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Genetic basis of differences in myxospore count between whirling disease-resistant and -susceptible strains of rainbow trout

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ABSTRACT: We used a quantitative genetics approach and estimated broad sense heritability (h^2_b) of myxospore count and the number of genes involved in myxospore formation to gain a better understanding of how resistance to *Myxobolus cerebralis*, the parasite responsible for whirling disease, is inherited in rainbow trout *Oncorhynchus mykiss*. An *M. cerebralis*-resistant strain of rainbow trout, the German Rainbow (GR), and a wild, susceptible strain of rainbow trout, the Colorado River Rainbow (CRR), were spawned to create 3 intermediate crossed populations (an F1 cross, F2 intercross, and a B2 backcross between the F1 and the CRR). Within each strain or cross, h^2_b was estimated from the between-family variance of myxospore counts using full-sibling families. Estimates of h^2_b and average myxospore counts were lowest in the GR strain, F1 cross, and F2 intercross (h^2_b = 0.34, 0.42, and 0.34; myxospores fish^{-1} = 275, 9566, and 45 780, respectively), and highest in the B2 backcross and CRR strain (h^2_b = 0.93 and 0.89; myxospores fish^{-1} = 97 865 and 187 595, respectively). Comparison of means and a joint-scaling test suggest that resistance alleles arising from the GR strain are dominant to susceptible alleles from the CRR strain. Resistance was retained in the intermediate crosses but decreased as filial generation number increased (F2) or backcrossing occurred (B2). The estimated number of segregating loci responsible for differences in myxospore count in the parental strains was 9 ± 5. Our results indicate that resistance to *M. cerebralis* is a heritable trait within these populations and would respond to either artificial selection in hatcheries or natural selection in the wild.

KEY WORDS: *Myxobolus cerebralis* · *Oncorhynchus mykiss* · Parasite · Salmonid · Heritability · Quantitative genetics

INTRODUCTION

Infection with *Myxobolus cerebralis,* the parasite responsible for salmonid whirling disease, is the primary factor causing recruitment failure and population collapses in rainbow trout *Oncorhynchus mykiss* fisheries throughout the Intermountain West, USA (Walker & Nehring 1995, Nehring & Walker 1996, Schisler et al. 1999a,b, Nehring & Thompson 2001). Due to the complex life cycle of the parasite and its durability in the environment, control of the parasite in wild trout populations is problematic. One promising avenue for managing trout populations in the presence of the parasite is the propagation and release of genetically resistant rainbow trout (Price 1985, Schisler et al. 2006).

Intentionally and unintentionally, hatchery managers have increased disease resistance in fish stocks through the continued propagation of survivors of disease as brood stock (Herman 1970); this selection

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process presumably led to the production of hatchery-derived rainbow trout strains that are resistant to *Myxobolus cerebralis*. El-Matbouli et al. (2002) found that, under experimental laboratory conditions, a German strain of rainbow trout (German Rainbow, GR) was at least as resistant to *M. cerebralis* as brown trout *Salmo trutta*, which evolved with the parasite in its European home range (Hoffman 1970). The GR strain was also found to be more resistant to *M. cerebralis* than either the North American Trout Lodge (TL) or Colorado River Rainbow (CRR) trout strains (Hedrick et al. 2003, Schisler et al. 2006). Resistance likely arose through the growth and reproduction of the GR strain under continuous exposure to the parasite in Bavarian hatcheries (Hedrick et al. 2003).

Resistance to disease in animals is often a complex, polygenic trait (Grenfell & Dobson 1995) that results from a series of complex interactions among the host, pathogen, and environment (Snieszko 1974, Hedrick 1998). The mechanisms for resistance to *Myxobolus cerebralis* seen in the GR strain, like those seen in trout resistant to a similar myxosporean, *Ceratomyxa shasta*, are suspected to be polygenic (Hedrick et al. 2001). Studies examining differential gene expression in resistant and susceptible strains of rainbow trout have identified several genes potentially involved in resistance (Severin & El-Matbouli 2007, Baerwald et al. 2008, Severin et al. 2010). Baerwald et al. (2011) discovered a major quantitative trait locus (QTL) influencing resistance that explains between 50 and 86% of the genetic variation relating to resistance in the GR strain, indicating that a single large-effect gene confers the bulk of the resistance in the hatchery-selected strain. However, other minor-effect genes may also be contributing, as at least one other QTL was identified by Baerwald et al. (2011), and a microarray gene expression study found up-regulation of the metallothionein-B gene in the same strain (Baerwald et al. 2008). Until specific genetic and immune mechanisms of resistance are known, we must rely on multiple approaches to explore how resistance is inherited. Such approaches may include selectively breeding disease-resistant populations and using quantitative genetic experiments to measure heritability within fish brood stocks (Price 1985).

Heritabilities are used as a guide for predicting the response to selection when certain individuals are spawned, and how selected traits will change in subsequent generations. Heritability estimates the proportion of phenotypic variation in a population that may be attributed to genetic causes. Statistical and experimental techniques for estimating heritability are well known and are often used in selective breeding programs (Becker 1992, Falconer & MacKay 1996, Lynch & Walsh 1998). The objective of our study was to gain a better understanding of how whirling disease resistance characteristics were inherited in rainbow trout. We used a quantitative genetics approach to estimate broad sense heritability (h^2_b) of myxospore count, a measure of susceptibility to *Myxobolus cerebralis*, and the number of genes involved in myxospore formation (Becker 1992, Lynch & Walsh 1998).

MATERIALS AND METHODS

Brood stock development

In 2004, the Colorado Division of Wildlife (CDOW) began a selective breeding program using the hatchery-selected GR strain and the CRR strain, a wild rainbow trout strain that had been historically used to establish rainbow trout populations in Colorado (Schisler et al. 2006). The CRR strain is susceptible to infection by *Myxobolus cerebralis* and has experienced severe population declines in areas where the disease has been introduced (Nehring & Thompson 2001). Resistant GR fish were crossed with susceptible CRR fish to incorporate whirling disease resistance from the GR strain into a population that retained many of the desired characteristics influencing the ability to survive and reproduce in the wild, which may have been lost in the highly domesticated GR strain (Schisler et al. 2006). Besides being domesticated, the GR strain is inbred and exhibits low heterozygosity (El-Matbouli et al. 2006), which may compromise its survival and adaptation in natural environments. Brood stocks developed during earlier studies were used to create the populations evaluated in this study.

Generation of experimental population

The experimental families examined in our study were spawned at the CDOW Bellvue Fish Research Hatchery (BFRH). Experimental fish included those from the original resistant GR and susceptible CRR parental strains, with crossing between the two to generate an F1 population, intercrosses among F1 fish to create an F2 population, and backcrosses between the F1 cross and CRR strain to create a B2 population. For each parental strain and crossed population, replicate full-sibling families were spawned

from unique male–female pairings to maintain a diverse experimental population. Offspring of each family were kept separate throughout the rearing process.

Eggs were placed in incubators at the BFRH or Colorado Cooperative Fish and Wildlife Research Unit (CFWRU) wet lab and held until they were eyed. Once eyed, eggs were placed in 76 l (20 gallon) flow-through tanks (0.5 l min^{-1}), supplied by a combination of well water and charcoal-filtered municipal water, at the CFWRU wet lab where they were hatched.

Myxobolus cerebralis exposure experiment

Laboratory design

In the exposure experiment, 100 tanks were used, each containing one family. A full-sibling design was chosen to complete both the exposure experiment and to meet other experimental goals for testing physiological responses to parasite exposure (Fetherman et al. 2011). A group of 80 full-sibling families was exposed to *Myxobolus cerebralis* and comprised 10 GR families, 10 CRR families, 20 F1 families, 20 F2 families, and 20 B2 families. The 20 F1 families were spawned from 10 CRR males individually crossed with GR females, and reciprocally, 10 GR males crossed with CRR females. The 20 B2 families were spawned from 10 CRR males individually crossed with F1 females, and reciprocally, 10 F1 males crossed with CRR females. Reciprocal families were included to test for differences in myxospore count following exposure to the pathogen, based on directionality of spawning. F2 intercross families were spawned from F1 males individually crossed with F1 females. As a result of both parents of an F2 intercross family originating from F1 cross fish, reciprocal families could not be created; replicate F2 families were used to equalize sample sizes among experimental populations (20 families each of the F1, F2, and B2 crosses). Due to a restriction on the number of brood stock available for spawning, only 80 total families could be tested. Ten exposed families per strain were needed to calculate heritability (*a priori* power calculation based on an additive genetic model of inheritance; Lynch & Walsh 1998), and space constraints limited the number of tanks that could be accommodated.

Prior to exposure, 4 families from each parental strain or cross were split and used as both exposed and unexposed control families, for a total of 20 unexposed control families. Unexposed families were placed together on the top shelf of a 2-tier shelving unit to avoid potential contamination due to overflow and spills from exposure tanks. Otherwise, exposed and unexposed families were randomly assigned to tanks, with 25 fish $tank^{-1}$, using a random number generator.

Pathogen exposure

In each of the 80 exposure tanks, 25 fish were exposed to *Myxobolus cerebralis* at an average (±SD) of 678 (± 44) degree-days (°C) post-hatch (following Schisler et al. 2006), and an average age of 9.8 (± 1.0) wk post-hatch. Mean individual weights (g) at exposure were 0.67 for GR, 0.49 for CRR, 0.71 for F1, 0.51 for F2, and 0.55 for B2 (Fetherman et al. 2011). The GR, CRR, F2, and B2 did not differ in mean weight at exposure; however, F1 hybrids were significantly larger than the F2, B2, and CRR fish (see Fetherman et al. 2011 for statistical details).

Triactinomyxons (TAMs), the infectious waterborne stage of *Myxobolus cerebralis*, were produced using Mt. Whitney *Tubifex tubifex* (origin, Mount Whitney Fish Hatchery, Lone Pine, California, USA). The concentration of viable TAMs was estimated by mixing 1000 µl of filtrate containing the TAMs and 60 µl of crystal violet; 84.6 µl of this mixture was then placed on a slide and the number of TAMs per slide was counted. Ten counts per filtrate were conducted in this fashion to account for a possible uneven distribution of the TAMs within the filtrate. An average of the 10 counts was taken, and this number was used to calculate TAMs ml^{-1} of culture. Fish were infected with 2000 TAMs $ind.^{-1}$, for a total of 50 000 TAMs $tank^{-1}$ (~715 TAMs l^{-1}).

Prior to the addition of TAMs, water flow to each aquarium was stopped for 1 h, and each aquarium received aeration to ensure mixing of the TAMs and even exposure of all fish. The amount of filtrate needed to deliver 50 000 TAMs was added to each aquarium in 2 doses, each dose containing half of the necessary filtrate. Using 2 doses helped ensure equal distribution of TAMs in the tank and accounted for a possible unequal distribution of TAMs within the filtrate. Variation in propagation of the disease among tanks was not possible because the secondary host (*Tubifex tubifex*) was not present in the experimental system. As a control, 20 tanks, 4 replicate tanks containing a family from each of the 5 parental strains or crosses, were not exposed to the pathogen, but were treated in the same manner as the exposure tanks.

After exposure, fish were reared for approximately 6 mo to ensure the full development of myxospores. During this time, developing signs of disease and mortalities were recorded daily, and the effects of *Myxobolus cerebralis* exposure on growth and swimming performance were also evaluated (Fetherman et al. 2011).

Post-exposure evaluations

Exposure evaluations began when fish reached approximately 2240 (± 38) degree-days (°C) post-exposure. At the time of evaluation, 10 individuals from each tank were removed, sacrificed using an overdose of tricane methanesulfonate (MS-222), and used for myxospore enumeration (O'Grodnick 1975) using the pepsin-trypsin digest method (Markiw & Wolf 1974). The heads were severed from the body just behind the operculum and pectoral fins, placed into individually labeled bags, and sent to the CDOW Brush Fish Health Laboratory in Brush, Colorado, USA, for myxospore enumeration. Lengths (cm) and weights (g) were recorded for each individual at the time of evaluation. Percent mortality occurring between exposure and evaluation was calculated for each parental strain or cross.

Statistical analyses

Statistical analyses were conducted using SAS Proc GLM (SAS Institute 2008). Myxospore counts were analyzed using a 2-factor analysis of variance (ANOVA), with exposure and parental strain or cross as factors. Mortality percentages were arcsine-square root transformed prior to analysis. Transformed values were then analyzed using a 2-factor ANOVA, with exposure and parental strain or cross as factors. Values for all analyses were reported from the Type III sum of squares to account for the unbalanced design of the experiment. If significant main effects were identified ($p \leq 0.05$), a Bonferroni adjusted least squares means method was used to determine which parental strain or cross differed from the others.

Quantitative genetics approach

Heritability

Broad-sense heritability (h^2_b) of myxospore count following exposure to *Myxobolus cerebralis* was calculated using a random, single-pair mating design (Becker 1992) within each unique parental strain (GR, CRR) and crossed population (F1, F2, B2). Single-pair mating generated full-sibling families, and heritability was estimated using covariance in traits within full-sibling families (COV_{FS}). This covariance includes half of the additive variance (V_A), one-fourth of the dominance variance (V_D), and the variance arising from a common environment (V_{EC}; Becker 1992, Falconer & MacKay 1996).

Heritability was estimated from variance components σ^2_w and σ^2_s, calculated from ANOVA as $\sigma^2_w = MS_W$, and $\sigma^2_s = (MS_S - MS_W)/k$ (see Table 1). MS_W is the mean squared error of myxospore counts associated with variance among progeny within families, and MS_S is the mean squared error among families. Both MS_S and MS_W were estimated using ANOVA, with k individuals in each family (Becker 1992). In our case $k = 10$, which, though low, lies within the range of acceptable k for calculating heritability (Lynch & Walsh 1998). Heritability was calculated as $h^2_b = (2\sigma^2_s) / (\sigma^2_w + \sigma^2_s)$, with standard errors and 95% confidence intervals calculated following Becker (1992). Myxospore counts were $\log_{10}$-transformed to normalize the data prior to performing quantitative genetic analyses.

Modes of inheritance

A joint-scaling test (Lynch & Walsh 1998) was used to calculate expected myxospore counts (χ^2 statistic using $\log_{10}$-transformed myxospore count) in each of the crosses and the GR and CRR parental strains based on purely additive (A) and additive-dominance (AD) models of inheritance. Given *a priori* expectations for myxospore count based on a purely additive model, a joint-scaling test determines whether observed values deviate from those expected, and whether the additive or additive-dominance model better describes the mode of inheritance for a given trait (Lynch & Walsh 1998).

The resistant GR strain was expected to exhibit low myxospore count with small variance, resulting in low heritability estimates, because resistant alleles were expected to be nearly fixed in this strain. The CRR strain was expected to exhibit high myxospore counts, also with small variance, because resistance should be minimal based on previous laboratory exposure trials by Schisler et al. (2006). The F1 cross was expected to exhibit low variation and a response intermediate to the GR and CRR strains, because F1 individuals should be heterozygous for resistant

alleles from the GR and susceptible alleles from the CRR. If resistance alleles displayed dominance, however, the myxospore counts in the F1 would be similar to the GR. The F2 intercross population was expected to exhibit a similar average myxospore count as the F1 cross fish, but with higher variance due to segregation and recombination of GR and CRR parental genes among F2 individuals. Finally, the B2 backcross between F1s and CRRs was expected to exhibit intermediate myxospore counts and variance, again due to segregation and recombination of parental alleles.

Number of independently segregating loci

Analysis of line crosses was used to estimate the effective number of segregating loci (n_e) responsible for differences in myxospore counts between the GR and CRR strains. Low n_e values suggest that relatively few genes on relatively few chromosomes underlie the differences, and higher values suggest that genes are spread over many chromosomes (Lynch & Walsh 1998).

Composite effects, which characterize additive and dominance genetic effects responsible for differences between parental strains and crosses, were calculated using the $\log_{10}$-transformed myxospore counts. Composite effects were used to estimate n_e and its standard error using the Castle-Wright estimator,

$$n_e = \frac{[\bar{z}(P_1) - \bar{z}(P_2)] - Var[\bar{z}(P_1)] - Var[\bar{z}(P_2)]}{8Var(S)} \quad (1)$$

where $\bar{z}(P_t)$ are the observed means, $Var[\bar{z}(P_t)]$ are the sampling variances of the means for the ith parental line, and $Var(S)$ is the segregational variance estimate (Lynch & Walsh 1998).

The assumption of independent assortment among loci was tested using haploid chromosome number (N = 29; Hartley & Horne 1982, Thorgaard 1983, Ocalewicz et al. 2004) and genetic linkage maps (Young et al. 1998, Sakamoto et al. 2000, Nichols et al. 2003) for rainbow trout. The average haploid chromosome number of 29 was chosen as representative for the rainbow trout population used in this study; however, due to polymorphism (Thorgaard 1983), haploid chromosome number in rainbow trout is known to vary from this average (Guyomard et al. 2006). The average recombination frequency between loci influencing the trait $\bar{c}$ was estimated to test for independent assortment, using the equation

$$\bar{c} = \frac{M-1}{2M} \quad (2)$$

where M is the haploid chromosome number (29). The maximum value $\bar{c}$ = 0.5 indicates independent assortment, with lower values indicating linkage among genes on a chromosome (Lynch & Walsh 1998).

RESULTS

Exposure metrics

Mean myxospore count differed among parental strains and crosses ($F_{4,793}$ = 61.39, p < 0.001; Fig. 1). CRR individuals had the highest mean myxospore count (187 595 myxospores fish^{-1}), and largest variation in myxospore count (SD = 115 355). Mean myxospore count did not differ among F1 and GR fish (9566 and 275 myxospores fish^{-1}, respectively), as expected if resistance alleles from the GR strain are dominant. GR fish showed the lowest variation in myxospore count (SD = 464; Fig. 1). F2 fish had a significantly higher mean myxospore count (45 780 myxospores fish^{-1}) than the F1 or GR, with more than double the variation in myxospore count (SD = 40 816) than F1 fish (SD = 14 164). As expected if resistance alleles differed between the GR and CRR parental strains, the B2 fish exhibited a higher

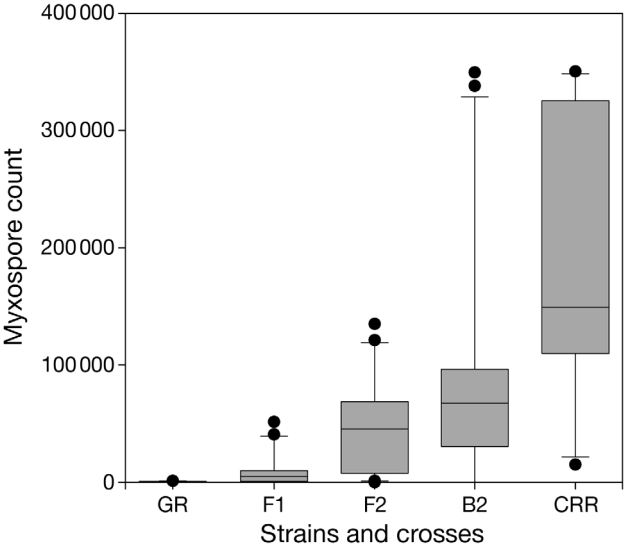

Fig. 1. *Oncorhynchus mykiss*. Myxospore count distribution for the 2 parental strains (German Rainbow, GR; Colorado River Rainbow; CRR) and crossed populations (F1, F2, B2) exposed to *Myxobolus cerebralis*. The box plots display the distribution of the mean myxospore counts per family within a strain or cross, with 10 families represented in the GR and CRR parental strains and 20 families represented in the F1, F2, and B2 crosses. Boxes: 25th and 75th percentiles; bars: 10th and 90th percentiles; dots: outliers

myxospore count (97 865 myxospores fish^{-1}), and larger variation in myxospore counts (SD = 102 362), than the GR, F1, or F2 fish.

Myxospore counts differed among families of the CRR strain and the F1 cross, F2 intercross, and B2 backcross ($p \leq 0.001$). No differences were seen among GR strain families ($p = 0.183$). No differences were seen between reciprocal families of the F1 cross ($p = 0.117$) or B2 backcross ($p = 0.233$), indicating that myxospore count is not influenced by maternal effects or other sex-linked genetic factors. Fish from unexposed control families did not develop myxospores.

Mortality did not differ between exposed and unexposed fish or among parental strains and crosses ($F_{9,88} = 1.38$, $p = 0.210$). Average mortality was low, 2.6% in unexposed control fish and 6.6% in exposed fish (Fetherman et al. 2011).

Quantitative genetics

Heritability

Variation in myxospore count, both within and between families, indicated that broad-sense heritability (h^2_b) was estimable for all strains and crosses. Because reciprocal families in the F1 cross and B2 backcross did not differ (see above), all families of these crosses were used in heritability calculations. The GR strain, and F1 and F2 crosses had relatively low h^2_b estimates, while the B2 backcross had a higher h^2_b estimate (Table 1). Interestingly, the CRR strain h^2_b was also greater than either the F1 or F2 crosses (Table 1).

Table 1. *Oncorhynchus mykiss*. Variance components (σ^2_s and σ^2_w) for $\log_{10}$-transformed myxospore counts, broad sense heritability estimates (h^2_b) of myxospore count as a result of exposure to *Myxobolus cerebralis*, standard errors (in parentheses, representing 2 SE; Becker 1992), and 95% confidence intervals (CI, mean ± 2 SE), for the parental strains (German Rainbow, GR; Colorado River Rainbow, CRR; N = 10 each) and crosses (N = 20 each) of rainbow trout used in the *M. cerebralis* exposure experiment

Strain/cross	σ^2_s	σ^2_w	h^2_b Myxospore count	95% CI
GR strain	0.14	0.68	0.34 (0.21)	(0.13, 0.55)
F1 cross	0.84	3.20	0.42 (0.23)	(0.19, 0.64)
F2 intercross	0.90	4.64	0.34 (0.21)	(0.13, 0.55)
B2 backcross	2.54	2.90	0.93 (0.28)	(0.66, 1.21)
CRR strain	0.33	0.69	0.89 (0.28)	(0.61, 1.17)

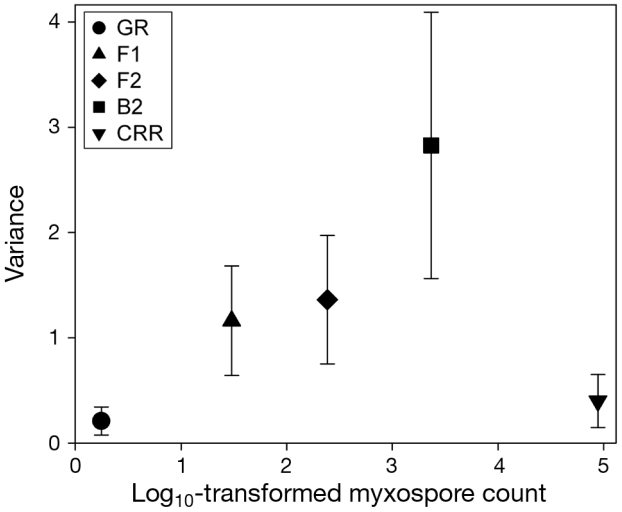

Fig. 2. *Oncorhynchus mykiss.* Line variance (± 2 SE) versus $\log_{10}$-transformed myxospore count for each of the parental strains (German Rainbow, GR; Colorado River Rainbow, CRR) and crosses exposed to *Myxobolus cerebralis*. Notice that the outline of the form made by connecting the variances plotted against myxospore counts should be a triangle with F2 at the apex, but that variance of the B2 cross is greater than expected from a pure additive model, suggesting that dominance genetic variance may have also influenced myxospore counts in this *M. cerebralis* exposure experiment (Lynch & Walsh 1998)

Modes of inheritance

Joint-scaling tests of means and variances of myxospore counts ($\log_{10}$-transformed) in the parental strains and crosses indicated that values did not deviate from the expectations of either additive ($\chi^2_{A, df=3} = 3.19$, $p = 0.36$) or additive-dominance ($\chi^2_{AD, df=2} = 0.19$, $p = 0.91$) modes of inheritance (Fig. 2), and that the 2 modes could not be distinguished from each other ($p = 0.084$).

Number of independently segregating loci

The effective number of factors (n_e) by which the GR and CRR strains differed in relation to myxospore count was 9 ± 5. The estimate of recombination among loci was $\bar{c} = 0.48$ (0.50 is considered independent segregation).

DISCUSSION

Our results indicate that resistance to *Myxobolus cerebralis* is a heritable trait and would respond to selection. Additionally, our joint-scaling analysis and

estimated number of segregating factors suggest that relatively few loci (n_e = 9) are involved in resistance to *M. cerebralis* in the GR strain. The patterns of variation, both within and among the parental strains and intermediate crosses, suggest that resistance alleles from the GR strain are dominant, that differences between the CRR and GR strains are heritable, and that the CRR strain is susceptible, but also genetically variable.

Although the GR strain was expected to have low heritability in myxospore count, because resistance alleles should be fixed, this strain nonetheless showed a significant h^2_b (Table 1). An overestimate of heritability may have arisen because common environmental variation between tanks influenced some families, i.e. tank effects may have arisen as a result of raising a single family in one tank. Although environmental variance can be considered small because environmental conditions, including temperature, water quality, and feed amount, were kept constant for all groups, it still likely influenced the estimates since the environment could not be completely controlled. Heritability estimates in the F1 cross and F2 intercross were similar to the GR strain, but h^2_b was higher in the B2 backcross, suggesting that differences between families also had a genetic basis.

High h^2_b in the CRR strain was unexpected because this strain was expected to show little variation in susceptibility alleles, and all families were expected to exhibit high myxospore counts. High h^2_b indicates that the CRR is more variable for susceptibility alleles than expected and/or has evolved some level of resistance. CRR fish exhibited high variation in myxospore count in other laboratory experiments (Schisler et al. 2006, 2007), again indicating that some individuals have a degree of natural genetic resistance to the parasite. However, in both this and previous experiments, environmental factors may also have contributed to the variation in myxospore count. In the future, we suggest that narrow sense heritability of myxospore count be evaluated for the CRR strain, using a paternal half-sib mating design and larger sample sizes, to determine the additive genetic variance for the resistance trait; this could be coupled with an exposure experiment similar to the one described here to determine whether resistance is increasing in the CRR population as it continues to be exposed to whirling disease.

Overall, it appears that resistance to *Myxobolus cerebralis* can evolve in rainbow trout, either as a response to artificial selection in hatcheries or natural selection in the wild. Resistance in a similar rainbow trout population from Harrison Lake, Montana, USA, has increased in the wild with continued exposure to the parasite (Miller & Vincent 2008). Miller & Vincent (2008) suggested that as more resistant young from the population age and predominate during annual spawning events, it may be possible for the population to return to levels observed prior to parasite establishment. A similar response to selection would be expected in residual CRR populations; however, recovery is expected to be slow, given the low survival of *M. cerebralis*-infected fish in wild CRR populations (Nehring & Thompson 2003). Quicker recovery could be facilitated with the introduction of one of the crosses examined in this study, for example, the F1 cross. If F1 fish were used in reintroductions, spawning between 2 F1 individuals (F2 offspring) would be most likely; however, there is a potential that F1 fish could spawn with residual CRR fish in some areas (B2 offspring). In both cases, our results suggest that offspring should exhibit a range of genetic variability for resistance, and it is expected that those offspring that survive exposure to *M. cerebralis* and recruit to the adult population would be those that exhibit the best combination of survival and resistance characteristics as a result of natural selection.

Development of *Myxobolus cerebralis*-resistant rainbow trout is still being evaluated, but quantitative genetic approaches and selective breeding programs have been used to change population characteristics in several other salmonid species. Challenge tests and selective breeding experiments for resistance to a similar myxosporean parasite of rainbow trout, *Ceratomyxa shasta,* indicated that resistance to *C. shasta* was also heritable (Hemmingsen et al. 1986, Ibarra et al. 1992, 1994), but that the mechanism of resistance is not the same for the 2 parasites (Hedrick et al. 2001). Heritability estimates indicate that selective breeding could facilitate an increase in resistance to salmon louse in Atlantic salmon *Salmo salar* (Kolstad et al. 2005). Selective breeding programs have also been used to increase resistance to furunculosis in brook trout *Salvelinus fontinalis* (Cipriano et al. 2002).

The line-crossing estimate of the number of segregating factors that account for the differences between the CRR and GR strains was n_e = 9 (± 5). In this experiment, the number of freely segregating loci cannot exceed the number of independently segregating chromosomal segments (108; Hartley & Horne 1982, Thorgaard 1983, Ocalewicz et al. 2004), or 2 to 3 times the haploid chromosome number (29; Hartley & Horne 1982, Thorgaard 1983, Ocalewicz et al. 2004). Our estimate of average recombination

between randomly-selected loci ($\bar{c}$ = 0.48) confirms independent segregation and that we are not likely underestimating the number of genes underlying resistance to *Myxobolus cerebralis* in rainbow trout; however, conclusions could change depending on haploid chromosome number used, if different from the average (Guyomard et al. 2006). Additionally, male rainbow trout have a lower mean rate of recombination in relation to females (e.g. Baerwald et al. 2011), thus the estimate of n_e may be low. This is not likely to have a large effect in this experiment, as the estimate of n_e (9) is much lower than the number of chromosomes in rainbow trout (29).

Our estimate of loci involved in resistance is also complementary to the previous study of Baerwald et al. (2011), who found one major QTL on chromosome Omy9 that explained 50 to 86% of the variance in resistance in 4 F2 mapping families derived from crosses between GR and CRR. We discuss 4 reasons that a single major gene probably underestimates the total genetic variation in resistance in rainbow trout. First, a candidate resistance gene identified in other studies of resistance to *Myxobolus cerebralis* does not generally map to the chromosomal region Omy9 (Baerwald et al. 2011; and see below). Second, trout tested by Baerwald et al. (2011) were at a younger age than ours, so the major gene may be especially influential through that stage of life, with additional genes having an effect later on. Third, the QTL study examined 4 F2 mapping families in depth (Baerwald et al. 2011). The greater number of families in our study (80) may have uncovered additional genetic variation that did not segregate in the 4 families in the QTL study. Fourth, significant variation among families (and h^2_b) in the parental CRR strain, and among families of the F1 cross, F2 intercross, and B2 backcross, further point to the presence of additional genetic variation even within the CRR parental strain. A single large-effect gene may be conferring the bulk of the resistance, but our estimates indicate that other minor-effect genes also contribute.

Genetic mechanisms of resistance to *Myxobolus cerebralis* in rainbow trout continue to be investigated, with some evidence that transforming growth factor beta (Severin & El-Matbouli 2007), metallothionein B (Baerwald et al. 2008), immune-regulatory genes arginase-2, and inducible nitric oxide synthase (Severin et al. 2010) may be involved. Our results suggest that these factors are part of an additive genetic mechanism of resistance to *M. cerebralis* in rainbow trout involving many minor-effect genes, and that a continued search for alleles involved in resistance is likely to be informative. Price (1985) suggested that once genotypes that confer resistance have been identified, measures to increase their frequency in the populations could be implemented, but cautions that extrapolating results from one environment to others may fail because genotypes may not confer high fitness in the same manner in different environments.

CONCLUSIONS

Resistance characteristics are important traits when considering rainbow trout population reestablishment and brood stock development. The low heritability of myxospore count of the GR strain indicates that introgressing genetic variation from the GR into wild populations may succeed in reestablishing and managing wild rainbow trout sport-fish populations where they were extirpated by whirling disease. Lower myxospore counts should also result in a reduction of the overall myxospore load in the drainages where these fish are introduced. Our and others' results suggest that F1 cross fish may be the best candidates for reintroducing rainbow trout because they may have an initial combination of resistance and wild rainbow trout characteristics that would favor survival in the wild (Schisler et al. 2006, 2007). The effects of exposure to *Myxobolus cerebralis* on the performance of important survival-related characteristics on the GR strain and F1 cross, the survival and reproductive characteristics of GR and F1 fish in a natural setting, and the survival and resistance characteristics of wild-produced offspring need further evaluation.

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