sylvestrus sylvestrus*) (Nussberger et al., 2014), Greater Spotted Eagles (*Aquila clanga*) (Väli et al., 2010), Iberian wolves (*Canis lupus signatus*) (Godinho et al., 2014, 2011), and red wolves (*C. rufus*) (Gese et al., 2015) over time, providing insight into the dynamics of those systems relevant for basing management.

The next challenge is developing models that can be used to quantify extinction risk. Predicting the fate of a hybrid system requires a variety of data, ranging from quantification of hybridization rates, spatial segregation and abundance of parentals, assortative mating, reproductive fitness of hybrids, and more. Adapting models of hybrid zones, genetic clines, and hybrid swarms (Barton and Hewitt, 1985; Baskett and Gomulkiewicz, 2011; Gilman and Behm, 2011; Moore, 1977; Rand and Harrison, 1989) can provide predictions into the future dynamics of a system. These models are appropriate for making predictions about system stability or collapse. Modeling the invasion of a non-native waterfrog (*Pelophylax ridibundus*) in Europe generated predictions of risk from genetic swamping for multiple native frog species that displayed varying levels of hybrid fertility (Quilodrán et al., 2015). These same models were used to model the efficacy of expanding available habitat as a conservation strategy. The next step is for these types of models to be integrated into general natural resource monitoring, similar to traditional population and harvest models. When the threat evolves into a concern for small threatened populations, these same data and variables can be adapted to a PVA-framework to quantify the risk of extinction due to hybridization. For example, PVAs, coupled with modeling of management strategies, prompted the development of a strategy to limit hybridization in the world's only wild population of red wolves (Fredrickson and Hedrick, 2006; Kelly et al., 1999). Such models can be incorporated into IUCN Red List assessments.

5. How can we prevent hybridization from becoming a problem?

Prevention differs for natural versus artificial systems. For natural systems, mixing often occurs in ‘hybrid zones’ (also called ‘contact zones’) where the ranges of the species, subspecies, etc. overlap, facilitating interbreeding. Sometimes these zones can be extensive, and in some situations the parental groups may be sympatric across the majority of their range. For natural systems the management goal should not be preventing hybridization but understanding mechanisms that regulate interbreeding (or lack thereof) and promote their stability. Integrating models of hybrid zone dynamics will be a major step towards understanding the forces that maintain them and the role of human activity, both positive and negative, in keeping them stable. Even in systems that appear to be stable, practices aimed to maintain that stability would provide safeguards to limit future problems. An example exists in southeastern Canada where eastern wolves (*Canis lycaon*) are restricted to a few protected areas and interbreed with eastern coyotes

(*C. latrans* var) and Great Lakes-Boreal wolves (*C. lupus* var) populations surrounding them. High wolf mortality outside protected areas disrupted social units (Rutledge et al., 2010b), facilitating hybridization (Rutledge et al., 2012). Managers and policymakers implemented harvest restrictions in a buffer zone around the core protected area of Algonquin Provincial Park: subsequently eastern wolves displayed natural patterns of social structure and maintained their genomic character despite occasional hybridization (Rutledge et al., 2010a, 2010b). Other studies have shown how ecological disruption can alter patterns of hybridization (Hasselman et al., 2014; Hoban et al., 2012; Seehausen et al., 2008). Management targets may need to shift from individual species to “hybrid complexes” or “species complexes” instead (Fitzpatrick et al., 2015).

On the other end of the spectrum, just as hybrid zones where species interbreed are natural, so are mosaics of species isolated geographically but not intrinsically. The obvious examples are species separated by oceans or continents, but even continuous landscapes are filled with reproductively compatible species that do not intermix because of geographic barriers. Human activity can link isolated groups and facilitate artificial hybridization. As an example, man-made shipping canals allowed white perch (*Morone americana*), a freshwater perciform, to disperse from coastal Atlantic, USA watersheds into the Great Lakes and Mississippi watersheds and hybridize with previously allopatric species (Irons et al., 2002). Protecting these barriers through landscape-level planning would prevent hybridization from becoming a potential problem. Islands and aquatic ecosystems in particular would benefit from inventories of reproductively-compatible species and protection of natural patterns of isolation. For any given species the list of species it can potentially interbreed with may be extensive and governments may choose to proactively ban certain species or varieties from import if they fear it could cause undesired introgression. An added nuance for hybridization in terms of introductions: even if the introduced species or variety fails to establish or is eradicated its legacy can live on through introgression, which emphasizes the importance of prevention.

This concept of natural patterns of gene flow and isolation guiding preventative management extends to the intraspecific level as well (Laike et al., 2010). Natural resource management has made strides in considering these patterns when managing wild populations. Activities such as artificial translocation, stocking, and water diversions can link previously isolated populations: establishing policies to limit the potential for novel gene exchange is critical. Genetic information plays a key role in these discussions, discerning patterns of population structure that guide management practices. For example, programs have been developed to assess the natal origin of migratory bull trout (*Salvelinus confluentus*) captured below hydroelectric dams using genetic markers (DeHaan et al., 2011). This then allows fisheries managers to transport fish to their appropriate breeding locations upstream, maintaining natural patterns of genetic structure and minimizing risk of homogenization.

6. How can we protect populations threatened by hybridization?

Once hybridization is identified as a legitimate conservation threat, the issue becomes identifying potential strategies to deal with it. The tools available to managers will differ depending on the characteristics of the system, such as artificial versus natural system, whether hybridization is on-going, the extent of introgression, and the role of human disturbance in promoting the interactions. Other reviews and perspectives have stressed the importance of thoroughly evaluating the justifications and ethical concerns associated with intensively managing hybrid systems (Allendorf et al., 2001; Jackiw et al., 2015; Kyle et al., 2007; Wayne and Shaffer, 2016). Sticking to the theme of this review, I will focus primarily on the feasibility aspect: are there strategies available for managing hybrid systems to achieve a desired conservation outcome?

6.1. Limited hybridization/introgression

The best case scenario involves early-stage artificial systems in which hybridization and introgression are not yet prevalent. Assuming parental groups can be reliably distinguished these systems mirror a typical invasive species removal project, with the goal of removing the invader (and its genes) before becoming established. Culling, assuming ethical concerns are addressed, is a valuable tool to prevent hybridization from becoming a potential problem. For example, in Europe a multi-nation initiative involves policies to cull and limit releases of the non-native ruddy duck (*Oxyura jamaicensis*) to prevent hybridization with the native white-headed duck (*O. leucocephala*) (Cranswick and Hall, 2010). Another example is Crater Lake National Park in Oregon, USA where endangered bull trout were temporarily removed from rivers to allow the application of poison to kill non-native brook trout (*S. fontinalis*); following removal the bull trout were released back into the waterways (Buktenica et al., 2013). An important point emphasized by both examples: culling was not haphazard but served a strategic purpose of a larger comprehensive strategy with measurable goals and outcomes. Policies to cull ruddy ducks were designed to augment policies preventing future releases. With the bull trout in Oregon, the nature of system made culling a one-time practical solution: artificial barriers blocked upstream dispersal of brook trout from other portions of the watershed once they were eliminated. Removal and culling programs for artificial systems are only practical if coupled with preventative measures that allow the development of clear goals and timelines.

6.2. No hybridization but lingering introgression

Trickier are systems in which the actual mating between groups and production of hybrids no longer occurs but introgression from past interactions persists. If the remaining population still has desirable qualities for conservation purposes and culling is impractical, the best option may be to accept introgression as a permanent characteristic (e.g. Campton and Kaeding, 2005; Searcy et al., 2016). For small, easily manageable populations, however, culling can be feasible on a defined spatial and temporal scale. In the United States herds of American bison (*Bison bison*) are being screened for the presence of cattle mitochondrial DNA (mtDNA) and individuals with domestic haplotypes are being culled (Dratch and Gogan, 2010). There is no way to phenotypically distinguish individuals with cattle mtDNA: genetic surveys revealed the pervasiveness of introgression (Halbert and Derr, 2007; Polziehn et al., 1995; Ward et al., 2001). Since many bison herds are under close human supervision, it is simple to test and cull individuals with clearly non-native haplotypes. Captive populations would also fall within this framework, assuming introgression can be reliably identified. Again, pursuing this as an option should be rooted in sound scientific, management, and ethical grounds, along with defined standards for classifying admixed individuals. For example, the decision to cull is often justified when introgressed individuals have lower fitness, impacting the future viability of the population. This has been observed for bison (Derr et al., 2012) and westslope cutthroat trout (Muhlfeld et al., 2009), which has influenced management decisions to remove admixed individuals.

Paradoxically, it is possible to use introgressed individuals as a means to restore lost lineages via selective breeding. Amador et al. (2014) presented a framework to recover desired genotypic units from an admixed population, called “de-introgression”. Using data from genetically distinct breeds of sheep, they simulated selectively breeding admixed individuals based on genomic characters to recreate the genomic profile of the original breeds. Currently this is a strategy being proposed for restoring “extinct” subspecies of Galapagos tortoises (*Chelonoidis nigra*) to their native islands (Edwards et al., 2013; Garrick et al., 2012). Such an approach accepts that a “recovered” population has a history of admixture but acknowledges the value of these re-created genotypes/phenotypes to conservation efforts. De-introgression would be practical for captive or semi-wild populations in which

individual organisms can be tracked and managed. Whether it can be implemented with wild populations via combinations of culling, introductions, and other manipulations remains to be seen (see westslope cutthroat trout example below). It is also uncertain whether bulk release of native genotypes with the goal of “swamping” non-native ancestry offers the precision and effectiveness of the formal framework presented by de-introgression.

6.3. Active hybrid systems

Most challenging are active hybrid systems, whether artificial or natural, in which parentals and hybrids are actively interbreeding within the landscape. Deciding which management strategies to implement ultimately depends on goals of the management program and the feasibility of the various options. Implementing culling in active hybrid systems is problematic. Accurately identifying the parental groups and their hybrids can be daunting. In many cases individuals cannot be readily classified in the field, requiring coordination between field personnel and genetic labs. Ideally, in small, closed systems culling could theoretically remove the undesired parental group and hybrids, protecting the threatened group from future threat. In large, open systems in which the prospect of eliminating hybridization seems infeasible, culling may not be an appropriate tool for long-term management.

There are also variations on the theme of culling. An innovative technique is to sterilize organisms so they are reproductively inactive yet serve as territorial place-holders. This concept has been pioneered with canids in which sterile individuals occupy space and prevent immigration from reproductive individuals by defending territory (Conner et al., 2008). It has been a vital component of the red wolf program, not only preventing hybridization but allowing wolves to colonize territory by displacing placeholder coyotes (Gese and Terletzky, 2015; Gese et al., 2015). Whether this is successful in the long-term for other systems requires further investigation and testing: at this point its most effective use may be as a tool to protect small populations at risk of swamping.

When culling is infeasible, there are other strategies to protect populations from hybridization when non-native genotypes proliferate. When native populations are small, they can be isolated to prevent genetic swamping. For example, Muhlfeld et al. (2012) assessed whether placing barrier dams in streams would be an effective strategy to protect cutthroat trout populations by preventing dispersal of non-native rainbow trout (*Oncorhynchus mykiss*) present in other portions of the watershed. The US Fish and Wildlife Service released captive-bred red wolves onto isolated barrier islands to allow them to “practice” being wild animals (Parker, 1987). In an innovative approach, Debeljak et al. (2015) used habitat modeling to predict how protected areas would minimize introgression from domestic varieties into wild populations of European black poplar (*Populus nigra*) by providing a buffer between wild and commercial stands. Strategies such as these can allow evolutionarily valuable native populations to persist in the wild under natural regimes while minimizing the risks of hybridization. Spatial isolation may be a particularly effective tool in freshwater aquatic systems. The downside to this approach is that it results into “park”-style of management in which parentals become restricted to a few isolated portions of the landscape, reducing their ecological and evolutionary relevance. Captive breeding is the most extreme form of this approach. This may be appropriate when culling and related techniques are impractical and risk is high.

With natural systems the optimal solution is addressing the forces altering hybridization dynamics. Habitat degradation facilitates hybridization by unsettling patterns of ecological segregation (Crispo et al., 2011; Hoban et al., 2012; Lamont et al., 2003) and mate choice (Seehausen et al., 2008). In highly social animal species, disrupting social dynamics through human-associated mortality has can increase the rate of interspecific pairing (Bohling and Waits, 2015; Rutledge et al., 2012, 2010b). Managers may be faced with “stabilizing” these

systems by minimizing disrupting forces. Aside from the canid system in southeastern Canada, there are few examples where environmental changes that facilitate hybridization were reversed, meaning more work remains to assess the feasibility of these approaches. Stabilization of hybrid systems is a long-term strategy linked with other proactive conservation efforts designed to improve the prospects of a species, such as habitat restoration or sustainable harvest strategies. It would likely be a preferred option at scales in which culling and monitoring the ancestry of individual organisms are impractical. Stabilization may also be relevant to artificial systems in which human activity either facilitates the advance of the invader and/or decline of the native.

6.4. Examples

Ultimately managing active hybrid systems requires integration of multiple management tools to effectively limit hybridization, as has been shown by the few examples that exist. Two of the most comprehensive management programs have been developed for westslope cutthroat trout in the Flathead River basin of Montana and the critically endangered red wolf population in eastern North Carolina, USA. Cutthroats have been impacted by decades of intermixing with introduced rainbow trout. Beginning in 2006 the Montana Department of Fish, Wildlife, and Parks along with its management partners embarked on a program to remove non-native genetic ancestry from populations in the South Fork Flathead River (MDFWP, 2016). Genetic monitoring facilitated the identification of native populations; waterbodies containing hybrid populations were poisoned. Fish with native genotypes were collected to seed these now fishless systems. Hatchery programs were developed to raise watershed-specific strains of cutthroats for restocking. Native cutthroat strains were also stocked to “swamp” some populations with non-native ancestry. Long-term success is facilitated by the presence of dam downstream that blocks passage of non-native genes from lower portions of the basin. This program has utilized genetic monitoring, culling, captive breeding, de-introgression, and spatial separation as tools to manage this artificial hybrid system. This program is now nearing its conclusion and further data collection is needed to evaluate its effectiveness; however, it serves a model for protecting a species of conservation interest actively threatened by hybridization involving the collaboration of various management partners and scientists from different disciplines.

The red wolf reintroduction program in eastern North Carolina also exploited a variety of tools to manage hybridization with coyotes. Culling was implemented based on modeling exercises suggesting hybridization was an imminent threat and removing coyotes and hybrids could stave off genetic swamping (Fredrickson and Hedrick, 2006; Kelly et al., 1999). Phenotypes, especially for hybrids, are difficult to distinguish. The development of intense trapping regimes, methods to rapidly assess ancestry using molecular genotypes, and coordination between field biologists and geneticists have limited the impact of hybridization (Adams et al., 2007; Gese et al., 2015; Miller et al., 2003; Stoskopf et al., 2005). These efforts have also benefitted from non-invasive genetic sampling facilitating surveys of large spatial scales efficiently (Adams and Waits (2006); Adams et al., 2007). Decisions regarding culling, sterilization, wolf releases, etc. are placed within an adaptive management framework that tests the efficacy of their use. Annual data collection has allowed for the adjustment of policies and procedures in response to changing conditions (Stoskopf et al., 2005).

The red wolf program, with its successes, also presents the most challenging type of scenario for managers. Elimination of coyotes from the red wolf reintroduction site is unlikely because there is no barrier preventing further colonization. Over a decade of intensive management has managed to maintain a genetically distinct red wolf population (Bohling et al., 2016; Gese et al., 2015). However, implementing such management has been resource-intensive and there are doubts as to whether it is an effective long-term strategy (Wildlife Management Institute, 2014) or even desirable (Kyle et al., 2007). This

is the greatest dilemma for the red wolf and similar systems: whether hybridization can ever be limited enough to allow persistence of the parental species. Addressing these situations demands greater discussion among the conservation community, for currently there are no guidelines for the long-term management of active hybrid systems in which one or more parental groups are at serious risk of extinction.

6.5. Massive release of non-native genotypes

A unique twist on active hybrid systems are those involving the mixing between native populations and genotypes massively released into the environment for commercial or recreational purposes. In these situations intermixing is maintained by the steady release of non-native genotypes that swamp native populations (Laikre et al., 2010). In other words, much of the hybridization could be “turned-off” if releases were eliminated. Managers may be in the difficult situation of minimizing the threat of hybridization without the ability to eliminate non-native genotypes, which requires a different suite of tools. The ideal solution would be to keep domestic or commercial strains physically separated from wild populations. In agroforestry, modifying the spatial placement of artificial plantings has been proposed as a strategy to limit introgression into rare native tree stands (Millar et al., 2012). Quantitative approaches incorporating spatial information into introgression probability such as demonstrated by Millar et al. (2012) have the potential to facilitate landscape-level planning that reduces introgression risk.

There are other novel strategies that could limit the potential for introgression in these situations. For example, incredible numbers of hatchery-origin salmon are stocked in the Pacific Northwest, causing concern these fish will interbreed with wild populations. One strategy to reduce the impacts of stocked fish on wild populations is marking hatchery-origin fish, often with a physical mark such as removal of the adipose fin, so they can be recognized by fishers (Naish et al., 2008; Satterthwaite et al., 2015). Regulations will specify that only fish bearing these marks can be harvested; wild-origin fish are released. Despite the wide-scale adoption of this practice along western North America, there has been no theoretical or empirical testing on whether this reduces hybridization between native and hatchery salmon populations. Much more evaluation of “mass-marking” is needed before it can be accepted as a technique that limits hybridization.

If non-native strains are going to be released into the environment, perhaps the most efficient way to limit hybridization is to make sure they are sterile. Crosses between certain species can produce sterile hybrids (e.g. tiger trout [*Salmo trutta* × *Salvelinus fontinalis*], “wiper” bass [*Morone saxatilis* × *M. chrysops*], tiger muskie [*Esox masquinongy* × *E. lucius*]): releasing these crosses can satisfy public demand while eliminating the potential for genetic introgression. Sterile strains can be produced by selective breeding (Piferer et al., 2009). Genetic engineering has already provided strategies to prevent commercial strains of domestic crops (Daniell, 2002; Gressel, 1999) and fish (Wong and Van Eenennaam, 2008) from reproducing. Keeping the gene pools of domestic, commercial, and wild populations separated could benefit from further discussion about the feasibility of such approaches.

7. Conclusion

The challenges posed by hybridization have limited the development of tools and recommendations that can be exploited by managers and policymakers. There are few institutions with formal policies or action plans specifically addressing hybridization. Most that does exist focuses on prevention. For example, the IUCN Reintroduction Specialist Group has recommendations for using native sources for reintroduction and translocation programs (IUCN/SSC, 2013). Review of salmon hatchery practices in the Pacific Northwest of the United States promoted a series of propagation strategies to minimize genetic homogenization of wild salmon populations (Möbrand et al., 2005; Paquet et al., 2011).

A limited number of government agencies and conservation organizations based in Canada and the United States have formal policies regarding hybrid management (Jackiw et al., 2015). Formal adoption of recommendations and policies regarding preventative hybrid management, especially in the context of natural systems, would enhance conservation efforts. Even more pressing is the need to develop guidelines for actually managing systems when hybridization is a conservation concern. Many tactics, such as culling and spatial isolation, have been implemented but there are few recommendations about their utility and feasibility under different scenarios.

To improve our ability to respond to hybridization, the most important step for the management and policy communities is to acknowledge hybridization as a ubiquitous force in nature and adjust programs and policies to reflect this. Integrating hybridization into natural resource management will facilitate the development of relevant monitoring and modeling techniques along with experimentation of strategies designed to control hybridization. This includes characterizing background levels of hybridization and introgression in natural hybrid systems to set baselines and guide management goals. Reaching this point requires integrating principles and experts from a variety of disciplines into the management process, including genetics, evolutionary biology, reproductive biology, and animal behavior. Some of the most comprehensive programs (e.g. American bison, red wolf, cutthroat trout) have been successful for this reason.

Many of the strategies and concepts discussed in this review require further investigation to develop a suite of tools for managing hybrid systems. Examples include the conditions under which strategies such as mass marking, culling, de-introgression, genetic engineering, and spatial management are successful at limiting gene flow. Ultimately large-scale approaches, such as incorporating patterns of reproductive isolation into landscape planning and system stabilizing, hold the most promise for long-term success but require much more assessment in order to produce sound recommendations. Given the rate at which human activity is altering patterns of hybridization across the globe, more thorough vetting of prospective strategies and standardization of techniques is needed.

Acknowledgements

I would like to thank Christian Smith, Linda Rutledge, and three anonymous reviewers for comments that greatly improved the manuscript. The findings and conclusions in this article are those of the author and do not necessarily represent the views of the U.S. Fish and Wildlife Service.

References

Adams, J.R., Waits, L.P., 2006. An efficient method for screening faecal DNA genotypes and detecting new individuals and hybrids in the red wolf (*Canis rufus*) experimental population area. *Conserv. Genet.* 8, 123–131.

Adams, J.R., Lucash, C., Schutte, L., Waits, L.P., 2007. Locating hybrid individuals in the red wolf (*Canis rufus*) experimental population area using a spatially targeted sampling strategy and faecal DNA genotyping. *Mol. Ecol.* 16, 1823–1834.

Allendorf, F.W., Leary, R.F., Spruell, P., Wenburg, J.K., 2001. The problems with hybrids: setting conservation guidelines. *Trends Ecol. Evol.* 16, 613–622.

Allendorf, F.W., Luikart, G., Aitken, S.N., 2013. *Conservation and the Genetics of Populations*. 2nd Edition Wiley-Blackwell Publishing, USA, p. 602.

Amador, C., Hayes, B.J., Daetwyler, H.D., 2014. Genomic selection for recovery of original genetic background from hybrids of endangered and common breeds. *Evol. Appl.* 7, 227–237.

Arnold, M.L., 1997. *Natural Hybridization and Evolution*. Oxford University Press, USA, p. 232.

Barton, N., Hewitt, G., 1985. Analysis of hybrid zones. *Annu. Rev. Ecol. Syst.* 16, 113–148.

Baskett, M.L., Gomulkiewicz, R., 2011. Introgressive hybridization as a mechanism for species rescue. *Theor. Ecol.* 4, 223–239.

Bohling, J.H., Waits, L.P., 2015. Factors influencing red wolf-coyote hybridization in eastern North Carolina. *U. S. A. Biol. Conserv.* 184, 108–116.

Bohling, J.H., Dellinger, J., McVey, J.M., Cobb, D.T., Moorman, C.E., Waits, L.P., 2016. Describing a developing hybrid zone between red wolves and coyotes in eastern North Carolina. *U. S. A. Evol. Appl.* 9, 791–804.

Buktenica, M.W., Hering, D.K., Girdner, S.F., Mahoney, B.D., Rosenlund, B.D., 2013. Eradication of nonnative brook trout with electrofishing and antimycin-A and the response

of a remnant bull trout population. *N. Am. J. Fish. Manag.* 33, 117–129.

Campton, D.E., Kaeding, L.R., 2005. Westslope cutthroat trout, hybridization, and the U.S. Endangered Species Act. *Conserv. Biol.* 19, 1323–1325.

Conner, M.M., Ebinger, M.R., Knowlton, F.F., 2008. Evaluating coyote management strategies using a spatially explicit, individual-based, socially structured population model. *Ecol. Model.* 219, 234–247.

Cranswick, P.A., Hall, C., 2010. Eradication of the Ruddy Duck *Oxyura jamaicensis* in the Western Palaearctic: a review of progress and a revised action plan 2010–2015. *WWT Report to the Bern Convention*, p. 51.

Crispo, E., Moore, J.-S., Lee-Yaw, J.A., Gray, S.M., Haller, B.C., 2011. Broken barriers: human-induced changes to gene flow and introgression in animals: an examination of the ways in which humans increase genetic exchange among populations and species and the consequences for biodiversity. *BioEssays* 33, 508–518.

Daniell, H., 2002. Molecular strategies for gene containment in transgenic crops. *Nat. Biotechnol.* 20, 581–586.

Debeljak, M., Ficko, A., Brus, R., 2015. The use of habitat and dispersal models in protecting European black poplar (*Populus nigra* L.) from genetic introgression in Slovenia. *Biol. Conserv.* 184, 310–319.

DeHaan, P.W., Bernall, S.R., Dossantos, J.M., Lockard, L.L., Ardren, W.R., 2011. Use of genetic markers to aid in re-establishing migratory connectivity in a fragmented metapopulation of bull trout (*Salvelinus confluentus*). *Can. J. Fish. Aquat. Sci.* 68, 1952–1969.

Derr, J.N., Hedrick, P.W., Halbert, N.D., Plough, L., Dobson, L.K., King, J., Duncan, C., Hunter, D.L., Cohen, N.D., Hedgecock, D., 2012. Phenotypic effects of cattle mitochondrial DNA in American bison. *Conserv. Biol.* 26, 1130–1136.

Dratch, P., Gogan, P., 2010. *Bison conservation initiative: bison conservation genetics workshop: report and recommendations*. Natural Resource Report NPS/NRPC/BRMD/NRR-2010/257. Fort Collins, CO, p. 50.

Edwards, D.L., Benavides, E., Garrick, R.C., Gibbs, J.P., Russello, M.A., Dion, K.B., Hyseni, C., Flanagan, J.P., Tapia, W., Caccone, A., 2013. The genetic legacy of Lonesome George survives: giant tortoises with Pinta Island ancestry identified in Galápagos. *Biol. Conserv.* 157, 225–228.

Ellstrand, N.C., Biggs, D., Kaus, A., Lubinsky, P., McDade, L.A., Preston, K., Prince, L.M., Regan, H.M., Roriver, V., Ryde, O.A., Schierenbeck, K.A., 2010. Got hybridization? A multidisciplinary approach for informing science policy. *Bioscience* 60, 384–388.

Fitzpatrick, B.M., Ryan, M.E., Johnson, J.R., Corush, J., Carter, E.T., 2015. Hybridization and the species problem in conservation. *Curr. Zool.* 61, 206–216.

Fredrickson, R.J., Hedrick, P.W., 2006. Dynamics of hybridization and introgression in red wolves and coyotes. *Conserv. Biol.* 20, 1272–1283.

Garrick, R.C., Benavides, E., Russello, M.A., Gibbs, J.P., Poulakakis, N., Dion, K.B., Hyseni, C., Kajdacs, B., Márquez, L., Bahan, S., Ciofi, C., Tapia, W., Caccone, A., 2012. Genetic rediscovery of an “extinct” Galápagos giant tortoise species. *Curr. Biol.* 22, R10–R11.

Gese, E.M., Terletzky, P.A., 2015. Using the “placeholder” concept to reduce genetic introgression of an endangered carnivore. *Biol. Conserv.* 192, 11–19.

Gese, E.M., Knowlton, F.F., Adams, J.R., Beck, K., Fuller, T.K., Murray, D.L., Steury, T.D., Stoskopf, M.K., Waddell, W.T., Waits, L.P., 2015. Managing hybridization of a recovering endangered species: the red wolf *Canis rufus* as a case study. *Curr. Zool.* 61, 191–205.

Gilman, R.T., Behm, J.E., 2011. Hybridization, species collapse, and species reemergence after disturbance to premating mechanisms of reproductive isolation. *Evolution* 65, 2592–2605.

Godinho, R., Llana, L., Blanco, J.C., Lopes, S., Álvares, F., García, E.J., Palacios, V., Cortés, Y., TALEGÓN, J., Ferrand, N., 2011. Genetic evidence for multiple events of hybridization between wolves and domestic dogs in the Iberian Peninsula. *Mol. Ecol.* 20, 5154–5166.

Godinho, R., López-Bao, J.V., Castro, D., Llana, L., Lopes, S., Silva, P., Ferrand, N., 2014. Real-time assessment of hybridization between wolves and dogs: combining non-invasive samples with ancestry informative markers. *Mol. Ecol. Resour.* 15, 317–328.

Gressel, J., 1999. Tandem constructs: preventing the rise of superweeds. *Trends Biotechnol.* 17, 361–366.

Halbert, N.D., Derr, J.N., 2007. A comprehensive evaluation of cattle introgression into US federal bison herds. *J. Hered.* 98, 1–12.

Harbicht, A., Wilson, C.C., Fraser, D.J., 2014. Does human-induced hybridization have long-term genetic effects? Empirical testing with domesticated, wild and hybridized fish populations. *Evol. Appl.* 7, 1180–1191.

Harrison, R.G., 1990. Hybrid zones: windows on evolutionary process. In: Futuyma, D., Antonovics, J. (Eds.), *Oxford Surveys in Evolutionary Biology* 7. Oxford University Press, New York, pp. 69–128.

Hasselman, D.J., Argo, E.E., McBride, M.C., Bentzen, P., Schultz, T.F., Perez-Umphrey, A.A., Palkovacs, E.P., 2014. Human disturbance causes the formation of a hybrid swarm between two naturally sympatric fish species. *Mol. Ecol.* 23, 1137–1152.

Hoban, S.M., McCleary, T.S., Schlarbaum, S.E., Anagnostakis, S.L., Romero-Severson, J., 2012. Human-impacted landscapes facilitate hybridization between a native and an introduced tree. *Evol. Appl.* 5, 720–731.

Irons, K.S., Hara, T.M.O., McClelland, M.A., Pegg, M.A., Ave, N.S., 2002. White perch occurrence, spread, and hybridization in the middle Illinois River, upper Mississippi River system. *Trans. Illinois State Acad. Sci.* 95, 207–214.

IUCN/SSC, 2013. *Guidelines for Reintroductions and Other Conservation Translocations*. Version 1.0. Gland. IUCN Species Survival Commission, Switzerland (viii + 57 pp).

Jackiw, R.N., Mandil, G., Hager, H.A., 2015. A framework to guide the conservation of species hybrids based on ethical and ecological considerations. *Conserv. Biol.* 29, 1040–1051.

Kelly, B.T., Miller, P.S., Seal, U.S., 1999. *Population and Habitat Viability Assessment Workshop for the Red Wolf (*Canis rufus*)*. Conservation Breeding Specialist Group (SSC/IUCN), Apple Valley, MN, p. 93.

Koen, E., Bowman, J., Lalor, J., Wilson, P., 2014. Continental-scale assessment of the hybrid zone between bobcat and Canada lynx. *Biol. Conserv.* 178, 107–115.

- Kovach, R.P., Muhlfeld, C.C., Boyer, M.C., Lowe, W.H., Allendorf, F.W., Luikart, G., 2015. Dispersal and selection mediate hybridization between a native and invasive species. *Proc. R. Soc. B* 282, 20142454.
- Kyle, C.J., Johnson, A.R., Patterson, B.R., Wilson, P.J., White, B.N., 2007. The conspecific nature of eastern and red wolves: conservation and management implications. *Conserv. Genet.* 9, 699–701.
- Laike, L., Schwartz, M.K., Waples, R.S., Ryman, N., GeM Working Group, 2010. Compromising genetic diversity in the wild: unmonitored large-scale release of plants and animals. *Trends Ecol. Evol.* 25, 520–529.
- Lamont, B.B., He, T., Enright, N.J., Krauss, S.L., Miller, B.P., 2003. Anthropogenic disturbance promotes hybridization between *Banksia* species by altering their biology. *J. Ecol.* 16, 551–557.
- Lenormand, T., 2002. Gene flow and the limits to natural selection. *Trends Ecol. Evol.* 17, 183–189.
- Mallet, J., 2008. Hybridization, ecological races and the nature of species: empirical evidence for the ease of speciation. *Philos. Trans. R. Soc. B* 363, 2971–2986.
- Millar, M.A., Byrne, M., Nuberg, I.K., Sedgley, M., 2012. High levels of genetic contamination in remnant populations of *Acacia saligna* from a genetically divergent planted stand. *Restor. Ecol.* 20, 260–267.
- Miller, C.R., Adams, J.R., Waits, L.P., 2003. Pedigree-based assignment tests for reversing coyote (*Canis latrans*) introgression into the wild red wolf (*Canis rufus*) population. *Mol. Ecol.* 12, 3287–3301.
- Mobrand, L.E., Barr, J., Blankenship, L., Campton, D.E., Evelyn, T.T.P., Flagg, T.A., Mahnken, C.V.W., Seeb, L.W., Seidel, P.R., Smoker, W.W., 2005. Hatchery reform in Washington State. *Fisheries* 30, 11–23.
- Montana Fish, Wildlife & Parks, 2016. South Fork Westslope Cutthroat Trout Project. URL <<http://fwp.mt.gov/regions/r1/wctproject/>>.
- Moore, W., 1977. An evaluation of narrow hybrid zones in vertebrates. *Q. Rev. Biol.* 52, 263–277.
- Muhlfeld, C.C., Kalinowski, S.T., McMahon, T.E., Taper, M.L., Painter, S., Leary, R.F., Allendorf, F.W., 2009. Hybridization rapidly reduces fitness of a native trout in the wild. *Biol. Lett.* 5, 328–331.
- Muhlfeld, C.C., D'Angelo, V., Kalinowski, S.T., Landguth, E.L., Downs, C.C., Tohtz, J., Kershner, J.L., 2012. A fine-scale assessment of using barriers to conserve native stream salmonids: a case study in Akokala Creek, Glacier National Park. *U. S. A. Open Fish Sci. J.* 5, 9–20.
- Muhlfeld, C.C., Kovach, R.P., Jones, L.A., Al-Chokhachy, R., Boyer, M.C., Leary, R.F., Lowe, W.H., Luikart, G., Allendorf, F.W., 2014. Invasive hybridization in a threatened species is accelerated by climate change. *Nat. Clim. Chang.* 4, 620–624.
- Muñoz-Fuentes, V., Darimont, C.T., Paquet, P.C., Leonard, J.A., 2010. The genetic legacy of extirpation and re-colonization in Vancouver Island wolves. *Conserv. Genet.* 11, 547–556.
- Naish, K.A., Taylor, J.E., Levin, P.S., Quinn, T.P., Winton, J.R., Huppert, D., Hilborn, R., 2008. An evaluation of the effects of conservation and fishery enhancement hatcheries on wild populations of salmon. *Adv. Mar. Biol.* 53, 61–194.
- Nussberger, B., Wandeler, P., Weber, D., Keller, L.F., 2014. Monitoring introgression in European wildcats in the Swiss Jura. *Conserv. Genet.* 15, 1219–1230.
- Paquet, P.J., Flagg, T., Appleby, A., Barr, J., Blankenship, L., Campton, D.E., Delarm, M., Evelyn, T., Fast, D., Gislason, J., Kline, P., Maynard, D., Mobrand, L., Nandor, G., Seidel, P.R., Smith, S., 2011. Hatcheries, conservation, and sustainable fisheries—achieving multiple goals: results of the Hatchery Scientific Review Group's Columbia River Both Basin and Review should be capitalized. basin review. *Fisheries* 36, 547–561.
- Parker, W.T., 1987. A strategy for establishing and utilizing red wolf populations on islands. Red Wolf Management Series Technical Report No. 2. US Fish and Wildlife Service, Asheville, NC, p. 10.
- Piferrer, F., Beaumont, A., Falguière, J.-C., Flajšhans, M., Haffray, P., Colombo, L., 2009. Polyploid fish and shellfish: production, biology and applications to aquaculture for performance improvement and genetic containment. *Aquaculture* 293, 125–156.
- Poelstra, J.W., Vijay, N., Bossu, C.M., Lantz, H., Ryll, B., Muller, I., Baglione, V., Unneberg, P., Wikelski, M., Grabherr, M.G., Wolf, J.B.W., 2014. The genomic landscape underlying phenotypic integrity in the face of gene flow in crows. *Science* 344, 1410–1414.
- Polziehn, R., Strobeck, C., Sheraton, J., Beech, R., 1995. Bovine mtDNA discovered in North American bison populations. *Conserv. Biol.* 9, 1638–1643.
- Quilodrán, C.S., Montoya-Burgos, J.L., Currat, M., 2015. Modelling interspecific hybridization with genome exclusion to identify conservation actions: the case of native and invasive *Pelophylax* waterfrogs. *Evol. Appl.* 8, 199–210.
- Rand, D., Harrison, R., 1989. Ecological genetics of a mosaic hybrid zone: mitochondrial, nuclear, and reproductive differentiation of crickets by soil type. *Evolution* 43, 432–449.
- Rhymer, J., Simberloff, D., 1996. Extinction by hybridization and introgression. *Annu. Rev. Ecol. Syst.* 27, 83–109.
- Rutledge, L.Y., Garroway, C.J., Loveless, K.M., Patterson, B.R., 2010a. Genetic differentiation of eastern wolves in Algonquin Park despite bridging gene flow between coyotes and grey wolves. *Heredity* 105, 520–531.
- Rutledge, L.Y., Patterson, B.R., Mills, K.J., Loveless, K.M., Murray, D.L., White, B.N., 2010b. Protection from harvesting restores the natural social structure of eastern wolf packs. *Biol. Conserv.* 143, 332–339.
- Rutledge, L.Y., White, B.N., Row, J.R., Patterson, B.R., 2012. Intense harvesting of eastern wolves facilitated hybridization with coyotes. *Ecol. Evol.* 2, 19–33.
- Satterthwaite, W., Anderson, E., Campbell, M.R., Garza, J.C., Mohr, M., Narum, S.R., Speir, C., 2015. Multidisciplinary Evaluation of the Feasibility of Parentage-based Genetic Tagging (PBT) for Management of Pacific Salmon. Pacific Salmon Commission, p. 135.
- Schwartz, M.K., Pilgrim, K.L., McKelvey, K.S., Lindquist, E.L., Claar, J.J., Loch, S., Ruggiero, L.F., 2004. Hybridization between Canada lynx and bobcats: genetic results and management implications. *Conserv. Genet.* 5, 349–355.
- Schwartz, M.K., Luikart, G., Waples, R.S., 2007. Genetic monitoring as a promising tool for conservation and management. *Trends Ecol. Evol.* 22, 25–33.
- Searcy, C.A., Rollins, H.B., Shaffer, H.B., 2016. Ecological equivalency as a tool for endangered species management. *Ecol. Appl.* 26, 94–103.
- Seehausen, O., Takimoto, G., Roy, D., Jokela, J., 2008. Speciation reversal and biodiversity dynamics with hybridization in changing environments. *Mol. Ecol.* 17, 30–44.
- Stoskopf, M., Beck, K., Fazio, B., Fuller, T., Gese, E., Kelly, B., Knowlton, F., Murray, D., Waddell, W., Waits, L., 2005. Implementing recovery of the red wolf integrating research scientists and managers. *Wildl. Soc. Bull.* 33, 1145–1152.
- Väli, Ü., Dombrovski, V., Treinys, R., Bergmanis, U., Daróczy, S.J., Dravecky, M., Ivanovski, V., Lontkowski, J., Maciorowski, G., Meyburg, B.-U., Mizera, T., Zeitz, R., Ellegren, H., 2010. Widespread hybridization between the greater spotted eagle *Aquila clanga* and the lesser spotted eagle *Aquila pomarina* (Aves: Accipitriformes) in Europe. *Biol. J. Linn. Soc.* 100, 725–736.
- Ward, T.J., Skow, L.C., Gallagher, D.S., Schnabel, R.D., Nall, C.A., Kolenda, C.E., Davis, S.K., Taylor, J.F., Derr, J.N., 2001. Differential introgression of uniparentally inherited markers in bison populations with hybrid ancestries. *Anim. Genet.* 32, 89–91.
- Wayne, R.K., Shaffer, H.B., 2016. Hybridization and endangered species protection in the molecular era. *Mol. Ecol.* 81, 778–793.
- Wildlife Management Institute, 2014. A Comprehensive Review and Evaluation of the Red Wolf (*Canis rufus*) Recovery Program. p. 171.
- Wolf, D.E., Takebayashi, N., Rieseberg, L.H., 2001. Predicting the risk of extinction through hybridization. *Conserv. Biol.* 15, 1039–1053.
- Wong, A.C., Van Eenennaam, A.L., 2008. Transgenic approaches for the reproductive containment of genetically engineered fish. *Aquaculture* 275, 1–12.