Journal of Aquatic Animal Health
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/uahh20

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Published online: 28 Feb 2014.

To cite this article: Maureen K. Purcell, Jeffrey J. Hard, Kathleen G. Neely, Linda K. Park, James R. Winton & Diane G. Elliott (2014) Genetic Variation in Bacterial Kidney Disease (BKD) Susceptibility in Lake Michigan Chinook Salmon and Its Progenitor Population from the Puget Sound, Journal of Aquatic Animal Health, 26:1, 9-18

To link to this article: http://dx.doi.org/10.1080/08997659.2013.860061

Journal of Aquatic Animal Health 26:9–18, 2014
American Fisheries Society 2014
ISSN: 0899-7659 print / 1548-8667 online
DOI: 10.1080/08997659.2013.860061

ARTICLE

Genetic Variation in Bacterial Kidney Disease (BKD) Susceptibility in Lake Michigan Chinook Salmon and Its Progenitor Population from the Puget Sound

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Abstract

Mass mortality events in wild fish due to infectious diseases are troubling, especially given the potential for long-term, population-level consequences. Evolutionary theory predicts that populations with sufficient genetic variation will adapt in response to pathogen pressure. Chinook Salmon *Oncorhynchus tshawytscha* were introduced into Lake Michigan in the late 1960s from a Washington State hatchery population. In the late 1980s, collapse of the forage base and nutritional stress in Lake Michigan were thought to contribute to die-offs of Chinook Salmon due to bacterial kidney disease (BKD). Previously, we demonstrated that Lake Michigan Chinook Salmon from a Wisconsin hatchery have greater survival following BKD challenge relative to their progenitor population. Here, we evaluated whether the phenotypic divergence of these populations in BKD susceptibility was due to selection rather than genetic drift. Comparison of the overall magnitude of quantitative trait to neutral marker divergence between the populations suggested selection had occurred but a direct test of quantitative trait divergence was not significant, preventing the rejection of the null hypothesis of differentiation through genetic drift. Estimates of phenotypic variation (V_P), additive genetic variation (V_A) and narrow-sense heritability (h^2) were consistently higher in the Wisconsin relative to the Washington population. If selection had acted on the Wisconsin population there was no evidence of a concomitant loss of genetic variation in BKD susceptibility. The *Renibacterium salmoninarum* exposures were conducted at both 14°C and 9°C; the warmer temperature accelerated time to death in both populations and there was no evidence of phenotypic plasticity or a genotype-by-environment (G × E) interaction. High h^2 estimates for BKD susceptibility in the Wisconsin population, combined with a lack of phenotypic plasticity, predicts that future adaptive gains in BKD resistance are still possible and that these adaptive gains would be stable under the temperature range evaluated here.

It is now recognized that infectious diseases can have an impact on the population dynamics of fish in the Great Lakes (Holey et al. 1998). Ecological perturbations can shift the relationship between host and pathogen resulting in increased incidence or severity of disease (Hedrick 1998), which can lead to large mortality events. Dramatic die-offs are troubling, but the long-term, population-level consequences of selection may be of more significance. Evolutionary theory predicts that

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Received October 21, 2013; accepted October 24, 2013

populations with sufficient genetic variation will adapt in response to pathogen-driven selection (Spielman et al. 2004). In addition to genetic variability, phenotypic plasticity is an important contributor to adaptive potential, especially over the short term. Phenotypic plasticity is the ability of an animal of a particular genotype to respond to changing conditions by altering its phenotype through physiological or behavioral adjustments (Falconer and MacKay 1996). The ability to respond and adapt to a changing environment is critical to the long-term sustainability of any population.

Introductions of fall-run (ocean-type) Chinook Salmon *Oncorhynchus tshawytscha* eggs from Puget Sound, Washington, into the Lake Michigan basin began around 1966 (Hansen and Holey 2002). As early as 1967, there were reports of bacterial kidney disease (BKD) caused by the gram-positive bacterium *Renibacterium salmoninarum* in Lake Michigan Chinook Salmon and other Pacific salmon species (MacLean and Yoder 1970). Chinook Salmon density in Lake Michigan increased over the following decades reaching its peak density in the mid-1980s. In the late 1980s, a collapse of the Chinook Salmon's primary prey item, the Alewife *Alosa pseudoharengus*, led to severe nutritional stress, which likely precipitated large fish kills due to BKD (Holey et al. 1998; Hansen and Holey 2002). Previously, we reported that the contemporary population of Chinook Salmon from a Lake Michigan hatchery in Wisconsin had greater survival following *R. salmoninarum* challenge relative to several Pacific Northwest populations, including the documented progenitor population from Green River, Washington (Purcell et al. 2008; Metzger et al. 2010). One hypothesis to account for this phenotypic divergence in BKD susceptibility is selection imposed by the Lake Michigan BKD epizootics or other factors encountered in the novel environment of the Great Lakes (Purcell et al. 2008). If true, a possible trade-off to the fitness gains achieved by selection can be a reduction in the overall genetic variation at the trait, thereby limiting future evolutionary potential (Roff 1997).

Although the previous laboratory challenge studies conducted on the Wisconsin and Washington populations documented a phenotypic difference in BKD susceptibility, these studies lacked sufficient families and individuals to confirm a genetic rather than environmental basis for this difference. In the present study, we used sets of full- and half-sibling families to evaluate phenotypic and genetic variation for two traits associated with BKD susceptibility (mortality and days to death) in both the Washington and Wisconsin populations. Additionally, we evaluated the hypothesis that selection was responsible for the phenotypic difference between the populations. We performed the disease challenges at two different water temperatures to also assess whether there was significant phenotypic plasticity in these traits. Temperature was chosen as the environmental parameter to evaluate plasticity because it strongly influences the physiological systems of poikilothermic animals (Brett 1956) and can dramatically alter the host–pathogen relationship by affecting the replication of the pathogen and kinetics of the host's immune response (Le Morvan et al. 1998). The results presented here provide insight into potential for future adaptive changes in BKD susceptibility in the Wisconsin Chinook Salmon population.

METHODS

Broodstock and creation of full-sibling families.—Gametes were collected from adult Chinook Salmon returning to the Strawberry Creek weir, Wisconsin, (Wisconsin Department of Natural Resources) or Soos Creek Hatchery, Washington, (Washington Department of Fish and Wildlife) on October 12, 2009, and September 30, 2009, respectively. Kidney and ovarian fluids were collected from spawning females; kidney samples were screened for *R. salmoninarum* antigen by double-polyclonal antibody enzyme-linked immunosorbent assay (ELISA II) (Pascho et al. 1991) and ovarian fluid samples were screened for *R. salmoninarum* cells by the membrane-filtration fluorescent antibody test (MF-FAT) (Elliott and Barila 1987).

Creation of full- and half-sibling families.—Because infection of progeny by vertical transmission of *R. salmoninarum* from female parents would confound experimental results, eggs were only used from females with *R. salmoninarum* negative kidney ELISA values and low ovarian fluid ELISA values (negative optical density cutoff was defined as two SDs above the mean of the negative control sample). Approximately 20% percent of the females used did have very low levels of detectable *R. salmoninarum* cells in the ovarian fluid as measured by MF-FAT. Milt from 20 Washington and 20 Wisconsin Chinook Salmon males was used to fertilize eggs from 40 Washington and 40 Wisconsin females, respectively, to initially create 80 families in a paternal half-sibling nested breeding design (one male : two females). The Wisconsin eggs were thiamine-treated during water hardening with 750 mg/L thiamine hydrochloride (Sigma-Aldrich) buffered to neutral pH with sodium bicarbonate (Sigma-Aldrich) for 2 h, to ameliorate thiamine deficiency that occurs in this stock. The Wisconsin and Washington eggs were disinfected with 100 mg/L iodophore (Argent Chemicals) for 15 min. The fertilized eggs were reared at the Western Fisheries Research Center (WFRC) in Seattle, Washington, using sand-filtered and ultraviolet-light-treated freshwater. All animal experiments were approved by the WFRC Institutional Animal Care and Use Committee (IACUC protocol 2008-08).

PIT tagging.—A total of 61 full-sibling families (36 Washington and 25 Wisconsin families) survived in sufficient numbers to a size suitable for tagging with PIT tags; 6,512 progeny fish representing these families were tagged over a 3-d period starting on May 4, 2010, (approximately 6 months posthatch) at a mean size of 7.2 g. Tagged fish representing both populations were pooled and transferred to common rearing tanks for the final grow-out phase. As a prophylactic precaution, fish were fed oxytetracycline-medicated feed (3% Terramycin, BioOregon) before and after tagging (for a total of 21 d).

Bacterial challenge.—Prior to *R. salmoninarum* challenge, a total of 30 tagged fish were sampled and kidney tissues were tested by ELISA and real-time quantitative PCR (qPCR; Chase et al. 2006, as modified by Elliott et al. 2013) to ensure that fish were negative for *R. salmoninarum* antigen or nucleic acids. PIT-tagged progeny were divided into two groups and acclimated to challenge temperatures of either 14°C or 9°C over a 2-week period. The *R. salmoninarum* intraperitoneal (i.p.) injection challenges were initiated approximately 2 months posttagging and challenges were conducted as previously described (Purcell et al. 2008). For the 14°C challenge, a total of 1,912 tagged progeny were each given an injection of *R. salmoninarum* strain ATCC 33209 suspended in 100 μL of 0.01 M phosphate-buffered saline (PBS), pH 7.4, containing 0.1% (v/v) peptone (PBS–peptone). After injection, the fish were divided among three replicate 276-L tanks (Table 1). For a sham challenge, another 630 fish were each injected with 100 μL PBS–peptone, placed in a single replicate tank, and held at 14°C. For the 9°C challenge, a total of 1,943 fish were injected with *R. salmoninarum*, as described above, and distributed among three replicate tanks; another 636 fish were injected for the sham challenge and placed in a single replicate tank. The injection dose of *R. salmoninarum* strain 33209 was estimated initially by MF-FAT counts. Final plate counts on KDM2 agar medium (Evelyn 1977) indicated the dose for both challenges was 9.4×10^6 CFU/fish. Unpaired *t*-tests were used to test for differences in mortality and mean days to death between the two populations (SPSS version 18; SPSS, Chicago).

Genetic analyses.—The statistic Q_{ST} is analogous to the F_{ST} statistic commonly used in population genetics to partition the total genetic variation observed in two or more populations into the portion attributable to the among-population component and the portion attributable to the within-population component. Similarly, Q_{ST} estimates the genetic component of phenotypic differentiation among two or more populations in one or more quantitative characters and was estimated by:

$$Q_{ST} = \sigma^2_{GB}/\left(\sigma^2_{GB} + 2\sigma^2_{GW}\right),$$

where $\sigma^2_{GB}$is the additive genetic variance among populations and σ^2_{GW} the additive genetic variance estimated within populations (Wright 1951; Spitze 1993).

Additive genetic variances and narrow-sense heritabilities of the two traits, mortality ("death") and days to death ("day"), were estimated from the combined pedigree–phenotype data for this study using an animal model. An animal model is a form of generalized linear mixed model (Bolker et al. 2009) that explicitly incorporates the breeding value of each individual (i.e., an individual's contribution to the trait phenotype in a population, measured as the deviation of its relatives from the

TABLE 1. Summary of results from sham and *Renibacterium salmoninarum* (*Rs*) challenges in Chinook Salmon by temperature. Mortality was monitored until 97 d postinfection. A total of 2,542 fish were challenged and held at 14°C, and 2,579 were challenged and held at 9°C. CPM = cumulative percent mortality; CPS = cumulative percent survivors; *Rs* average (shown in bold text) represents the mean ± SD of the three replicate *R. salmoninarum* tanks for each population and temperature.

Population	Temperature (°C)	Treatment	Replicate tank	Total fish	Number of mortalities (CPM)	Number of survivors (CPS)	Mean days to death
Washington	14	Sham		413	12 (3%)	401 (97%)	38.7
		Rs	Rep 1	416	376 (90%)	40 (10%)	52.5
			Rep 2	397	369 (93%)	28 (7%)	53.2
			Rep 3	366	349 (95%)	17 (5%)	54.9
			***Rs* average**		**93% ± 2%**	**7% ± 2%**	**53.5 ± 1.2**
Wisconsin	14	Sham		217	45 (21%)	172 (79%)	33.9
		Rs	Rep 1	217	188 (87%)	29 (13%)	51.8
			Rep 2	234	206 (88%)	28 (12%)	55.0
			Rep 3	282	245 (87%)	37 (13%)	56.7
			***Rs* average**		**87% ± 1%**	**13% ± 0.7%**	**54.5 ± 2.5**
Washington	9	Sham		441	3 (1%)	438 (99%)	21.0
		Rs	Rep 1	430	390 (91%)	40 (9%)	68.6
			Rep 2	400	361 (90%)	39 (10%)	70.0
			Rep 3	329	294 (89%)	35 (11%)	69.6
			***Rs* average**		**90 ± 1%**	**10% ± 1%**	**69.4 ± 0.7**
Wisconsin	9	Sham		195	16 (8%)	179 (92%)	57.4
		Rs	Rep 1	225	189 (84%)	36 (16%)	67.2
			Rep 2	250	216 (86%)	34 (14%)	70.7
			Rep 3	309	268 (87%)	41 (13%)	70.7
			***Rs* average**		**86% ± 1%**	**14% ± 1%**	**69.5 ± 2.0**

population mean) as a random factor to provide an estimate of a trait's genetic variance and heritability, or genetic covariance and correlation between traits (Wilson et al. 2010).

A full-probability Bayesian approach as implemented in the R package MCMCglmm ("Markov Chain Monte Carlo generalized linear mixed models": Hadfield 2010) was applied to the data to evaluate the phenotypes in the breeding design (a mixture of half- and full-sibling families distributed between the two populations). The variance components underlying each trait were estimated using a Markov chain Monte Carlo (MCMC) method incorporating a specific prior distribution to evaluate the posterior distribution. For mortality, the prior was based on a partitioning of the phenotypic variance between genetic and residual effects using the chi-square distribution; for days to death, the prior was based either on the inverse Wishart distribution or the Cauchy distribution, but incorporating parameter expansion (see Hadfield 2010). The days-to-death trait was analyzed as a continuous (Gaussian) trait, and mortality was analyzed as a threshold (binary) trait. A threshold trait is defined as a trait that is expressed as two or more discrete phenotypes (mortality or survival) but is governed by an underlying, usually hidden, quantitatively distributed state.

Variance components and heritabilities were estimated separately for each population, accounting for temperature (9°C or 14°C) as a fixed factor, and challenge tank as a random factor. This analysis therefore applied an animal model of the form:

$$y_{ijk} = \mu + a_i + t_j + v_k + e_{ijk},$$

where y_{ijk} is the phenotype for trait y in individual i, μ is the population mean, a_i is the breeding value of individual i (the contribution of i to the distribution of y relative to μ), t_j is the fixed effect of temperature j, v_k is the random effect of tank k, and e_{ijk} is the residual term associated with y_{ijk}. Heritabilities of mortality and days to death were also estimated separately within each temperature treatment using simpler models to evaluate whether the estimates were different between the temperature treatments.

Variance, covariance, and heritability estimates were made from the animal and residual ("units") effect variances using single Markov chains 600,000–1,500,000 iterations long, with 200,000–300,000 burn-in iterations, and thinning rates of 1 in 1,000. Gelman–Rubin diagnostics were inspected to ensure that lag autocorrelation and chain convergence were sufficient in each analysis. The 95% credible (highest posterior density [HPD]) intervals for each estimate were obtained from the posterior densities.

Unpaired t-tests were used to test for differences in body weight between the two populations. Correlations between body weight at tagging, mean day to death, and mortality were assessed by examining the pairwise phenotypic correlations (r_P) and by estimating the genetic correlations (r_G) through the correlations of mean family values (r_M). The r_P coefficients were estimated with the Hmisc package in R. P-values were adjusted for multiple comparisons by the Holm–Bonferroni correction method (Holm 1979). The r_M values were estimated by determining the variances and covariances of the three traits (weight at tagging, mortality, and days to death) using conventional quantitative genetic methods (Via 1984; Roff and Preziosi 1994).

To evaluate phenotypic plasticity in mortality and days to death, several analyses were conducted. For a given trait, phenotypic plasticity typically manifests itself as a genotype's differential phenotypic expression in distinct environments. At the family level, reaction norms—the family-specific relationships between phenotype and environment—were first inspected to evaluate whether the patterns were parallel or not. This was followed by a more direct test. Each trait expressed at the two different temperatures was considered a separate trait; for both mortality and days to death, this permits a test for the presence of genotype × environment (G × E) interaction within each population. For each trait, if the same genotype has differential phenotypic expression at different temperatures, the genetic variances at the two temperatures are expected to differ significantly and the genetic correlation between them is expected to be less than +1 (McAdam and Boutin 2003; Charmantier and Garant 2005).

RESULTS

Do the Washington and Wisconsin Populations Differ in BKD Susceptibility?

Minimal mortality (<2%) was observed after tagging. Kidney tissue samples taken from progeny of Washington and Wisconsin stocks ($n = 30$ per stock) prior to challenge tested negative for *R. salmoninarum* antigen and DNA by ELISA and qPCR screening, respectively. The onset of mortality and mean days to death following *R. salmoninarum* challenge were earlier in the 14°C challenge relative to the 9°C challenge (Figure 1). Cumulative percent mortality (CPM) in the sham-challenged fish ranged from 1% to 21% depending on stock and temperature (Table 1). The sham-challenged Wisconsin fish held at 14°C experienced a higher level of mortality early after challenge (3–20 d postinjection [dpi]) that was not associated with obvious clinical signs. One explanation for this early mortality was handling stress, but early mortality was not observed in the groups injected with *R. salmoninarum* and subjected to equivalent handling. Alternatively, the elevated mortality in the sham-treatment tank may have been associated with a tank effect, since only a single replicate tank was used for the sham challenge. In the *R. salmoninarum* challenged groups held at 14°C, onset of mortality associated with clinical BKD signs was 17 dpi and the CPM in the Washington and Wisconsin stocks was 93% and 87%, respectively (Figure 1A; Table 1). In the *R. salmoninarum* challenged groups held at 9°C, onset of mortality associated with clinical BKD signs was 28 dpi and the CPM in the Washington and Wisconsin stocks was 90% and 86%, respectively (Figure 1B; Table 1). Although the magnitude of CPM difference

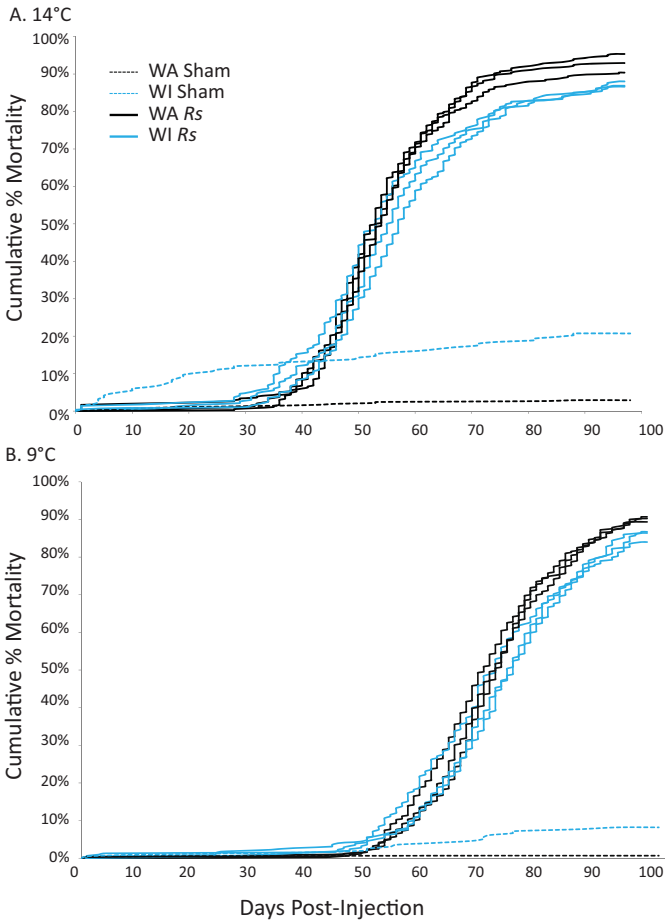

FIGURE 1. Cumulative percent mortality of sham-infected and *Renibacterium salmoninarum* (*Rs*)-infected Chinook Salmon from Washington (WA) and Wisconsin (WI) populations held at either (**A**) 14°C or (**B**) 9°C. A single replicate tank held the sham-infected fish while three replicate tanks were used for the *R. salmoninarum*-infected fish.

between the populations was small, the results were remarkably consistent, with Washington progeny experiencing higher mortality in all three replicate tanks relative to Wisconsin progeny for each temperature treatment (Figure 1). The final CPM was significantly different between the Washington and Wisconsin stocks at 14°C ($t_4 = 3.4$, $P = 0.03$) and at 9°C ($t_4 = 4.9$, $P = 0.008$), while mean days to death did not differ significantly between stocks at either temperature (Table 1).

Is the Difference in BKD Susceptibility Due to Past Selection or Genetic Drift?

To test the competing hypotheses of selection versus drift, we compared estimates of F_{ST} based on "neutral" variation within and between populations and Q_{ST} based on phenotypic variation within and between populations (Lynch and Walsh 1998; Whitlock 2008). Higher values of Q_{ST} relative to F_{ST} are expected if selection is acting to diversify populations at the phenotypes under analysis, while lower values of Q_{ST} relative to F_{ST} strongly imply that diversifying selection is not acting on the same phenotypes. Previously, we estimated F_{ST} at a value of 0.0075 between Green River (Washington) Chinook Salmon returning in 1998, 2003, and 2004 and Wisconsin Chinook Salmon returning in 2003 and 2005 using a set of 13 DNA microsatellite loci (Purcell et al. 2008). Here, we estimated Q_{ST} at 0.280 from the additive genetic variance for the combined traits of mortality and days to death; this estimate was not significantly different from zero ($F_{1,2} = 0.776$, $P > 0.25$). The larger Q_{ST} value relative to the F_{ST} estimate suggested that the two populations were more divergent with respect to the two quantitative traits studied here than at the neutral microsatellite loci. However, given that the estimate of Q_{ST} was not significantly different from zero, we cannot definitively conclude that past selection has acted to produce the differing response to infection by *R. salmoninarum* in the two populations.

Is There a Genetic Basis for the Variation in BKD Susceptibility?

We estimated the narrow-sense heritability (h^2) of two traits related to *R. salmoninarum* susceptibility, mortality and days to death, using an animal model that incorporated temperature as a fixed effect and tank as a random effect. The estimate of h^2 based on mortality was not significant in the Washington population but was significant in the Wisconsin population (Table 2). The

TABLE 2. Estimates of narrow-sense heritability (h^2) and genetic variation (V_A) for mortality and days to death in Chinook Salmon from Washington and Wisconsin challenged with *Renibacterium salmoninarum*. Estimates were obtained from a Bayesian full probability animal model, conditioned on temperature (9°C or 14°C) as a fixed effect and tank as a random effect. Significant h^2 and V_A estimates are shown in bold text. 95% credible highest posterior density (HPD) intervals are shown for each estimate.

Temperature	Population	h^2 mortality (95% HPD)	h^2 days to death (95% HPD)	V_A mortality (95% HPD)	V_A days to death (95% HPD)
Combined (fixed effect)	Washington	0.22 (0.00–0.39)	**0.15 (0.09–0.23)**	0.17 (0.00–0.65)	**0.02 (0.01–0.03)**
	Wisconsin	**0.69 (0.54–0.84)**	**0.31 (0.20–0.58)**	**1.67 (0.92–4.17)**	**0.05 (0.03–0.13)**
14°C only	Washington	0.01 (0.00–0.43)	<0.01 (0.00–0.01)	0.01 (0.00–0.80)	<0.01 (0.00–0.69)
	Wisconsin	**0.71 (0.50–0.85)**	<0.01 (0.00–0.01)	**1.65 (0.83–4.54)**	<0.01 (0.00–1.30)
9°C only	Washington	0.01 (0.00–0.39)	<0.01 (0.00–0.01)	0.01 (0.00–0.65)	<0.01 (0.00–0.76)
	Wisconsin	**0.61 (0.38–0.81)**	<0.01 (0.00–0.01)	**1.13 (0.31–3.84)**	<0.01 (0.00–1.32)

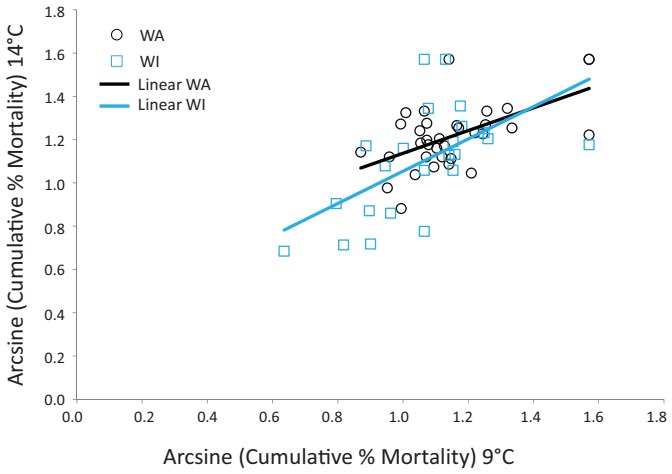

FIGURE 2. Association between cumulative percent mortality at 97 d postinjection of full-sibling Chinook Salmon families from Washington (WA) and Wisconsin (WI) populations infected with *Renibacterium salmoninarum* and held at either 9°C or 14°C.

estimates of h^2 based on days to death were significant in both the Washington and Wisconsin populations (Table 2). These estimates indicated a genetic basis for BKD susceptibility in the Wisconsin population and strongly suggested that the same is true for the Washington population.

Are There Similar Levels of Genetic Variation at Traits Associated with BKD Susceptibility?

We observed that the h^2 estimates for mortality and days to death were consistently higher in the Wisconsin population relative to the Washington population. As evidenced in Figure 2, we also observed higher levels of phenotypic variation (V_P) in the Wisconsin families in the form of a greater range in mean CPM values among the Wisconsin families at both temperatures relative to the Washington families. Narrow-sense heritability (h^2) is calculated as the fraction of that phenotypic variation (V_P) that is due to additive genetic variation (V_A) (Lynch and Walsh 1998). Thus, it is useful to compare V_A estimates independent of V_P to assess whether the two populations possess different levels of genetic variation. Use of an animal model that incorporated temperature as a fixed effect and tank as a random effect showed significant estimates of additive genetic variance (V_A) for mortality for the Wisconsin population but not for the Washington population (Table 2). Estimates of V_A based on days to death were significant but small for both populations (Table 2).

Are the Two Measures of BKD Susceptibility Controlled by the Same Genes?

We estimated both phenotypic (r_P) and additive genetic (r_A) correlations between mortality and days to death in both populations using a bivariate animal model with the estimates conditioned on temperature as a fixed effect and tank as a random effect. For Washington Chinook Salmon, mortality and days to death were negatively correlated at the phenotypic level ($r_P = -0.87$; 95% HPD interval, -0.90 to -0.84); these traits were also negatively correlated genetically, based on the correlations of family means ($r_M = -0.35$ at 9°C, $P < 0.05$; $r_M = -0.84$ at 14°C, $P < 0.05$). A similar pattern was observed in the Wisconsin fish ($r_P = -0.89$; 95% HPD interval, -0.92 to -0.82; $r_M = -0.41$ at 9°C, $P > 0.05$; $r_M = -0.79$ at 14°C, $P < 0.05$). The magnitudes of the negative correlations among family means for these traits indicated that the same genes were likely influencing both traits within each population.

Is BKD Susceptibility Correlated with Body Weight?

Weight of the Washington and Wisconsin Chinook Salmon at the time of PIT tagging was 7.2 ± 1.5 g (mean ± SD) and 7.2 ± 2.1 g, respectively, and did not differ significantly between the populations (*t*-test: $P = 0.50$). We estimated both phenotypic (r_P) and genetic family correlation (r_M) between weight at tagging and either mortality or days to death in both populations at both temperatures (Table 3). At the phenotypic level, there was no significant correlation between weight and mortality in the Washington population at either temperature, but a significant negative correlation was observed in the Wisconsin population at both 14°C ($r_P = -0.10$) and 9°C ($r_P = -0.09$) (Table 3). There was also a significant positive phenotypic correlation between weight and days to death in the Washington stock at 14°C ($r_P = 0.15$) and in the Wisconsin stock at both temperatures (14°C: $r_P = 0.20$; 9°C: $r_P = 0.28$). Thus, at the phenotypic level, greater body weight was associated with lower mortality in the Wisconsin population and was associated with increased time to death in both the Washington and Wisconsin populations. However, at the genetic level (r_M), there were no significant correlations between body weight at tagging and mortality or days to death (Table 3). These results indicated that

TABLE 3. Correlations between body weight of Chinook Salmon at time of tagging and mortality and time to death at the phenotypic level (r_P) and among family means (r_M). Values in bold text indicate significance ($P < 0.05$) after correction for multiple comparisons.

Temperature	Population	r_P mortality	r_P days to death	r_M mortality (95% CI)	r_M days to death (95% CI)
14°C	Washington	0.03	**0.15**	0.04 (−0.30–0.37)	0.02 (−0.32–0.36)
	Wisconsin	**−0.10**	**0.20**	−0.01 (−0.44–0.42)	0.09 (−0.35–0.50)
9°C	Washington	−0.01	0.09	−0.05 (−0.38–0.30)	0.15 (−0.20–0.47)
	Wisconsin	**−0.09**	**0.28**	−0.08 (−0.48–0.35)	0.24 (−0.20–0.60)

different genes were likely influencing body weight and BKD susceptibility.

Is There a Significant Effect of Temperature on Phenotypic and Genetic Variation?

Overall, higher water temperature (14°C) accelerated disease mortality relative to 9°C in both populations in a similar fashion, as evidenced in Figure 1. The mean family CPM at 9°C versus 14°C was significantly associated in both the Washington ($F_{1, 32} = 16.2$, $P = 0.0003$) and Wisconsin populations ($F_{1, 21} = 10.79$, $P = 0.0035$) (Figure 2). There was a trend towards a steeper slope in the Wisconsin population (slope = 0.76; 95% CI, 0.28–1.24) relative to the Washington population (slope = 0.53; 95% CI, 0.26–0.80), which suggests a less proportional response in the Washington fish. However, there was no significant direct or interactive effect of population on the relationship between mortality at the two different temperatures (population: $F_{1, 53} = 0.59$, $P = 0.45$; population × temperature: $F_{1, 53} = 0.82$, $P = 0.37$).

The h^2 estimates for mortality in the Washington population as well as the h^2 estimates for days to death in both populations were not significantly different from zero within each temperature environment (Table 2). The coefficients for this factor (lower to upper bounds of 95% HPD interval) were 0.13 (−0.65 to 0.89) in the Washington population and −0.01 (−0.61 to 0.72) in the Wisconsin population. Similarly, temperature had no detectable effect on the heritability of days to death in either population with coefficients of −0.06 (−0.33 to 0.22) in Washington fish and −0.13 (−0.37 to 0.34) in Wisconsin fish. Estimates of V_A based on mortality were significant in the Wisconsin population but not the Washington population within each temperature treatment (Table 2). Thus, the results were relatively similar whether we calculated across both temperature environments or within each temperature treatment, supporting the conclusion that temperature has no significant effect on the patterns of the disease response due to genetic variation.

Finally, we applied a general linear model incorporating an interaction effect between family and temperature to test for a genotype by environment (G × E) interaction for mortality or days to death. In the Washington Chinook Salmon population, the interaction between family and temperature for both mortality and days to death was not significant ($F_{33, 3116} = 0.81$, $P > 0.75$) and a similar pattern was observed for the Wisconsin population ($F_{22, 1872} = 0.21$, $P > 0.40$). Thus, we concluded that there was no evidence of a G × E interaction, indicating that the traits controlling susceptibility to *R. salmoninarum* responded in a predictable manner in both temperature environments.

DISCUSSION

This study demonstrated that Chinook Salmon from the representative Washington and Wisconsin populations, derived from the 2009 brood year, have differential mortality following *R. salmoninarum* challenge. These results were consistent with our previous challenge studies conducted on the 2003 and 2005 brood years of the same populations (Purcell et al. 2008), as well as the 2008 brood year (M. K. Purcell, unpublished data). Although the difference in overall mortality between the two populations was small, the response of each population was remarkably consistent, with the Wisconsin progeny experiencing lower mortality than the Washington progeny across all three "common garden" replicate tanks. The use of a "common garden" experimental design that mixed the populations in common tanks provided a more rigorous test of differential survival than our previous studies that placed each population into separate challenge tanks. Although the Wisconsin population consistently displayed lower mortality than the Washington population in all challenge trials conducted over the years, the magnitude of the difference between the populations has fluctuated over time. For instance, the difference in CPM between the populations was 50% in 2003, 20% in 2005, 24% in 2008 (Purcell, unpublished data), and 6% in the present study. Direct comparison of challenge studies across brood years must be done cautiously because the experimental designs varied from year to year. Additionally, the conditions used in the present study resulted in a more virulent challenge than in previous studies, as both populations experienced very high mortality. Despite these caveats, the results showed a trend towards a narrowing of the phenotypic difference between the Washington and Wisconsin populations over the 6-year testing period.

Previously, we hypothesized that selection, possibly related to the Lake Michigan BKD epizootics or other circumstance encountered in the novel environment of the Great Lakes, was responsible for the phenotypic divergence between the Washington and Wisconsin populations (Purcell et al. 2008). The point estimate of Q_{ST} based on the BKD susceptibility traits was an order of magnitude higher than the estimate of F_{ST} based on microsatellite markers. The higher Q_{ST} value strongly suggested that directional selection contributed to the phenotypic divergence in BKD susceptibility. However, a Q_{ST} estimate that was not significantly different from zero was obtained in the current study, preventing rejection of the null hypothesis that stochastic factors were the primary mechanism responsible for population differentiation. More direct and powerful methods exist to estimate the Q_{ST} parameter and its relationship to F_{ST} (Ovaskainen et al. 2011; Karhunen and Ovaskainen 2012), but these newer methods require individual genotyping of all individuals within an analysis, which was cost prohibitive for the present study.

The ability of a population to adapt is a function of the additive genetic variation present in the population. Here, narrow-sense heritability (h^2) was estimated by two traits associated with BKD susceptibility (mortality and time to death) to gain insights into the adaptive potential of these two populations. Previous estimates of h^2 of disease susceptibility in Chinook Salmon populations have varied widely in magnitude. A study of Columbia River Chinook Salmon reported h^2 estimates of 0.89 for BKD mortality and 0.35 for days to death (Hard et al. 2006), whereas a study of a British Columbia Chinook Salmon population estimated h^2 of BKD mortality (as a continuous trait) at 0.10

(Beacham and Evelyn 1992a). In contrast, a study comparing three different British Columbia Chinook Salmon populations reported varying estimates for h^2 of BKD mortality, assessed as a continuous trait, at 0.00, 0.42, and 0.66 (Beacham and Evelyn 1992b). Interestingly, there were also wide phenotypic differences in BKD susceptibility among these British Columbia populations with observed cumulative mortality reported as 6.9, 29.4, and 42.1% (Beacham and Evelyn 1992b). Our h^2 estimates based on mortality (or days to death) of 0.22 (0.15) for Washington and 0.69 (0.31) for Wisconsin populations are within the range reported by others. The results support the conclusion that additive genetic variation exists within the Wisconsin Chinook Salmon population for *R. salmoninarum* susceptibility and further adaptation could be possible by artificial or natural selection. The results were more equivocal for the Washington population as the h^2 estimates were not always consistently different from zero, despite having a greater number of families represented in the overall study.

Evolutionary theory that focuses on single traits predicts that strong and constant directional selection may result in overall loss of variation at the trait, leading to lower estimates of h^2 for that trait (Falconer and MacKay 1996). Here, we observed higher levels of both genetic and phenotypic variation for BKD susceptibility in the Wisconsin population relative to the Washington fish. Although seemingly contradictory to our hypothesis that the Wisconsin population experienced directional selection towards decreased BKD susceptibility, many empirical studies have found that high levels of genetic variance are maintained at quantitative traits despite strong selection (Crow 2008). Maintenance of this variation may be due to a constantly changing environment, such as that of the Great Lakes, in which a phenotype that is favored in one generation may not be optimal for the next. Natural fish populations are exposed to a milieu of potential pathogens that are likely physically or temporally patchy and, depending on the host's defense mechanisms involved, resistance to one pathogen may or may not be correlated with resistance to other pathogens. For example, resistance to the salmonid bacterial disease furunculosis was positively correlated at the genetic level with susceptibility to two viral diseases in Atlantic Salmon *Salmo salar* (Drangsholt et al. 2011) and with susceptibility to BKD (Gjedrem and Gjoen 1995); however, no genetic correlation in disease susceptibility was observed for Chinook Salmon between BKD and furunculosis (Beacham and Evelyn 1992b) or between BKD and vibriosis (Hard et al. 2006).

The lower levels of phenotypic and genetic variation for BKD susceptibility traits in the Washington Chinook Salmon population were unanticipated. One explanation for this result may have been the severe challenge conditions used in the study that resulted in over 90% mortality in the Washington progeny. The bacterial challenge dose may have overwhelmed differences among the hosts' defense mechanisms in this more susceptible population and compressed variation as the mortality curves approached their maximum value of 100%. A lower h^2 estimate is indicative of reduced adaptive potential for BKD survival, which suggests the Washington population is more vulnerable if the relationship with *R. salmoninarum* shifts due to changes in the pathogen or environment. However, there is a long coevolutionary history between Chinook Salmon and *R. salmoninarum* in the Pacific Northwest (Fryer and Lannan 1993) and previous studies have found a lack of phenotypic or genetic diversity among *R. salmoninarum* isolates worldwide (Bruno and Munro 1986; Starliper 1996; Grayson et al. 1999). Since 2003, we have screened adults returning to the Soos Creek Hatchery in Washington for selected years and we have not encountered fish with significant levels of *R. salmoninarum* antigen at spawning (Purcell, unpublished data), although clinical BKD is sometimes observed in juvenile fish (Chase et al. 2006). Furthermore, the Soos Creek Hatchery does not employ a BKD-specific management program that is commonly used in Columbia River basin hatcheries culturing Chinook Salmon (Munson et al. 2010). Like other Puget Sound Chinook Salmon populations, the Washington population likely encounters *R. salmoninarum* at various stages during their life cycle. For instance, prevalence of *R. salmoninarum* in Puget Sound Chinook Salmon populations in the estuarine environment was estimated to be as high as 67% in 2006, but the bacterial levels encountered were not levels typically associated with disease (Rhodes et al. 2006). Understanding the implications of the lower h^2 estimate for the Soos Creek Hatchery population would be best approached by comparing this population with other Puget Sound Chinook Salmon populations using a less virulent challenge dose.

There is a need to better understand how species will adapt to a changing environment resulting from altered habitats or climate change. Nearly all organisms have a physiological optimal range of temperatures beyond which acclimatization or adaption becomes difficult. In the present study, temperature altered the course of *R. salmoninarum* infection resulting in an earlier time to death in the fish held at 14°C relative to those at 9°C, and both the Washington and Wisconsin population responded in a similar fashion to the temperature shift. This is consistent with previous studies that found higher temperatures resulted in increased mortality and earlier time to death (Jones et al. 2007). However, lower mortality in Coho Salmon *O. kisutch* occurred following *R. salmoninarum* challenge in 15°C water relative to that in 12°C (Sanders et al. 1978), even though in vitro culture studies suggest the optimal replication temperature for *R. salmoninarum* is 15°C (Smith 1964). Overall, because we did not find evidence of phenotypic plasticity in either the Washington or Wisconsin population, or evidence for a genotype by environment interaction involving temperature, we predict that any adaptive gains in BKD resistance due to selection would be expressed equally across the range of temperatures from 9°C to 14°C.

A recent retrospective analysis of salmonid pathogen surveys conducted in the state of Michigan reported decreased *R. salmoninarum* prevalence and intensity between 2001 and 2010 (Faisal et al. 2012). This prevalence drop was likely due to the introduction of an integrated fish health management approach

that included methods to systematically control both vertical and horizontal transmission of *R. salmoninarum* in Lake Michigan salmon populations. Although our BKD challenge studies have been confined to a single population that returns to the Strawberry Creek weir in Sturgeon Bay, Wisconsin, historical records and genetic markers suggest that all Lake Michigan Chinook Salmon stocks were derived from the same Washington progenitor population (Weeder et al. 2005). However, more empirical study is required to establish whether all Chinook Salmon populations within Lake Michigan also have enhanced BKD resistance relative to the Washington State progenitor stock. It is tempting to speculate that the decrease in *R. salmoninarum* prevalence in Lake Michigan might have been associated with the narrowing of the phenotypic difference between the Wisconsin and Washington progenitor populations we observed in our laboratory studies conducted over the past decade. Unfortunately, our results were not sufficient to assess the relationship between these two observations.

Following the Chinook Salmon declines between 1988 and 1992 due to the forage fish collapse and associated BKD epizootics, some facilities imported eggs from elsewhere in the Great Lakes for restocking purposes. For the Strawberry Creek population, no outside stocks were imported, and the survivors of BKD epizootics that returned to the weir continued to be spawned. Based on tag returns and fin clips, there is very little straying into Strawberry Creek from other Wisconsin stocking locations or other states (S. Marcquenski, Wisconsin Department of Natural Resources, personal communication). The results presented here support the decision made for this population, as we observed higher BKD resistance in the Wisconsin population relative to the progenitor population, possibly due to selection, but no evidence of a concomitant loss of additive genetic variation. Previous studies have also found no evidence of reduced genetic variation at neutral markers in the Sturgeon Bay, Wisconsin, population (Purcell et al. 2008) or in other Michigan Chinook Salmon populations (Weeder et al. 2005) relative to the progenitor population. The high h^2 estimates for BKD susceptibility traits observed in the Wisconsin population, combined with a lack of phenotypic plasticity or G $\times$ E interaction, predict that future adaptive gains in BKD resistance are still possible and that these adaptive gains would be stable under the range of temperatures evaluated here.

ACKNOWLEDGMENTS

Funding for this study was provided by the Great Lakes Fisheries Trust (Project 2008.966), U.S. Geological Survey, and National Oceanic and Atmospheric Administration Fisheries Service. The authors acknowledge James C. Woodson, Samantha Badil, Taylor Alton, Rachel L. Thompson, Connie McKibben, Carla Conway, Orlay Johnson, and Jim Myers for assistance. Fish were provided by Susan Marcquenski (Wisconsin Department of Natural Resources) and Mike Wilson (Washington Department of Fish and Wildlife). Doug Marsh and Brian Beckman (Northwest Fisheries Science Center) kindly provided PIT tags for use in this study. The use of trade, firm, or corporation names in this publication is for the information and convenience of the reader. Such use does not constitute an official endorsement or approval by the U.S. Department of Interior, U.S. Geological Survey, U.S. Department of Commerce, or National Oceanic and Atmospheric Administration Fisheries Service of any product or service to the exclusion of others that may be suitable.

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