Hybridization rapidly reduces fitness of a native trout in the wild

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Human-mediated hybridization is a leading cause of biodiversity loss worldwide. How hybridization affects fitness and what level of hybridization is permissible pose difficult conservation questions with little empirical information to guide policy and management decisions. This is particularly true for salmonids, where widespread introgression among non-native and native taxa has often created hybrid swarms over extensive geographical areas resulting in genomic extinction. Here, we used parentage analysis with multilocus microsatellite markers to measure how varying levels of genetic introgression with non-native rainbow trout (*Oncorhynchus mykiss*) affect reproductive success (number of offspring per adult) of native westslope cutthroat trout (*Oncorhynchus clarkii lewisi*) in the wild. Small amounts of hybridization markedly reduced fitness of male and female trout, with reproductive success sharply declining by approximately 50 per cent, with only 20 per cent admixture. Despite apparent fitness costs, our data suggest that hybridization may spread due to relatively high reproductive success of first-generation hybrids and high reproductive success of a few males with high levels of admixture. This outbreeding depression suggests that even low levels of admixture may have negative effects on fitness in the wild and that policies protecting hybridized populations may need reconsideration.

Keywords: fitness; hybridization; introgression; invasive species; reproductive success; cutthroat trout

1. INTRODUCTION

Invasive species are one of the greatest threats to global biodiversity (Vitousek *et al.* 1997). One of the most pernicious threats to native species is hybridization and genetic introgression with exotic taxa (Rhymer & Simberloff 1996). These irreversible changes have contributed to the demise and extinction of many populations and species of plants and animals. Furthermore, hybridization is exacerbated

by habitat modification (Allendorf *et al.* 2001), so this problem will probably become more serious with increasing land use and global climate change. Consequently, effective conservation of native biota can be enhanced by an understanding of the ecological and evolutionary consequences of human-mediated hybridization. Such data for endangered and rare species in nature, however, are limited.

Hybridization is more common in fishes than in any other vertebrate taxa (Leary *et al.* 1995). Many fish species have external fertilization and similar mating behaviours, facilitating interbreeding. In undisturbed ecosystems, reproductive isolation is maintained primarily by spatial and temporal reproductive isolation rather than well-developed pre- and post-mating isolating mechanisms (Mayr 1963). However, such barriers to interbreeding may be lost because of habitat disturbance and introduction of non-native species (Allendorf *et al.* 2001). This is particularly true for salmonids, where widespread introgression among native and non-native taxa has replaced native species over large areas throughout their native ranges (Leary *et al.* 1995). Rainbow trout (*Oncorhynchus mykiss*), the most widely introduced salmonid in the world (Lever 1996), produces fertile offspring when crossed with cutthroat trout (*Oncorhynchus clarkii*), and introgression often continues until a hybrid swarm is formed and the native cutthroat genomes are lost (Allendorf & Leary 1988). A major consequence of such interspecific hybridization may be outbreeding depression due to the break-up of co-adapted gene complexes and disruption of local adaptations (Barton & Hewitt 1989). Introgression poses a serious threat to all 14 subspecies of cutthroat trout in western North America due to widespread stocking and invasion of non-native trout into historical cutthroat trout habitats (Trotter 2008); two subspecies are now extinct, five are listed as threatened under the Endangered Species Act (ESA) and seven have been petitioned for listing.

Interbreeding between westslope cutthroat trout (*Oncorhynchus clarkii lewisi*) with non-native rainbow trout exemplifies the conservation challenges of interspecific hybridization. Westslope cutthroat trout historically occupied aquatic habitats throughout the Columbia, Fraser, Missouri and Hudson Bay drainages of the United States and Canada. However, non-hybridized populations now persist in less than 10 per cent of their historic range, with many restricted to small, fragmented headwater habitats, where their long-term sustainability is uncertain (Trotter 2008). Distinguishing between hybrid and non-hybridized cutthroat trout based on morphology is difficult especially when individual fishes contain low levels of rainbow trout admixture. Owing to this morphological similarity, westslope cutthroat trout populations with less than 20 per cent non-native admixture have been included as part of the native taxon in a recent status review (Department of the Interior (DOI) 2003). The decision to use the 20 per cent threshold continues to be controversial because the potential fitness effects of this amount of admixture are unknown, and the threshold used will influence the decision to list by determining the number of populations classified as part of the native taxon (Haig & Allendorf 2006). Consequently, there

Table 1. GLMM analysis of male and female reproductive success.

factor	coefficient	s.e.	d.f.	t-value	p-value
using fractional allocation[a]					
female intercept	3.8540	0.5730	143	6.7100	0.0000
proportion rainbow trout admixture	−3.3530	0.4500	143	−7.4500	0.0000
sex-M	−3.0300	0.7600	143	−3.9900	0.0001
length	0.0028	0.0018	143	−1.5010	0.1354
sex-M : length	0.0065	0.0026	143	2.5410	0.0121
deleting tied assignments[b]					
female intercept	2.7100	0.6600	143	4.1000	0.0001
proportion rainbow trout admixture	−3.0400	0.4600	143	−6.5600	0.0000
sex-M	−2.7500	0.8500	143	−3.2200	0.0016
length	−0.0004	0.0020	143	−0.1980	0.8432
sex-M : length	0.0053	0.0028	143	1.9100	0.0576

[a]Random effect s.d.: year = 0.1091; individual (residual) = 2.1090.
[b]Random effect s.d.: year = 0.1863; individual (residual) = 1.8695.

is a need to better understand how introgression affects fitness. Here, we describe how a wide range of levels of genetic admixture affect reproductive success of a native trout in the wild.

2. MATERIAL AND METHODS

Mendelian exclusion was used for parentage identification (see the electronic supplementary material) to compare reproductive success of spawning trout in Langford Creek, MT, USA. From 2003 to 2007, we captured 61 females and 124 males migrating upstream in the creek to spawn and 648 emigrating juveniles (see table S1 in the electronic supplementary material). All fish were genotyped at 16 microsatellite loci for parentage analyses, and the proportion of rainbow trout admixture of each fish was estimated using eight diagnostic microsatellite loci (see table S2 in the electronic supplementary material). Based on the most conservative parentage analysis (see the electronic supplementary material), we assigned 397 outmigrating juveniles to a mother, 389 to a father and 149 of these juveniles to both parents. Reproductive success was measured as the number of offspring per parent for each spawning year. The fixed effects of the proportion of rainbow trout admixture, sex, fish length (covariates) and spawning year (random effect) on reproductive success (response variable) were evaluated using generalized linear mixed models (GLMMs) using a natural log-link function with a quasi-Poisson error distribution (see the electronic supplementary material).

3. RESULTS

The GLMM showed that the proportion of rainbow trout admixture among spawning females and males had a strong negative effect on reproductive success (table 1). Reproductive success declined sharply with an increase in the proportion of non-native rainbow trout admixture. A declining exponential regression fitted to the data ($y = 13.039\,e^{-3.040x}$) suggests that reproductive success declines by approximately 50 per cent, with 20 per cent admixture indicating that even small amounts of non-native rainbow trout introgression rapidly reduce reproductive success in later-generation hybrids (figures 1 and 2). Length had no detectable effect on female reproductive success, and only a small, but statistically significant effect on male success (table 1). Furthermore, the small standard deviation of the year random effect indicates that annual fluctuation in reproductive success was minimal (generally less than 25%).

Although there was an exponential decline in fitness with increasing levels of introgression, three males (with 0, 44 and 75% rainbow trout admixture) produced high numbers of juvenile offspring. We
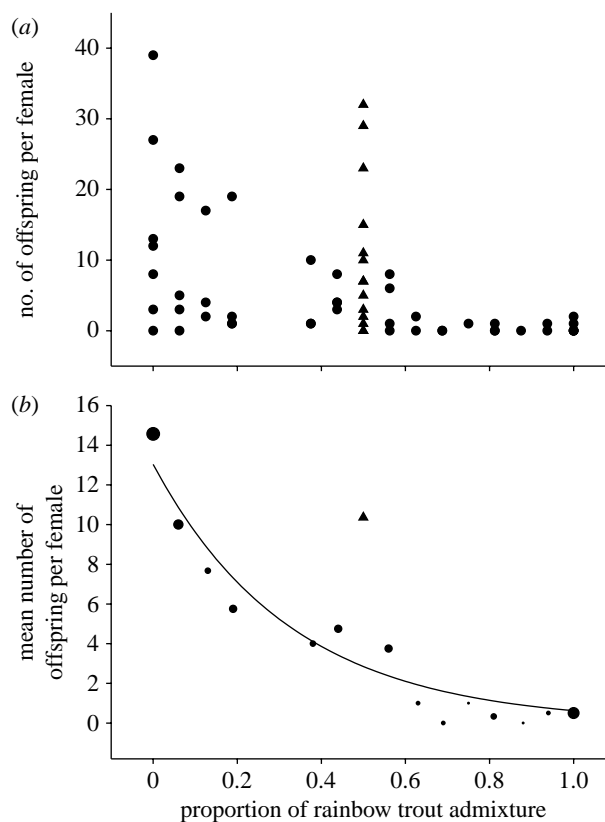

Figure 1. (*a*) Number of offspring per female versus the proportion of non-native rainbow trout admixture. The plot includes 61 mothers and 397 juvenile assignments using parentage analysis. Each point represents an estimate for an individual fish from a spawn year. Circles represent westslope cutthroat trout, rainbow trout and later-generation hybrids, and triangles represent first-generation hybrids. (*b*) Bubble plot of the mean number of offspring per female plotted against the proportion of rainbow trout admixture. The mean value for first-generation hybrids ($n = 14$) is shown as a triangle, but these points were not included in the regression.

considered that males were a mixture of two groups, and separated these three observations in the statistical analysis (see the electronic supplementary material). The two hybrid males had very high reproductive success, comprising a total of 30 per cent of the total offspring assigned. The male with 75 per cent rainbow trout admixture mated with four known non-hybridized
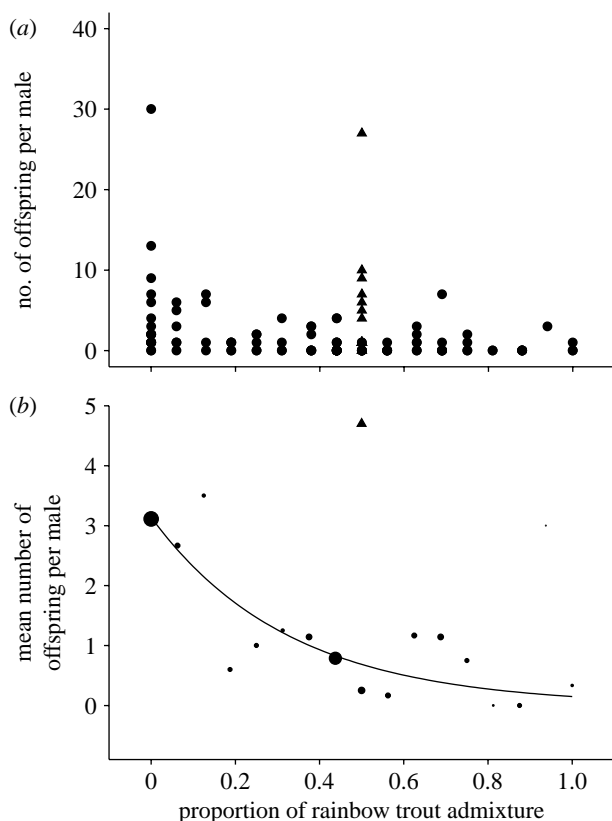
Figure 2. (*a*) Number of offspring per male versus the proportion of non-native rainbow trout admixture. The plot includes 124 fathers and 389 juvenile assignments using parentage analysis. Each point represents an estimate for an individual fish from a spawn year. Circles represent westslope cutthroat trout, rainbow trout and later-generation hybrids, and triangles represent first-generation hybrids. Two hybrids (with 44 and 75% rainbow trout admixture) produced 52 and 64 offspring, respectively, and are not included in the plot. (*b*) Bubble plot of the mean number of offspring per male plotted against the proportion of rainbow trout admixture. The intercept is adjusted for the mean of the sex-M : length interaction term. The mean value for first-generation hybrids ($n = 16$) is shown by a triangle, but these points were not included in the regression.

female cutthroat trout and produced the highest number of assigned juvenile offspring. Together, these data indicate that a few males with a high proportion of rainbow trout genes and high reproductive success are probably promoting the spread of hybridization.

The reproductive success of first-generation (F_1) hybrids was relatively high for both sexes and similar to that of non-hybridized cutthroat trout (figures 1 and 2). This result may be explained by the fact that F_1 hybrids have a maximum amount of genetic variation, and have intact haploid genomes from the parental species.

4. DISCUSSION

Our results present a paradox—genetic introgression reduces fitness but progresses rapidly in most situations, where rainbow trout are introduced into native cutthroat trout habitats. Our data present a partial explanation for this conundrum. First, we observed that F_1 hybrids can have relatively high fitness. This may facilitate subsequent generations of

backcrossing. Second, we observed that a few post-F_1 hybrid males had reproductive success as high as or substantially higher than non-hybridized cutthroat trout. This decreases the efficiency of selection against hybrid genotypes and may be a conduit through which genetic introgression proceeds. Third, hybridization is spreading upstream in the system via continent–island and stepping-stone invasion, and hybrids have higher straying rates than non-hybridized westslope cutthroat trout, further promoting the spread of hybridization (Boyer *et al.* 2008). Finally, even with severe fitness penalties against hybrid progeny, hybrid swarms can still be formed because all progeny of hybrids are hybrids (Epifanio & Philipp 2001).

Several important questions remain unanswered. First, we do not know the life stage(s) at which outbreeding depression occurred. Our estimate of reproductive success spans the stages of gamete production, mating, spawning, egg-to-fry emergence and juvenile survival, and selection could have acted at any or all of these times. Second, the extent to which outbreeding depression may be affected by the environment is unknown. Third, we do not know the genetic mechanism reducing reproductive success. Hybridization can reduce fitness by either introducing alleles to a population that are not suited to the local environment (extrinsic outbreeding depression) or disrupting co-adapted gene complexes (intrinsic outbreeding depression) (Templeton 1986). These mechanisms are not mutually exclusive, and identifying the contribution of each effect is difficult. However, the high reproductive success of F_1 hybrids relative to post-F_1 hybrids with similar amounts of admixture suggests that some of the outbreeding depression is intrinsic. Finally, we do not know the evolutionary fate of hybrid trout populations. If selection is strong enough, it may be able to purge deleterious rainbow trout alleles from hybridized populations, or it may select for combinations of rainbow and cutthroat alleles that have high fitness.

How much hybridization is acceptable to maintain the evolutionary legacy of a species, subspecies or evolutionarily significant unit? This question is important, as the legal status of hybrids is one of the most controversial questions in endangered species law (Allendorf *et al.* 2001; Beaumont *et al.* 2001). We addressed this issue by providing quantitative estimates of the fitness consequences of human-mediated hybridization using parentage assignment. This approach could be readily adapted to other species where level of hybridization has been contentious such as with the red wolf (*Canis rufus*; Fredrickson & Hedrick 2006) and spotted owl (*Strix occidentalis*; Haig *et al.* 2004).

In the USA, currently, there is no official policy for how to classify hybrids under the ESA. The most contentious issue has been establishing a threshold for deciding how much hybridization is permissible for hybrids to be considered part of the native taxon (Haig & Allendorf 2006). To date, federal agencies have addressed this issue on a case-by-case basis, and have relied heavily on morphological data. This ruling assumes that morphological similarity is indicative of

genetic and ecological similarities. Ideally, these decisions should be based on assessing the fitness consequences of different levels of hybridization, especially at low levels. Our results suggest that small amounts of non-native genetic admixture that may not be morphologically detectable may have negative effects on fitness of native trout in the wild and that conservation and policy programmes that protect hybridized populations may need reconsideration.

Our data illustrate that fitness can decline quickly in nature when previously allopatric species interbreed. These results and those of Boyer *et al.* (2008) suggest that remaining non-hybridized cutthroat trout populations may be at greater risk than previously thought. We suspect that non-hybridized populations will continue to be lost if hybrid source populations are not suppressed or eliminated, and that protection of fishes with even low levels of admixture may facilitate continued expansion of hybridization.

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