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2010-2011 Annual Report

INFLUENCE OF ENVIRONMENT AND LANDSCAPE ON SALMONID GENETICS

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ABSTRACT/SUMMARY

Environmental and landscape features can greatly contribute to population structure, life history diversification, and adaptation of salmonids. This ongoing project combines two studies from the Fish & Wildlife Program Accords with the following objectives: 1) Environment & Landscape Genetics – Evaluate genetic structure of natural populations of salmonids relative to their environment and identify candidate markers associated with traits that are related to adaptation of steelhead and Chinook salmon populations; and 2) Controlled Experiments – laboratory/hatchery experiments with controlled environmental variables to validate phenotypic response of fish with given genotypes.

During the performance period of July 1, 2010 to June 30, 2011, work was completed that addresses components of both Objective 1 and Objective 2. This included both empirical and theoretical approaches. For Objective 1, extensive analyses of landscape genetics of both Chinook salmon and steelhead were evaluated throughout the Columbia River Basin and were included in peer-reviewed publications (Blankenship et al. 2011; Matala et al. 2011). For Objective 2, theoretical work (Section 1) was done to compare common methods that are used to identify candidate markers under selection (Narum et al. 2011a) and empirical work was done to further advance our understanding multiple traits related to recovery of salmonids in the Columbia River. Investigations during the past performance period focused on thermal tolerance in *O. mykiss* (Section 2). However, preparations were also made for future studies to further investigate thermal tolerance, smoltification, and disease resistance in *O. mykiss*, and adult run-timing in Chinook salmon (Section 3).

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Introduction

Environmental and landscape features can greatly contribute to the population structure, life history diversification, and local adaptation of organisms in aquatic habitats (reviewed in Storfer et al. 2006). Geographic barriers to dispersal include recent events that may have been human induced (e.g., dams) as well as ancient events such as glaciations and formation of mountain chains (e.g., Castric et al. 2001). However, other environmental characteristics such as elevation, temperature, forest cover, and precipitation may influence distribution, adaptation, and gene flow of species (Dionne et al. 2008; Narum et al. 2008). For example, the geographic distributions of species ranges' are often determined by thermal tolerance (Brannon et al. 2004) and may necessitate adaptations for survival in extreme environments (Rodnick et al. 2004).

Screening with many genetic markers provides the opportunity to investigate local adaptation in natural populations and identify candidate genes under selection (Beaumont and Nichols 1996; Beaumont and Balding 2004; Excoffier et al. 2009). This has become a commonly employed approach in ecological and population genetics studies to detect outlier loci that are putatively under selection (e.g., Vasemagi and Primmer 2005; Nosil et al. 2008). Additionally, correlation methods can be highly informative to identify markers in coding and cis-regulatory regions of known functional genes that are associated with specific selective pressures or phenotypes (Lyman and Mackay 1998; Chase et al. 2009; Torgerson et al. 2009). With increasing genomic information available for non-model organisms, single nucleotide polymorphisms (SNPs) have begun to see increased use as genetic markers for population genetic studies (e.g., Morin et al. 2004). These sequence polymorphisms are densely scattered throughout the genome of most organisms, and are commonly observed in both coding and non-coding regions of functional genes making them ideal markers to study adaptive molecular variation (e.g., Akey et al. 2002). In a large suite of unlinked SNPs that are distributed across the genome (e.g., Campbell et al. 2009), it is possible to utilize both functionally neutral and adaptive markers within a single study. This combination of information provides a powerful approach to study questions in ecological genetics since both demographic processes (i.e., gene flow and genetic drift) and local adaptation (i.e., selection) may be inferred.

While candidate markers under selection can be used to address local adaptation in natural populations, the inclusion of neutral markers also provides the opportunity to evaluate gene flow among populations in relationship to geological or environmental barriers. The study will also provide resources for evaluating the maintenance of biologically relevant genetic diversity in hatcheries. A variety of statistical models have been developed to address specific questions related to genetic structure due to environment and landscape features (reviewed in Manel et al. 2003; Storfer et al. 2006). For example, ordination models with canonical correspondence analysis have been used as an alternative to Mantel tests to simultaneously evaluate drainage, altitude, and human impacts to genetic diversity of salmonid fishes (Angers et al. 1999; Costello et al. 2003). In this study, we plan to apply these approaches to better understand environmental genetics of steelhead and Chinook salmon in the Columbia River.

Report Structure

This report is divided into three sections. The first section reports on a theoretical evaluation of methods to identify candidate markers under selection, the second section reports on thermal adaptation, and the third section provides brief summaries for future work on each of the traits of interest in the study including thermal tolerance, smoltification, disease resistance, and run-timing.

SECTION 1: Theoretical Evaluation of Outlier Methods to Detect Candidate Markers Under Selection

Introduction

When a phenotype is known and quantified in study organisms, association studies can evaluate selection on genetic variation at a given locus (e.g., Chase *et al.* 2009). Alternatively, as shown in recent studies of human populations (e.g., Akey 2009), genome scans and outlier approaches can be effective at identifying genes under selection without known phenotypes. The benefits of outlier analyses include the ability to screen numerous markers in genome scans to identify candidate genes for further investigation. However, there are several challenges to address with outlier approaches including genotyping errors (Xue *et al.* 2009), population stratification and false positives (Pritchard and Rosenberg 1999; Price *et al.* 2006; Zhang *et al.* 2008; Excoffier *et al.* 2009), variation in mutation rate (Hedrick 2005), and limited sensitivity (false negatives; Kelley *et al.* 2006; Hermisson 2009). Further, candidate outlier loci often vary in pairwise population comparisons (e.g., Nosil *et al.* 2008) and therefore overall divergence (global F_{ST}) may not detect candidates that are under selection in only a portion of populations (Vitalis *et al.* 2001). Outlier methods are also well known to have limited power in detecting balancing selection (Beaumont & Balding 2004) and various forms of weak divergent selection (i.e., relaxed selection; Wachowiak *et al.* 2009).

One of the main features of outlier tests is the use of global F_{ST} and relative level of total heterozygosity to discern if a portion of the genetic markers in a study have higher values of genetic distance than expected from a neutral distribution. Therefore, global F_{ST} values have a major role in outlier tests, with markers that display the greatest values of F_{ST} often being identified as outliers and candidates for divergent selection (regardless of heterozygosity). However, demographic scenarios (i.e., bottlenecks) and hierarchical genetic structure can result in numerous false positive loci in outlier tests (Excoffier *et al.* 2009) and the outlier program implemented in Arlequin v.3.5 was designed to address this issue (Excoffier & Lischer 2010). However, additional scenarios remain to be investigated to more fully understand the limitations of various outlier tests, for example, evaluating sensitivity of outlier tests to detect markers experiencing differing strengths of selection, and ability to identify markers under selection when some complexity in the adaptive landscape is introduced. The latter scenario is relatively common to observe in natural systems where habitats and their respective local selective pressure is heterogeneously distributed with regards to a network of metapopulations. For example, selection on temperature tolerance in redband trout occupying a network of streams that experience a range of temperatures across desert-montane environment (Narum *et al.* 2010), or coat color variation in pocket mice inhabiting both dark- and light- rocky environments (Nachman *et al.* 2003). This type of complex adaptive landscape is in contrast with a more simplistic scenario in which a highly dispersive species occupies relatively continuous habitat and experiences clinal selection gradients (e.g. selection on coloration in European barn owls, Antoniazza *et al.* 2010; temperature tolerance along a latitudinal gradient for walleye Pollock, Canino *et al.* 2005).

In this study, we evaluated a wide variety of outlier tests to detect candidate loci under selection in four simulated data sets. Four methods were evaluated in this study including F_{ST} histograms (e.g., Luikart *et al.* 2003), FDIST2 (Beaumont and Nichols

1996), BAYESCAN (Foll & Gaggiotti 2008), Arlequin v.3.5 (Excoffier *et al.* 2009). Four sets of simulated data were generated to represent both strong and weak forms of divergent selection under the following two scenarios of one-dimensional, stepping-stone models of isolation-by-distance (IBD) gene-flow: 1) the range of phenotypic values of a quantitative trait under selection were assigned optimal fitness in a way that reflected a clinal selection gradient such that adaptive variation across populations matched the neutral variation influenced by IBD gene-flow, or 2) phenotypic values of a quantitative trait under selection were assigned optimal fitness randomly across populations such that adaptive variation contrasted the neutral variation influenced by IBD gene-flow. The strong and weak forms of divergent selection were intended to evaluate outlier test sensitivity to varying strength of selection, and randomization of which phenotypic value was optimal across populations added complexity to the adaptive landscape. Simulated data sets included 95 neutral and 5 selected markers in 10 populations. The various outlier tests were evaluated based on the relative levels of Type I (false positive) and Type II (false negative) error rates of each method for the four simulated scenarios.

Methods

Simulated data were used to evaluate four different outlier methods to detect candidate markers under selection. Simulations were completed with the program QUANTINEMO (Neuenschwander et al. 2008) in order to evaluate both Type I and Type II error of outlier tests. Four data sets were generated with 100 loci each (95 neutral and 5 divergent selection) in 10 populations, under scenarios of weak and strong divergent selection where adaptive variation either matched or contrasted with neutral patterns of IBD. Five additive quantitative traits were simulated (each with 1 locus) underpinning the phenotype under selection. To represent strong divergent selection, mean phenotypic values with optimal fitness per population were set to span a range of 10 units (interval of 1 per population) with selection intensity set to 4 units to restrict phenotypic variation per population to a width of 8 units (Figure 1). For weak divergent selection, the mean phenotype optima per population were set to a narrower range that spanned 5 units (0.5 interval per population), also with selection intensity set to 4 units (Figure 1). Thus, under strong selection the populations at the extreme ends of the phenotypic range had no overlap of phenotypic values that conferred positive individual fitness, while under weak selection the populations at the extreme ends of the range had substantially overlapping phenotype values conferring positive individual fitness (Figure 1). Both strong and weak selection were invoked in scenarios where patterns of adaptive variation matched or contrasted with an IBD pattern of neutral genetic variation. In order to create contrasting patterns of adaptive and neutral genetic variation (neutral ≠ adaptive), a linear stepping stone model of gene-flow was simulated, but selection optima were randomized across the populations. In the case of matching patterns (neutral = adaptive), selection optima in each population followed the same linear pattern of gene flow in an IBD model. Thus the four simulated scenarios were as follows: A) neutral ≠ adaptive variation (weak selection); B) neutral ≠ adaptive variation (strong selection); C) neutral = adaptive variation (weak selection); D) neutral = adaptive variation (strong selection). In all four of the scenarios, 10 populations (each with N = 500) were allowed to evolve over a period of 2000 generations with recombination, mutation rate of 1.0×10^{-4}, with 0.01 migrants per generation, and starting allele frequencies of 0.5 at time zero. The resulting allele frequencies of the selected loci after the 2000^{th} generation for each of the four

scenarios are shown in Figure 2. Loci were initially independent, but tests for pairwise linkage disequilibrium (LD) were completed in GENEPOP v. 3.3 (Raymond & Rousset 1995) since simulations allowed for recombination over 2000 generations.

Genotypes from simulated data sets were used to evaluate global F_{ST} histograms and three simulation approaches to detect candidate markers for selection. The three outlier methods included the FDIST2 approach of Beaumont & Nichols (1996) implemented in LOSITAN (Antao *et al.* 2008), BAYESCAN (Foll & Gaggiotti 2008), and Arlequin v.3.5 (Excoffier *et al.* 2009). Simple histograms of global F_{ST} were one of the initial outlier methods and are still commonly used in high density genome scans to detect regions with multiple markers with high F_{ST} (e.g., Akey *et al.* 2002). The Beaumont & Nichols (1996) method with FDIST2 was a more sophisticated approach since it incorporates heterozygosity and simulates a distribution for neutrally distributed markers. Variations on the FDIST2 method were added with inclusion of hierarchical genetic structure (Excoffier *et al.* 2009; implemented in Arlequin v.3.5) and the introduction of a Bayesian approach (BAYESCAN). Hierarchical structure for Arlequin (F_{CT}) was determined based on neutral variation under an IBD pattern where the ten populations were combined into five groups, each consisting of two adjacent populations. Only the hierarchical model with F_{CT} was tested in Arlequin since FDIST2 incorporates the alternative finite island model with F_{ST}. While the same one-dimensional stepping stone migration model (i.e., neutral variation follows an IBD pattern) was simulated for all four sets of data, the pattern of adaptive variation was either random (contrasting IBD) or followed a clinal gradient (matching IBD) for both scenarios of weak and strong selection (as described above). Pairwise population approaches such as DetSel (Vitalis *et al.* 2001) were not tested due to the large number of pairwise comparisons involved and the difficulty in interpreting inconsistent results when many loci and populations are evaluated.

Results

Results of Type I and Type II errors for simulated data sets were dense with numerous outcomes for three outlier tests, at two confidence levels, for balancing and divergent selection, under scenarios of weak vs. strong divergent selection. The text below highlights the major findings of our tests, but Table 1 provides a clear summary of results and supporting figures (3-6) are presented with detailed results for F_{ST} histograms and each outlier test.

Results for the four simulated data sets generated by QUANTINEMO with matching or contrasting patterns of neutral (IBD) and adaptive variation under both scenarios of weak and strong selection were evaluated with F_{ST} histograms and three outlier methods. Each of the simulated data sets included 95 neutral markers and 5 markers under divergent selection. Histograms of global F_{ST} correctly identified all five markers under divergent selection when the top 10% F_{ST} values were considered, with the exception of scenario A (neutral ≠ adaptive variation -weak selection) (Figure 3a-d). Under both cases of strong selection, global F_{ST} histograms had all five selected markers in the top 5% (Figure 3a, 3c) while weak selection scenarios had 2 or 3 neutral markers included in the highest 5% (Figures 3b and 3d, respectively). As expected, both scenarios of weak selection resulted in markers with lower F_{ST} values than strong selection and thus histograms were only highly reliable with strong selection. Under strong selection, tests for pairwise LD were highly significant ($p < 0.00001$) among 390

combinations of neutral and selected markers, but no pairs of neutral and selected markers were highly significant under weak selection.

Results with FDIST2 failed to identify any of the five markers under both cases of weak selection as candidates for divergent selection at the 99% confidence level, while at the 95% confidence level the method correctly detected two markers in scenario A and zero in scenario C (Figures 4a, 4c). Under both scenarios of strong selection, FDIST2 correctly identified all five selected markers at both the 95% and 99% confidence levels (Figures 4b, 4d). Several neutral markers were incorrectly identified as candidates (Type I error) with FDIST2 under all four scenarios. In scenario A, Type I error for divergent selection included 3 and 1 neutral markers at 95% and 99% respectively, and for balancing selection had 14 and 3 markers at 95% and 99% respectively (Table 1; Figure 4a). For scenario B, FDIST2 had 2 Type I errors for divergent selection at 95% but none at 99%, and for balancing selection there were 17 and 9 Type I errors at 95% and 99% respectively (Table 1; Figure 4b). For scenario C, Type I error for divergent selection included 5 and 3 neutral markers at 95% and 99% respectively, and for balancing selection had 20 and 9 markers at 95% and 99% respectively (Table 1; Figure 4c). Lastly in scenario D, Type I error for divergent selection included 3 and 1 neutral markers at 95% and 99% respectively, and for balancing selection had 30 and 13 markers at 95% and 99% respectively (Table 1; Figure 4d).

Analyses with Arlequin that accounted for hierarchical genetic structure (F_{CT}) had mixed performance depending upon whether simulation scenarios of adaptive variation were contrasting or matching neutral structure, and whether Type I or Type II error was considered. For Type II error, when adaptive variation was randomized among populations in a pattern contrasting neutral structure (scenarios A and B), Arelquin performed poorly regardless of whether selection was weak or strong. Specifically, Arlequin was not able to correctly identify any of the selected markers under the first two scenarios (i.e., neutral ≠ adaptive variation) and loci that were simulated under clear patterns of divergent selection (see Figure 1) were incorrectly identified by Arlequin as candidates for *balancing* selection in these cases (Figures 5a and 5b). This included three weak divergent selection markers identified as candidates for balancing selection at the 95% confidence level (Figure 5a) and all five strong divergent selection markers identified as a candidates for balancing selection at the 99% confidence level (Figure 5b). (The other two simulated loci under weak divergent selection were in the range for neutral loci at the 95% confidence level, but were also positioned near the threshold for balancing selection; Figure 5a). In contrast to the results observed in scenarios A and B, Arlequin had similar Type II error as other methods in scenarios C and D where adaptive variation matched neutral patterns. In these situations, Arlequin correctly identified all five markers for strong selection, and 3 of 5 markers under weak selection (Figures 5c and 5d) as candidates for divergent selection. In addition to these Type II errors, Arlequin consistently had the highest Type I errors compared to FDIST2 and BAYESCAN under all scenarios and confidence levels (Table 1).

Analyses with BAYESCAN at the equivalent 95% and 99% Bayes Factor thresholds were generally encouraging (Figure 6a-d), as this method did correctly identify all five markers under both cases of strong selection even at the 99% threshold (Figure 6b and 6d) and had much lower Type I error than the other methods (Table 1). However, BAYESCAN was unable to detect any of the five markers under scenario A and only 2 of 5 markers in scenario C (Figures 6a and 6c). False positives were observed in all four

scenarios, but at the lowest overall level relative to other methods with greatest advantage in Type I error for balancing but not necessarily divergent selection (Table 1).

In summary across the four simulation scenarios, Arlequin had the highest Type I and Type II error relative to FDIST2 and BAYESCAN. BAYESCAN had much lower Type I error than either of the other two methods, but BAYESCAN and FDIST2 had similar Type II error. Specifically, average Type I error at 95% confidence was 24.7% for FDIST2, 37.9% for Arlequin, and 9.7% for BAYESCAN. However, much of the Type I error for each method was for balancing selection, and when only divergent selection was considered, FDIST2 and BAYESCAN had similarly low average Type I error (3.4% and 4.7%, respectively) compared to Arlequin (11.6%). Average Type II error at 95% confidence was 40.0% (2 out of 5) for both FDIST2 and BAYESCAN, and 60.0% (3 out of 5) for Arlequin. As expected, increased stringency at 99% confidence levels generally decreased average Type I error and increased average Type II error for all methods (Table 1).

Discussion

Overall, we observed discordance of results among the three outlier methods tested in this study (Table 1). Of the three simulation based methods, FDIST2 and BAYESCAN generally had the lowest Type II error rate (fewest false negatives) and had similarly low Type I error rate (fewest false positives) for divergent selection. Type I error for balancing selection was high for FDIST2 and Arlequin, but relatively low for BAYESCAN. The hierarchical method in Arlequin generally performed the worst of the three methods as it typically had both the highest Type I and Type II error. Further, for scenarios A and B in which adaptive variation contrasted neutral structure, Arlequin often placed markers that were simulated under divergent selection in the region for balancing selection. This was not the case for scenarios C and D in which adaptive variation matched neutral variation in an IBD pattern, where markers were typically identified correctly as candidates for divergent selection. These observations suggest that Arlequin may not perform well in situations where adaptive variation contrasts with patterns of neutral variation, likely due to the effect of hierarchical grouping based on neutral markers. In fact, our simulated scenarios included hierarchical groups that contained populations with opposing selection forces (fitness optima) driving different mean phenotypes (i.e., local adaptation; Figure 7). While the hierarchical groupings were appropriate based on neutral genetic structure, the approach caused divergent markers to be incorrectly identified as candidates for balancing selection due to offsetting allele frequencies and extremely low F_{CT} values in the differently adapted populations included in the same group. While our study included a limited range of parameters for mutation, time, migration, and effective size, the issue identified regarding hierarchical groups in Arlequin would likely apply under a wide variety of scenarios with differing parameters. Given that patterns of adaptation variation relative to neutral structure are not typically known prior to an outlier test, extreme caution should be used in application of hierarchical approaches based on the results observed in this study.

False positives for candidate markers under divergent and balancing selection were abundant with all three methods, but some observations from this study offer useful guidelines for interpreting results. Accurate detection of balancing selection is an inherent weakness of outlier approaches (e.g., Beaumont & Nichols 1996), and the majority of Type I error indeed occurred for balancing selection with all three outlier

tests. This further emphasizes the need to cautiously interpret balancing selection detected from outlier tests. Additionally, some neutral markers had strong signals of divergent selection under all scenarios for each of the three outlier methods. This is likely due to divergence hitchhiking of neutral markers in linkage disequilibrium with actual QTL loci following simulations over 2000 generations with recombination. Thus, the neutral markers were not the actual loci under selection but rather indicators of nearby QTL regions. This emphasizes the point that loci detected as outliers may not be genes under selection but rather regions that warrant further investigation and validation to identify true adaptive variation in the genome (e.g., intensive pyrosequencing and common garden studies).

One intriguing issue of these results was the limited sensitivity of outlier approaches to identify markers that were under weak selection. This may be due to insufficient sensitivity of outlier methods to identify loci that demonstrate a gradient of selection across populations. Weak divergent selection may not cause large differences in allele frequencies of candidate loci among populations (Figure 2) resulting in lower values of global F_{ST}, and therefore loci under weak selection may not be identified as outliers. This is an inherent challenge for outlier methods. Possible solutions to address these incompatibilities include logistic regression analyses with specific environmental and habitat variables (Joost *et al.* 2007) or gene interactions (Park & Hastie 2008) that may minimize Type II error even in the context of weak selection. A recently introduced logistic regression approach called Spatial Analysis Method (SAM; Joost *et al.* 2008) may offer an advance in outlier detection since this approach is performed at the individual genotype level and may offer high power due to the number of data points included in analyses. However, this approach may not be possible for some applications because it requires adequate characterization and collection of environmental and habitat data (e.g., Narum *et al.* 2010).

The results of global F_{ST} histograms indicated that they were useful heuristic tools to identify candidate markers based on highest divergence in the data sets, and may be worthwhile for preliminary evaluations in conjunction with a simulation procedure (i.e., FDIST2 or BAYESCAN). Of the three simulation methods, FDIST2 and BAYESCAN appeared to provide the most power depending on the scenario and BAYESCAN had the least Type I error (fewest false positives). As with most analyses options, there are apparent trade-offs between Type I vs. Type II error with different methods and thus statistical procedures should be evaluated carefully and chosen based on the goals of a particular study. In many cases, simple histograms may be at least as useful as outlier tests that incorporate more sophisticated simulations. However, future opportunities exist for much more thorough simulations with evaluation of additional parameters such as larger numbers of loci, variable population sizes, and different hierarchy structures and population clusters. This is particularly clear since each of the F_{ST} outlier methods evaluated here have been shown to be effective under certain simulation parameters (e.g., Excoffier et al. 2009).

As genomics tools become further developed with the ability to sequence large portions of genomes in non-model species, it is expected that outlier detection will become common practice to identify adaptive molecular variation in ecological and conservation genetics studies. Given the results provided here, it will be important that outlier loci are interpreted cautiously and error rates of various methods are taken into consideration with the goals of a particular study.

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Table 1. Summary of Type I (false positive) and Type II (false negative) errors for outlier methods tested with simulated data for divergent selection.

Data Set	Threshold	FDIST2	Arlequin	Bayescan
Simulated: Neutral ≠ Adaptive Variation (Weak Selection)				
Correct candidates (5)				
	95%	2	0	0
	99%	0	0	0
False negatives				
	95%	3	5	5
	99%	5	5	5
False positives				
Divergent	95%	3	20	5
	99%	1	9	4
Balancing	95%	14	17	4
	99%	3	9	1
Simulated: Neutral ≠ Adaptive Variation (Strong Selection)				
Correct candidates (5)				
	95%	5	0	5
	99%	5	0	5
False negatives				
	95%	0	5	0
	99%	0	5	0
False positives				
Divergent	95%	2	7	5
	99%	0	6	5
Balancing	95%	17	22	4
	99%	9	11	2
Simulated: Neutral = Adaptive Variation (Weak Selection)				
Correct candidates (5)				
	95%	0	3	2
	99%	0	0	0
False negatives				
	95%	5	2	3
	99%	5	5	5
False positives				
Divergent	95%	5	8	3
	99%	3	2	2
Balancing	95%	20	23	7
	99%	9	15	2
Simulated: Neutral = Adaptive Variation (Strong Selection)				
Correct candidates (5)				
	95%	5	5	5
	99%	5	5	5
False negatives				
	95%	0	0	0
	99%	0	0	0
False positives				
Divergent	95%	3	9	5
	99%	1	3	1
Balancing	95%	30	38	4
	99%	13	37	2

Figure 1. Conceptual rendering of selection parameters used to simulate either a) weak or b) strong divergent selection among 10 populations. Populations at extremes (in bold) have overlapping phenotypes under weak selection while under strong selection the phenotypes are non-overlapping. For strong divergent selection, mean phenotype with optimal fitness per population were set to span a range of 10 units (interval of 1 per population) with selection intensity set to 4 units to restrict total phenotypic variation per population to a width of 8 units. For weak divergent selection, the mean phenotype optima per population were set to a narrower range that spanned 5 units (0.5 interval per population), also with selection intensity set to 4 units.

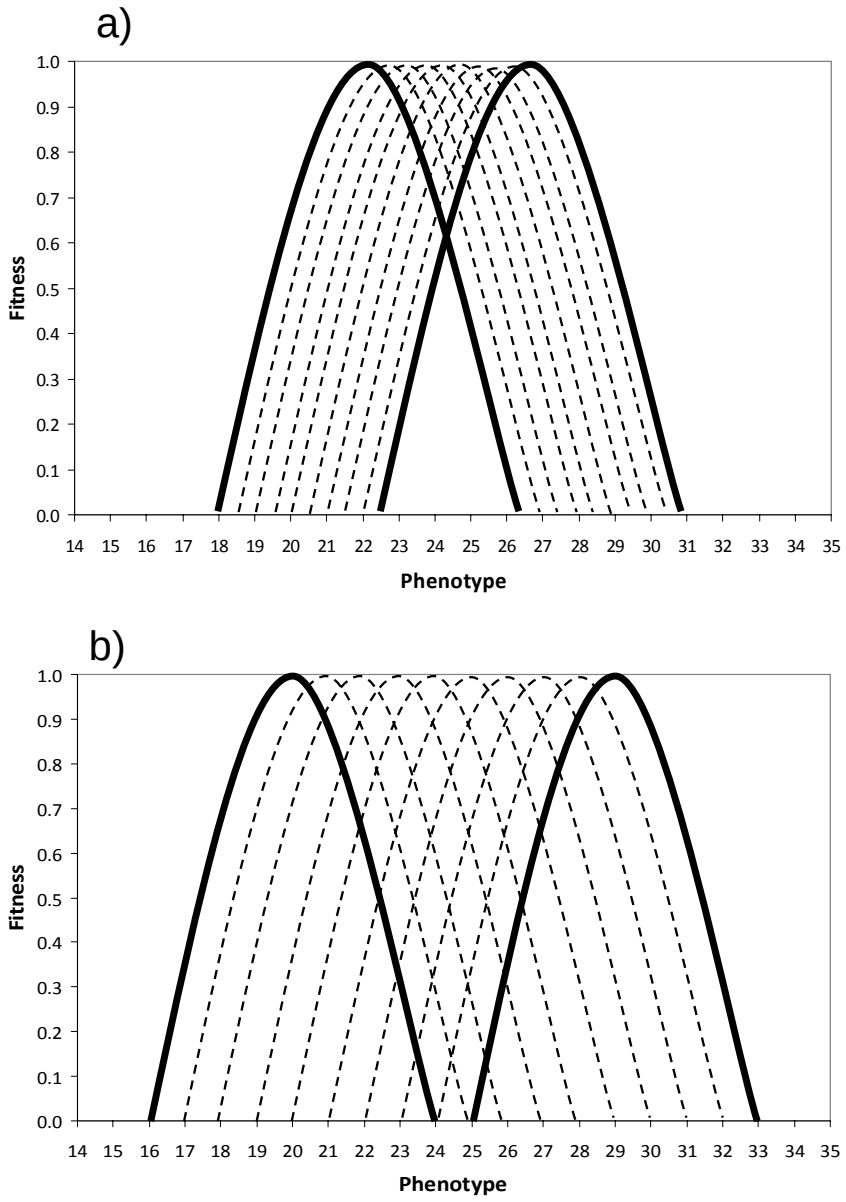


Figure 2. Subsequent allele frequencies of five loci under divergent selection after populations were allowed to "evolve" for 2000 generations with starting allele frequencies of 0.5 at time zero. Four scenarios are shown: a) neutral ≠ adaptive variation (weak selection); b) neutral ≠ adaptive variation (strong selection); c) neutral = adaptive variation (weak selection); d) neutral = adaptive variation (strong selection). Additional simulation parameters are available in the main text. A total of 100 loci (5 selected and 95 neutral) and 10 populations were included for each simulation.

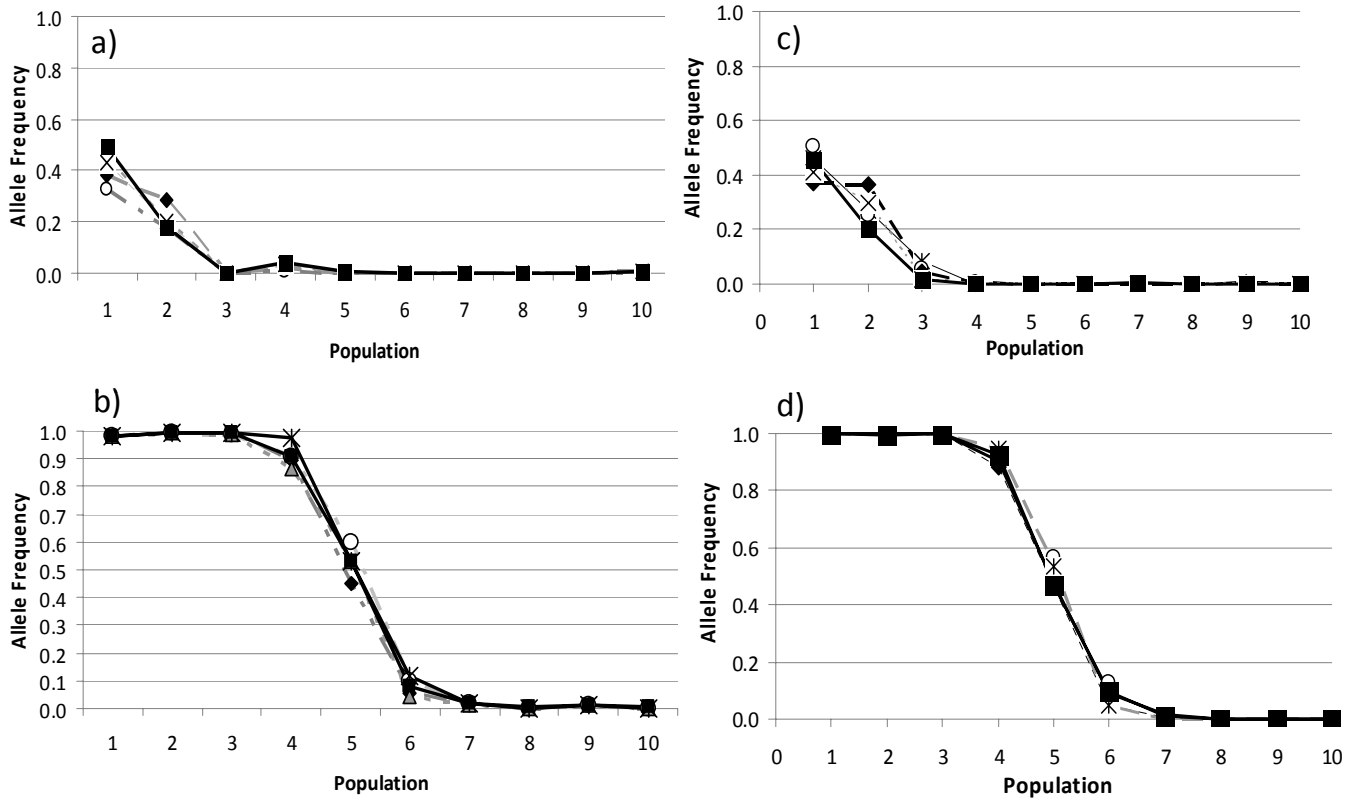
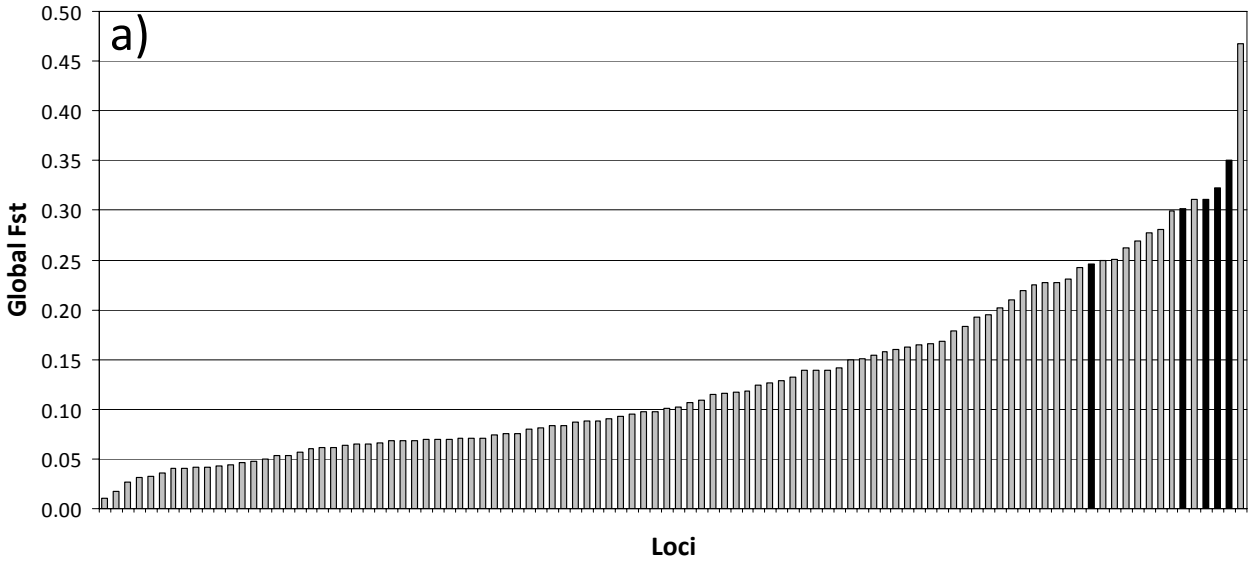
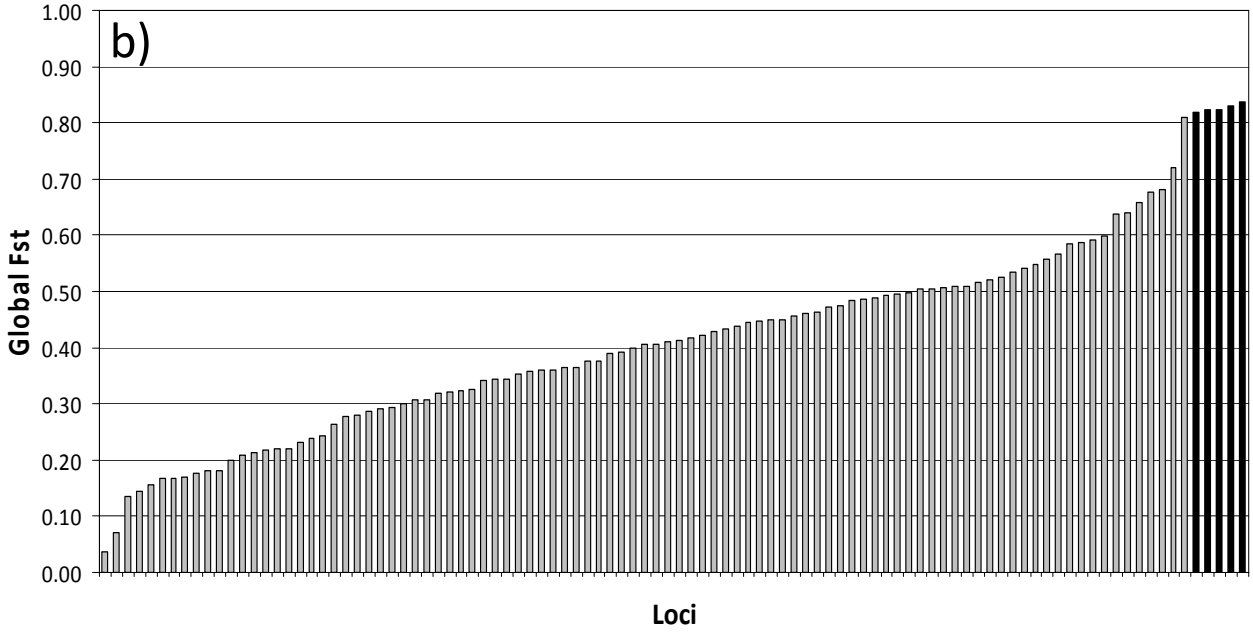
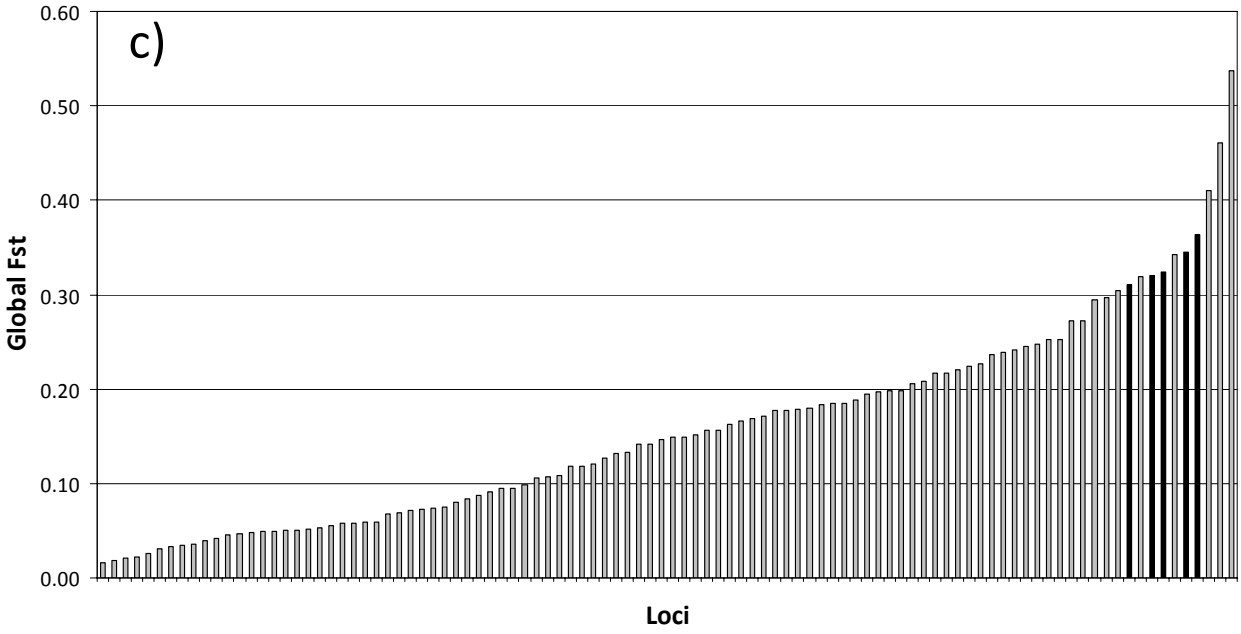


Figure 3. Histogram of global F_{ST} for simulated data sets a) neutral ≠ adaptive variation (weak selection); b) neutral ≠ adaptive variation (strong selection); c) neutral = adaptive variation (weak selection); d) neutral = adaptive variation (strong selection). Neutral markers are gray, divergent-selected markers are black.






c)
0.60
0.50
0.40
0.30
0.20
0.10
0.00
Global Fst
Loci

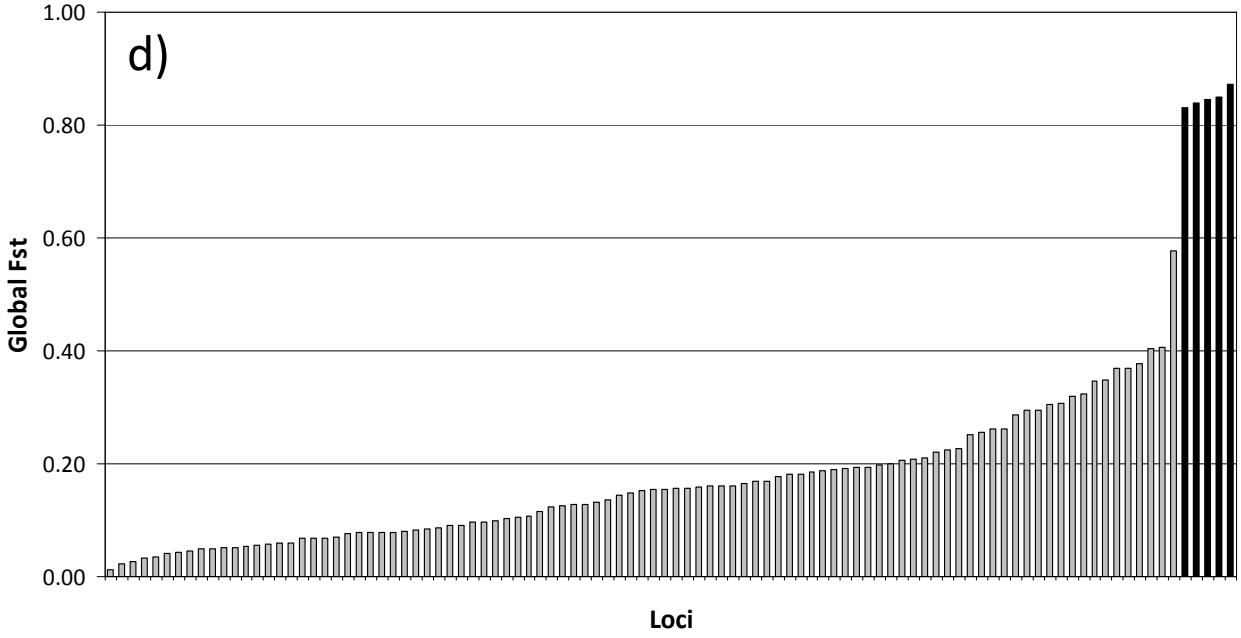
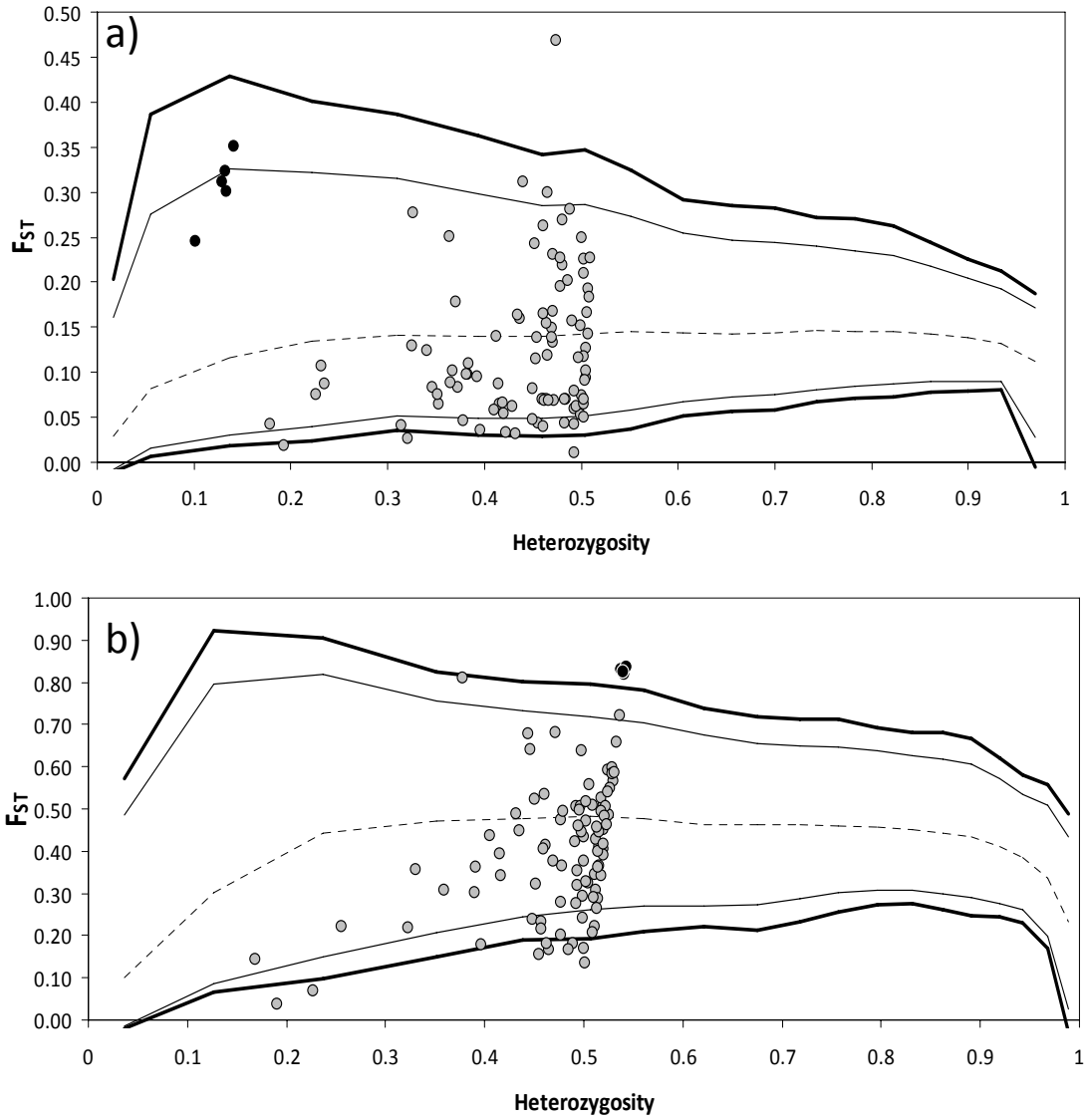
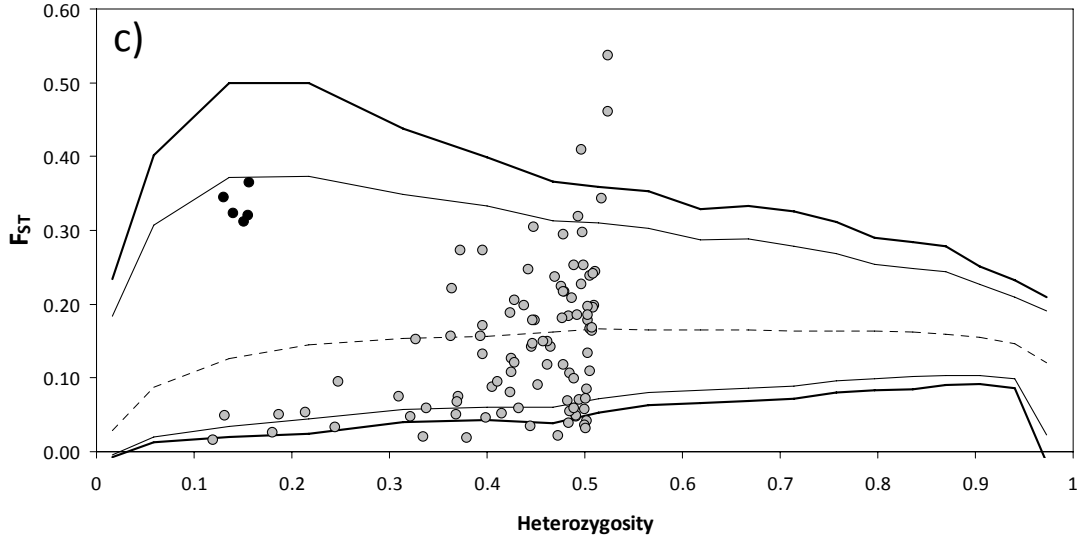

d)
1.00
0.80
0.60
0.40
0.20
0.00
Global Fst
Loci

Figure 4. FDIST2 results for simulated data under scenarios of a) neutral ≠ adaptive variation (weak selection); b) neutral ≠ adaptive variation (strong selection); c) neutral = adaptive variation (weak selection); d) neutral = adaptive variation (strong selection). 95% (thin line) and 99% (bold line) confidence levels are shown. Neutral markers are gray, divergent-selected markers are black.




c)
0.60
0.50
0.40
0.30
0.20
0.10
0.00
FST
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
Heterozygosity

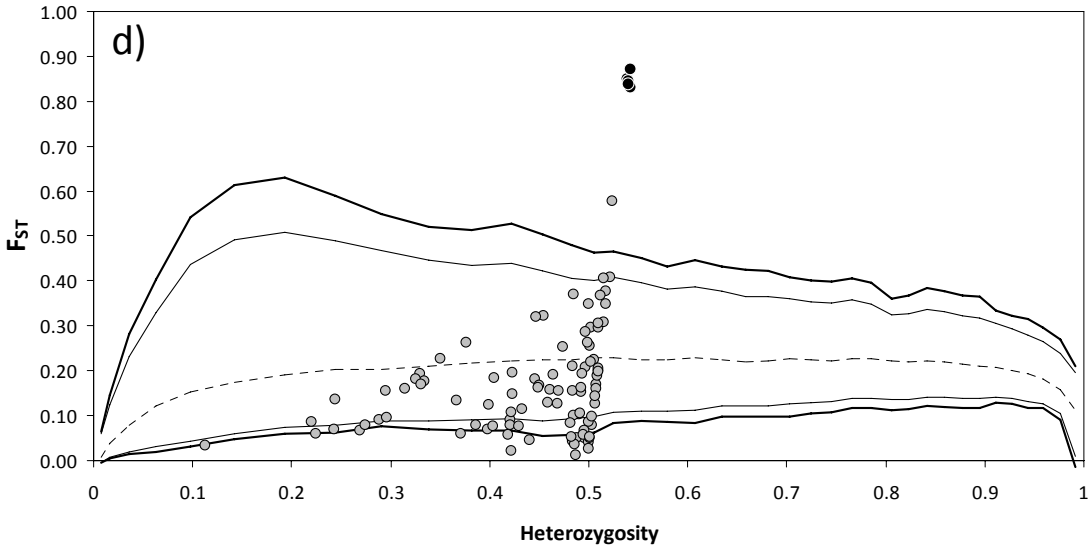
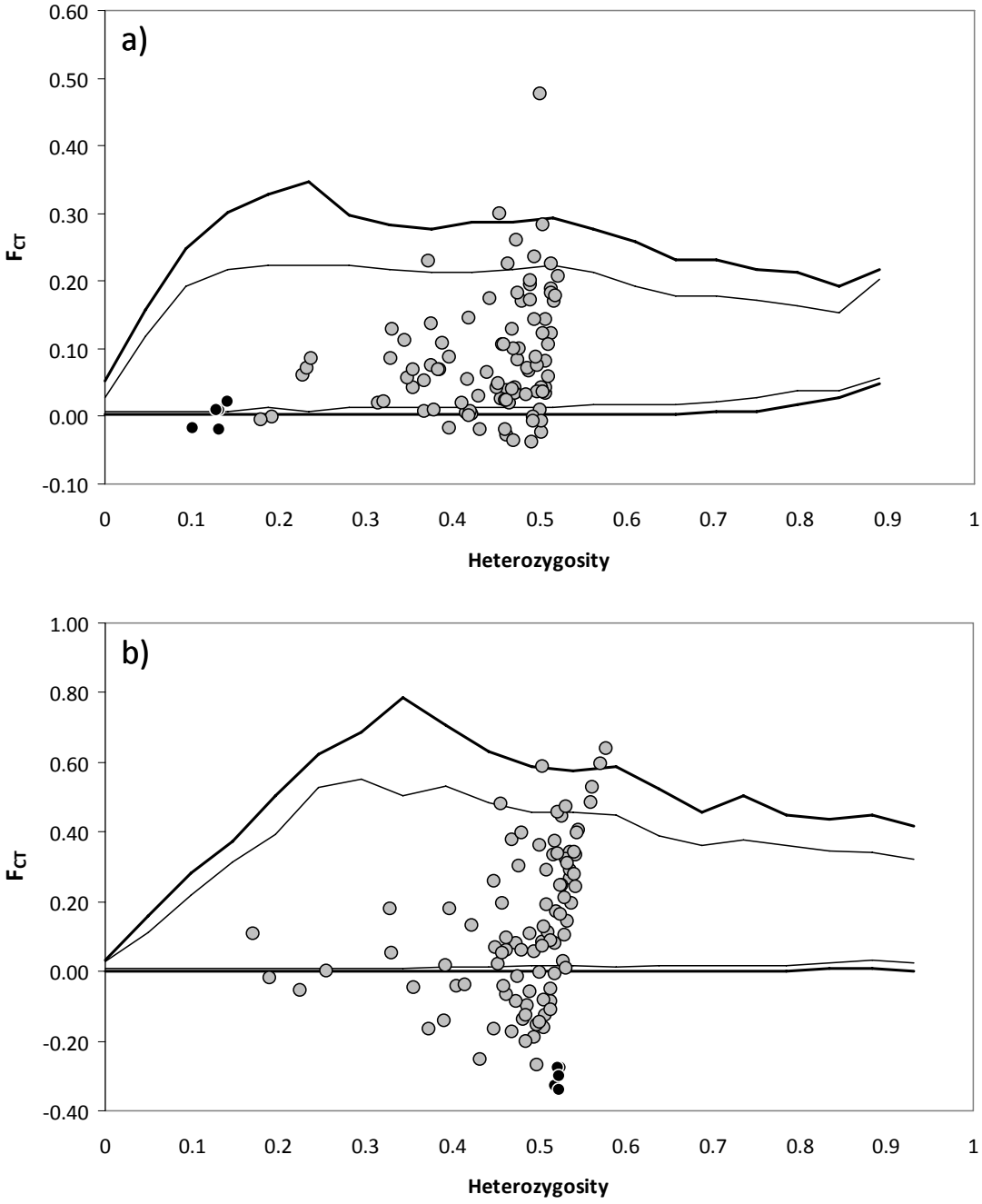
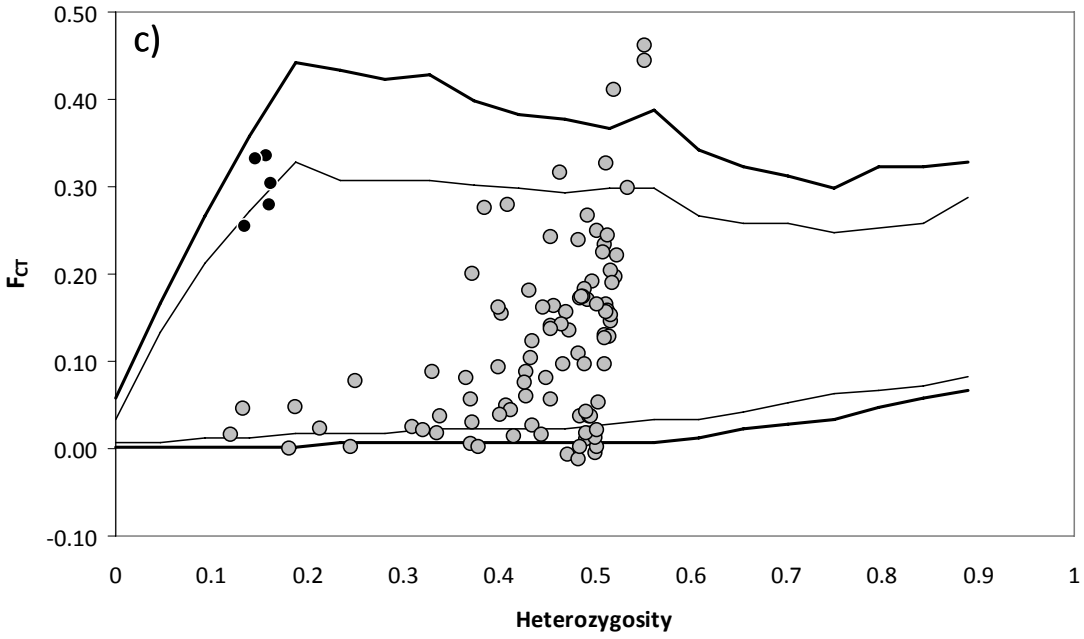

d)
1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
FST
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
Heterozygosity

Figure 5. Arlequin results for simulated data under scenarios of a) neutral ≠ adaptive variation (weak selection); b) neutral ≠ adaptive variation (strong selection); c) neutral = adaptive variation (weak selection); d) neutral = adaptive variation (strong selection). 95% (thin line) and 99% (bold line) confidence levels are shown. Neutral markers are gray, divergent-selected markers are black.




c)
0.50
0.40
0.30
0.20
0.10
0.00
-0.10
F_{CT}
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
Heterozygosity

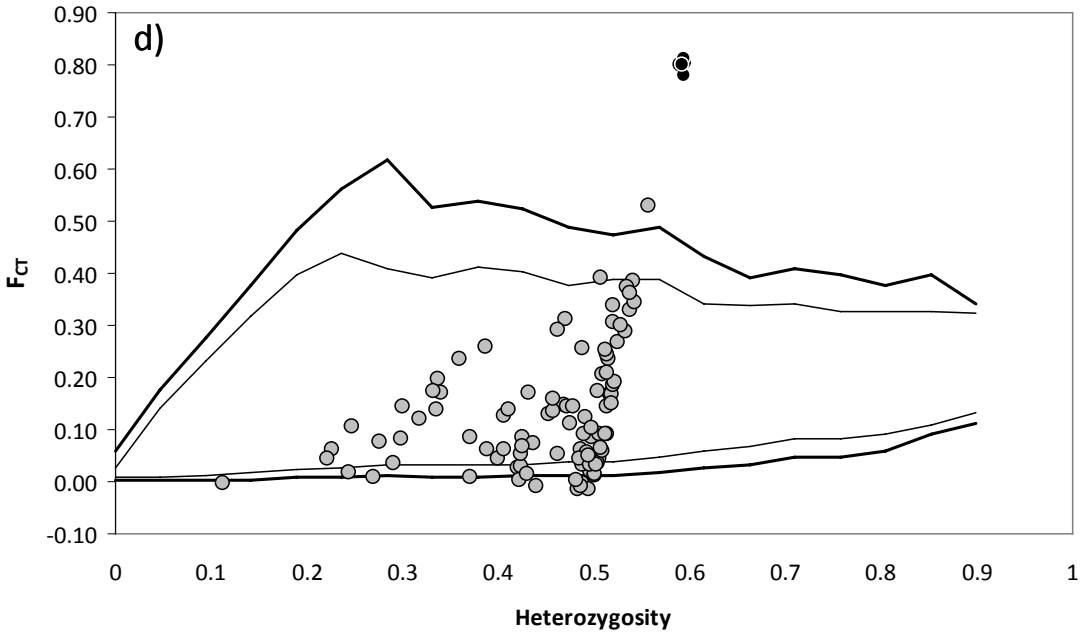

d)
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
-0.10
F_{CT}
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
Heterozygosity

Figure 6. BAYESCAN results for simulated data under scenarios of a) neutral ≠ adaptive variation (weak selection); b) neutral ≠ adaptive variation (strong selection); c) neutral = adaptive variation (weak selection); d) neutral = adaptive variation (strong selection). Dashed lines indicating equivalent Bayes Factor thresholds of 95% (1.5) and 99% (2.0). Neutral markers are gray, selected markers are black.

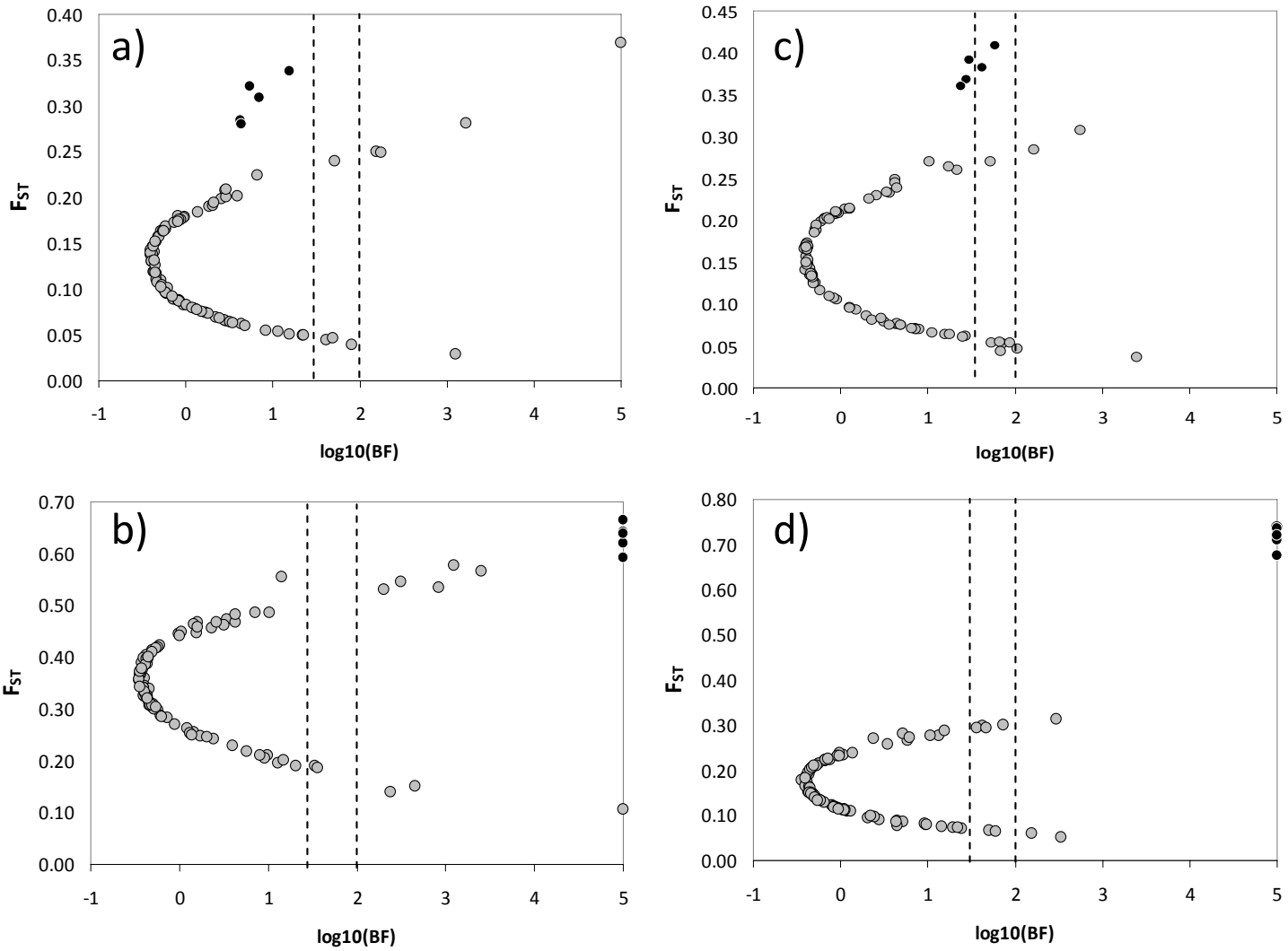
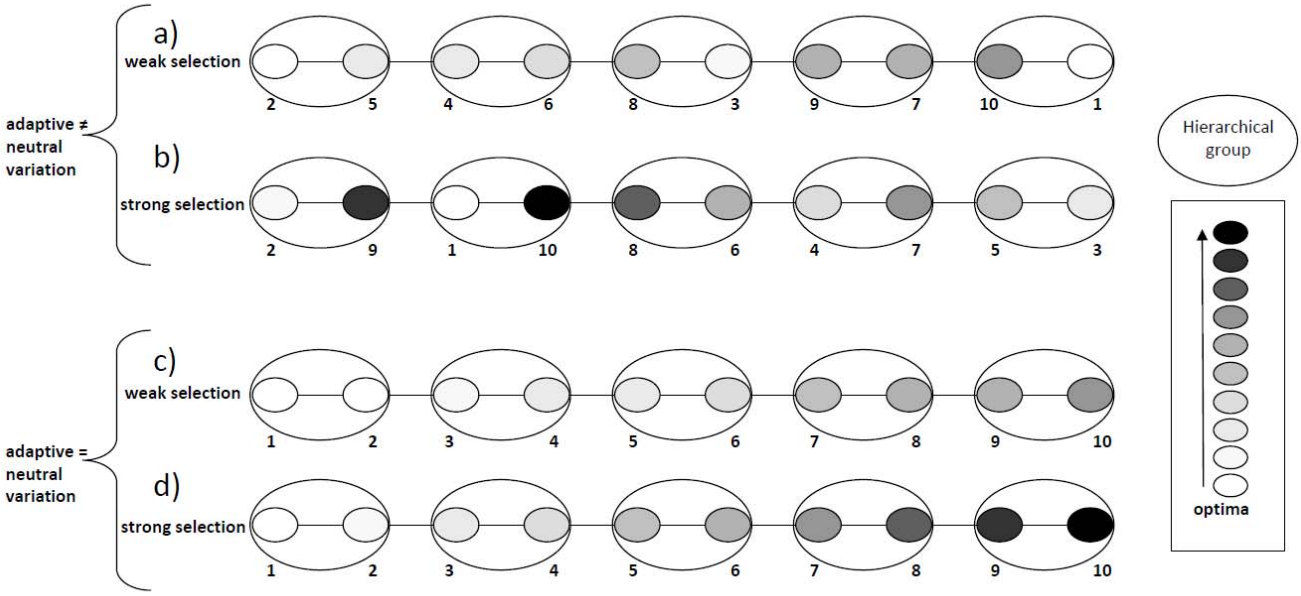


Figure 7. Conceptual figure that depicts the patterns of adaptive variation for each population (shaded ovals) under four scenarios: a) neutral ≠ adaptive variation (weak selection); b) neutral ≠ adaptive variation (strong selection); c) neutral = adaptive variation (weak selection); d) neutral = adaptive variation (strong selection). Hierarchical groupings used for analyses in Arlequin are shown for each scenario by the large ovals. Numbers below small ovals correspond to population numbers in Figure 2.



SECTION 2: Thermal Adaptation of *O. mykiss*

Introduction

Previous studies suggest that climate change may greatly impact seasonal cues in nature (e.g., Bradshaw & Holzapfel 2008) and cause shifts in species distributions and migration patterns (Berthold et al. 1992; Bradshaw et al. 2004; Hari et al. 2006). However, locally adapted reaction norms may be sufficiently plastic to allow for adaptation to different environments if phenotypic and genetic variation exists (e.g., Jensen et al. 2008). Thus, monitoring of candidate gene allele frequencies along genetic clines may prove to be effective for quantifying the influence of climate change on natural populations (e.g., Umina et al. 2005). Further, the potential to predict genetic adaptability of individuals and populations to changing climate conditions may have profound implications for many species that face extensive anthropogenic disturbances.

Redband trout (*Oncorhynchus mykiss gairdneri*) occupy a wide range of habitats including desert and montane streams, with significant differences in habitat characteristics such as elevation, gradient, substrate, shading and temperature (Meyer et al. 2010). Physiological differences have also been observed in redband trout occupying desert streams (Gamperl et al. 2002). Previous work has demonstrated local adaptation in populations occupying desert and montane habitats (Narum et al. 2010). To further elucidate the genetic mechanisms of thermal adaptation, gametes were collected from a single desert and montane population for rearing in a controlled environment.

Methods

Gametes, Rearing, and Tissue Samples

Gametes were collected from mature fish from desert and montane populations previously determined to be adapted to either warm (Little Jacks Cr. – "LJ") or cool/intermediate (Keithley Cr. – "K") climates, respectively (Narum et al. 2010). Tissue samples (fin clips) were collected from adults that provided gametes to identify parentage. Gametes were fertilized to produce progeny of a pure desert line (LJ x LJ), a pure montane line (K x K), and their F1 crosses (K_{female} x LJ_{male}). These lines are referred to hereafter as desert, montane, and F1 lines, respectively.

Fish from each of these lines were reared at constant temperatures (15°C spring water) at the Hagerman Fish Culture Experiment Station until they reached an average of 5 grams, and then were divided into treatment and control groups. Fish in treatment tanks experienced diel temperature cycles that reached a maximum of approximately 28.5°C in the afternoon and a minimum of 17.0°C at night (Figure 1) over the course of six weeks. Control tanks were held at a constant temperature of 15°C (spring water). Mortality was observed and recorded throughout the duration of the experiment from three replicate tanks for each line.

Tissue samples for DNA analyses were collected throughout the experiment, with fin clips collected from all deceased fish at date time of mortality, and all remaining survivors at the end of the experiment. For gene expression purposes, liver and gill tissues were collected

from three fish per tank in both control and treatment tanks at peak diel temperature at four periods of the experiment (24 hours, 72 hours, 7 days, 30 days). Fin clips for DNA analyses were stored dry on Whatman paper (LaHood et al. 2008) while liver and gill tissue were stored in RNALater and frozen at -80°C.

Laboratory and Statistical Analyses

DNA was extracted with Qiagen DNeasy® from fin clips of individuals that survived or died in treatment tanks and were screened with 192 SNP markers throughout the genome (Table 1). Markers were genotyped with Taqman chemistry (Applied Biosystems) and Fluidigm 96.96 dynamic array chips (reaction volumes of ~7nL) for SNP genotyping as described by Narum et al. (2010).

RNA was isolated from liver tissues and tested for gene expression at six heat shock genes (hsc71, hsp47, hsp70, hsp90, hsf1, hsf2) and tested with real-time quantitative PCR on an ABI 7900 instrument.

Differences in survival among lines were tested with one-way ANOVA. Parentage analysis (CERVUS; Kalinowski et al. 2008) was completed to identify family groups and test for differences in survival by maternal and paternal contributions. Allelic association tests with phenotype (survivor/mortality) were completed with PLINK (Purcell et al. 2007) by individual line and overall three lines. The following thresholds were applied to the dataset for PLINK analyses: loci removed if MAF was less than 0.05, SNPs removed if greater than 10% missing data, and individuals removed if greater than 10% missing data. The BY-FDR method was used to correct for multiple tests in individual lines and overall three lines. In the test overall three lines, association was determined once stratification was accounted for among lines in PLINK.

Results & Discussion

Survival results indicated that all three lines experienced the largest daily level of mortality upon first exposure to water temperatures exceeding 28°C, suggesting that upper thermal tolerance was exceeded for all groups. Interestingly, upon first exposure the desert and F1 lines had higher (but not significant) mortality than the montane line. However, in the weeks following first exposure, the montane line had the highest level of mortality relative to the desert and F1 lines, as would be expected if differences existed for thermal adaptation.

Parentage analyses confirmed a genetic basis for thermal tolerance as significantly higher mortality was observed in specific families from the montane and F1 lines. In the montane line from Keithley Cr., 11 females and 10 males contributed offspring to the study and 1 female and 1 male had offspring with significantly higher survival than expected, and 2 females and 1 male had offspring with lower survival than expected. In the F1 line, 4 females (montane origin) and 6 males (desert origin) contributed offspring to the study and 1 female and 1 male had offspring with significantly higher survival than expected, and 1 female and 3 males had offspring with lower survival than expected. There were no significant differences in family mortality of the desert line.

Tests for allelic association with phenotype (survivor/mortality) followed a similar pattern as the survival data as several markers were identified that were significantly associated in the montane (40 markers) and F1 (6 markers) lines, but only a single marker in the desert line. One marker, Omy_Ots249-227, was significant in two lines (montane and desert). Tests overall three lines indicated nine significant markers associated with phenotype, with the top candidate as Omy_hsp47-86 (BY-FDR adjusted $p = 0.00024$).

The combination of results from family survival and association tests within each of the individual lines suggest evidence for thermal adaptation in this study. While mortality was observed in the desert line, this appeared to be a plastic response without genetic basis. Further, mortality was greatly reduced in the desert line following the first exposure to high water temperatures as would be expected of a population adapted to warm climate. This is in contrast to observations in the montane line, where mortality had a strong genetic basis, many genetic markers were associated with phenotype, and mortality increased over time with daily exposure to high water temperatures. The F1 line had a similar pattern of survival as the desert line, but a genetic basis was observed in specific families and six markers were significantly associated with phenotype.

Further testing is ongoing to determine the relationship between allelic association of genes with thermal tolerance and gene expression data. Additionally, a more dense genome scan with thousands of SNPs will be applied to the samples tested in this study.

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Figure 1. Diel water temperature of treatment tanks over a six week period.

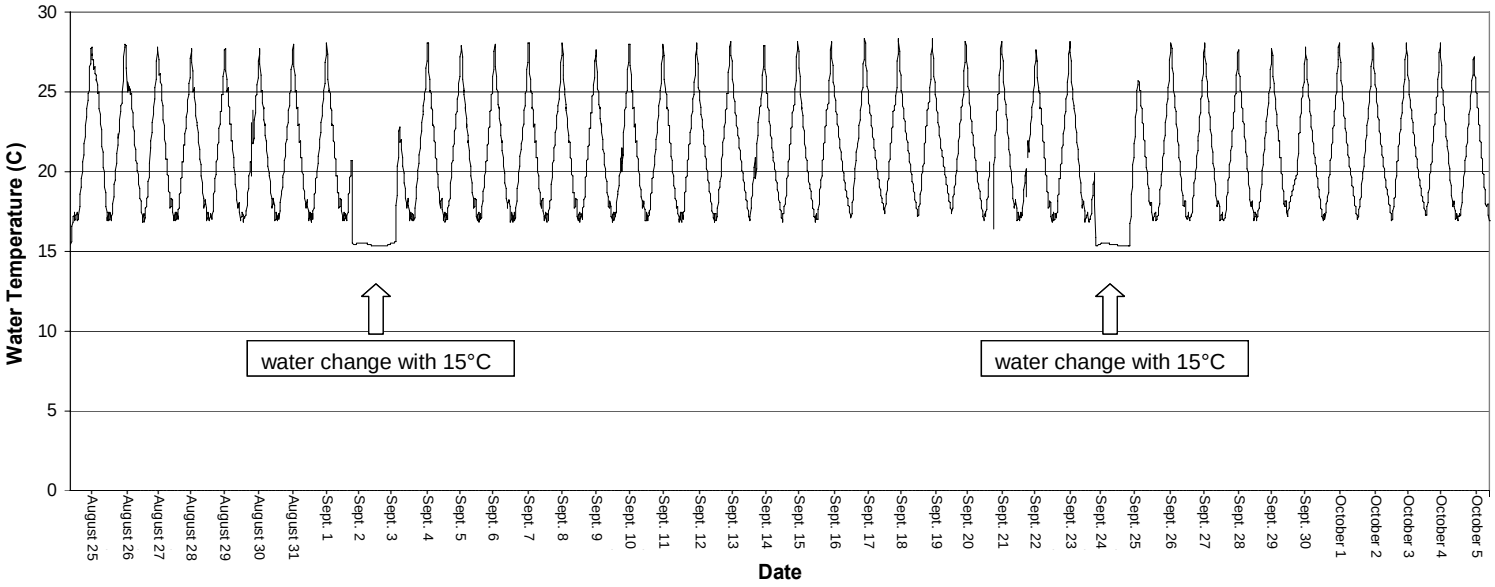
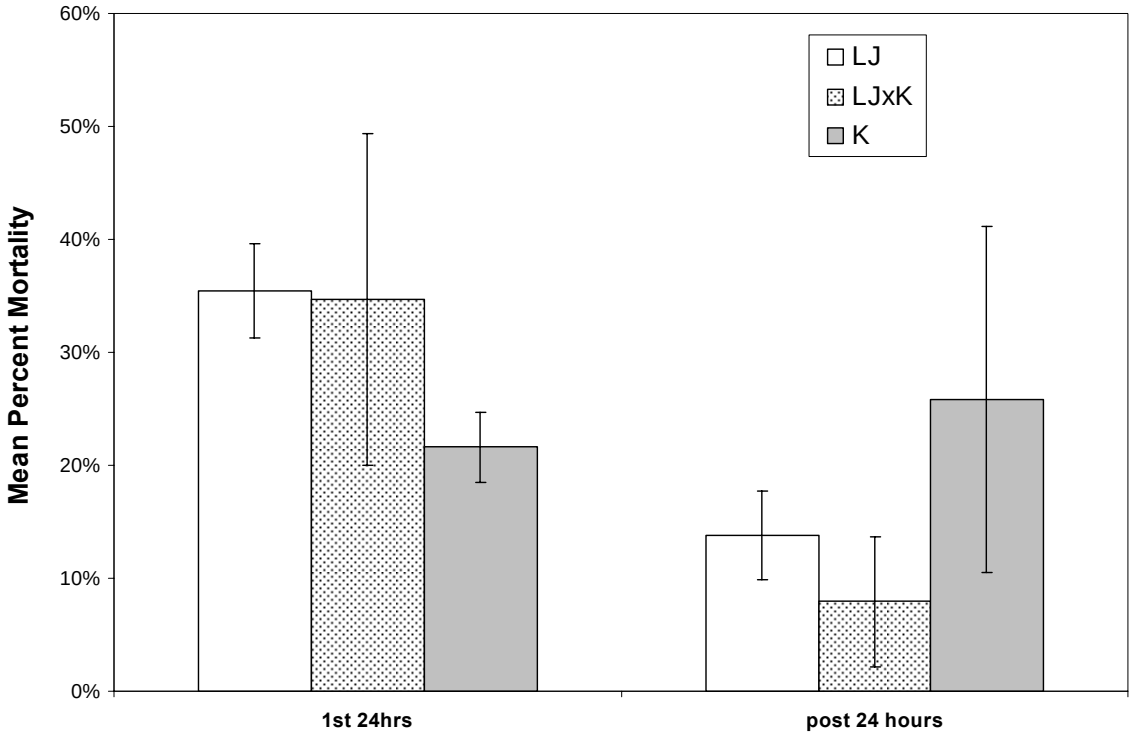


Figure 2. Mean percent mortality of the three lines of redband trout reared in diel water temperature cycles ranging from 17.0-28.5 C. Bars of mean mortality are broken into two groups, 1st 24 hours of exposure, and all days following the initial day of exposure. LJ = Little Jacks Cr. – warm adapted; K = Kiethley Cr. – cool/intermediate adapted, LJxK= F1 crosses. Tests of ANOVA were not significant between lines at either time period.



SECTION 3: Future Studies

As an ongoing Accords project, preparations are underway for further evaluation of multiple traits such as thermal tolerance, smoltification, and disease resistance in *O. mykiss*, and adult run-timing in Chinook salmon. More details are provided below regarding plans to investigate each of these traits in the upcoming performance period (July 1, 2011 – June 30, 2012).

For thermal tolerance, further testing is ongoing to determine the relationship between allelic association of genes with thermal tolerance and gene expression data. Additionally, a denser genome scan with thousands of SNPs will be applied to the samples tested in this study. Specifically, reduced representation RAD libraries will be sequenced on an Illumina HiSeq instrument to identify thousands of SNPs and provide genotypes for individuals. This dense genome scan approach is anticipated to better narrow the genomic regions responsible for thermal tolerance and adaptation, allowing for genetic markers to be developed to screen broad populations throughout the Columbia River Basin.

For smoltification/anadromy, we looked to expand on initial results that reported candidate genetic markers associated with anadromy in the Klickitat River (Narum et al. 2011b). Since markers associated with anadromy may vary across populations in different geographic regions, we identified collaborators to facilitate expanded research into the Yakima River (YKFP and WDFW), Klickitat River (YKFP), and Snake River basin (UI). Both resident and anadromous individuals from these sites were targeted for collection in previous years and in 2011. Tissue samples will be delivered from collaborators for testing in the next performance period.

For disease resistance, we coordinated with existing collaborators at USDA and Clear Springs Research to investigate markers associated with infectious haematopoietic Necrosis virus (IHNV) and bacterial water disease (CWD) as previous studies have not yet identified strong genetic correlations (Overturf et al. 2010). Controlled experiments were designed and completed at the Clear Springs facility to challenge *O. mykiss* from known family groups to both IHNV and CWD. Samples were collected from male and female parents and their offspring, and survival of offspring from each family were identified following direct exposure to the two diseases. Family groups displaying high and low levels of survival to the diseases will be tested in upcoming years of this project.

For run timing, a pedigree study of Chinook salmon from the Hood River has identified naturally occurring crosses of fall-run (Lower Columbia lineage) and spring-run (interior stream type lineage) that will be used to further investigate the genetic basis of this trait. Initial results have demonstrated that run-timing among these individuals is heritable within families.

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