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Size and Age-Structure Divergence and the Genetic Basis of Growth among Populations of Threespine Stickleback (*Gasterosteus aculeatus*)

A Dissertation Presented

by

Jennifer Lynn Rollins

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Abstract of the Dissertation

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Body size may have played a major role in the adaptive radiation of the Threespine Stickleback (*Gasterosteus aculeatus*) species complex as the basis for the assortative mating involved in maintaining divergence between marine and freshwater, benthic and limnetic, and lake and stream populations in sympatry. The trait is known to be heritable in sticklebacks and other fish species, and considerable size differences within and among populations suggest large genetic variation on which natural selection can act. This dissertation addresses fundamental questions about divergence in life history traits associated with body size in Threespine Stickleback among populations divergent for the trait on a small geographic scale (Cook Inlet drainages in Alaska, USA). Chapter 1 quantifies the degree of size divergence among populations in Cook Inlet drainages. Chapter 2 asks whether body size variation is explained by divergence in other life history traits, like age structure, average lifespan, or age and size at maturation. Chapter 3 identifies candidate growth genes under selection and associated with growth phenotypes within populations of Threespine Stickleback. I found significant divergence among populations in body size, age structure and lifespan, and size and age at maturation. Size differences among populations were substantial even after accounting for differences in age structure, and were not correlated with local environmental variables (e.g., lake size, productivity, and concentrations of important ions) or measures of geographic proximity or distance from the sea. I identified single nucleotide polymorphism (SNP) markers within four candidate growth genes associated with growth rate in a single population. SNP markers in all but two candidate growth genes were detected as loci under directional or stabilizing selection in an F_{ST} outlier analysis. Thus, I found evidence for (a) significant divergence in body size across populations of Threespine Stickleback within a small geographic region, (b) local adaptation to different life history regimes including differences in growth rate, age structure and lifespan, and age and size at maturation, and (c) a significant genetic component to potential local and convergent evolution of life histories among populations.

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Chapter 1: Size Divergence Among Populations of Threespine Stickleback

1.1: Introduction

Body size is an important life history trait because it (a) varies so widely across organisms within and among taxa (e.g., Peters 1983), (b) is known to be heritable and therefore subject to natural selection (e.g., Trivers 1985, Mousseau and Roff 1987, Stearns 1992), and (c) is often used as a proxy for fitness in studies where growth rates are thought to mediate the effect of morphological, physiological, or behavioral traits on survival and reproduction (e.g., Schluter 1994, 1995, 2003; Rundle *et al.* 2003; Bolnick and Lau 2008).

Body size is one of the most conspicuous differences among populations within the Threespine Stickleback (*Gasterosteus aculeatus*) species complex (e.g., McPhail 1977, Moodie and Reimchen 1976, Wootton 1984, Reimchen *et al.* 1985, Baker 1994, McKinnon and Rundle 2002). Threespine Stickleback are euryhaline fishes with marine, anadromous, and freshwater life habits in different populations (Bell and Foster 1994). Freshwater populations of stickleback, which are present throughout the Holarctic and are descended from marine or anadromous forms, are thought to have arisen in numerous independent colonizations between 9.5 and 15 KYA upon retreat of the glaciers and isostatic rebound following the most recent glaciation (e.g., McPhail and Lindsey 1970, Bell and Foster 1994, Reger and Pinney 1996). Most marine and anadromous populations are larger bodied than freshwater residents (Baker 1994, McPhail 1994). Dwarfism in landlocked or freshwater resident forms of salmonids and other fishes is often a plastic response (Roff 1992). However, body size variation and growth rates in Threespine Stickleback are highly heritable, with populations retaining their relative size differences when raised in a common laboratory environment (McPhail 1977; Snyder 1991a, b; McKinnon and Rundle 2002). Body size in Threespine Stickleback has been dubbed a magic trait due to its importance in intersexual selection and speciation in the stickleback radiation (e.g., Borland 1986, McPhail 1994, Nagel and Schluter 1998, Rundle and Schluter 1998, McKinnon *et al.* 2004, Ishikawa and Mori 2000, Maccoll 2009, and Matthews *et al.* 2010). In addition to a few freshwater stickleback populations that have independently evolved abnormally large body size and longer life spans than the supposed ancestral anadromous condition (e.g., Moodie 1972a, Moodie and Reimchen 1976, Bell 1984), there is an apparently large degree of general variation in body size among freshwater stickleback populations both globally and on regional scales (e.g., Moodie and Reimchen 1976, Reimchen *et al.* 1985, Baker 1994, Wootton 1994, McKinnon and Rundle 2002).

Preliminary collections from 1990 (unpublished observations) and previous work (Francis *et al.* 1986) suggested a degree of size divergence within a small geographic region, the Cook Inlet of Alaska, USA, that rivals the degree of size differences found globally for Threespine Stickleback. Quantification of population-level variation in body size is a necessary precursor to determining the primary causal factors of the variation. In this study, I quantified and compared body size differences among Threespine Stickleback from 21 lakes and one nearby anadromous population, which is thought to resemble the presumed “ancestor” of the lake populations in the region (e.g., Bell 1976, 1994). I predicted substantial and significant size divergence among populations. Since anadromous stickleback may live longer (3 years; Yershov and Sukhotin 2015, Rollins *et al.* 2017) than freshwater stickleback (1 or 2 years; reviewed in Baker 1994), and others have found that they can be up to twice as large as lake stickleback (Baker *et al.* 1998), I expected anadromous Threespine Stickleback to be about twice as large as most freshwater stickleback. Since preliminary observations suggest that some freshwater populations have stickleback that are close to or may exceed the size of anadromous fish in the Cook Inlet study region, I also expected significant size and growth rate differences among freshwater populations on the same order of magnitude as that between anadromous and lake stickleback found elsewhere (about a 2-fold difference in size).

1.2: Methods

I collected samples using unbaited minnow traps (1/8” or ¼” mesh size) between 2 June and 21 June, 2012 or 2013, from 21 lakes in the Matanuska-Susitna Valley and on the Kenai Peninsula, Alaska, USA (Table 1.1) that were thought (based on preliminary observations) to span the range of body sizes in Threespine Stickleback populations from these geographic regions. I also collected anadromous Threespine Stickleback samples from their freshwater breeding grounds at Rabbit Slough in the Matanuska-Susitna Valley.

Samples were fixed in 10% formalin and preserved in 50% isopropyl alcohol. Standard length (distance from the tip of the rostrum to the last vertebra) was measured using digital calipers to the nearest 0.01 mm. Since there is sexual dimorphism for body size in stickleback, I considered males and females separately. I cut a slit through the abdomen in the left side to examine the gonads under a dissection microscope and determine sex of the specimens.

Since many stickleback populations in Alaska, USA have a one- or two- year lifespan, size-frequency distributions are often bimodal and include both age 1 and age 2 (and rarely older) fish when collections are taken during the spawning season (Baker 1994; Fig. 1.1). Therefore, to quantify size divergence among populations, I first determined which populations had unimodal or bimodal frequency distributions as described in Rollins *et al.* (2017) using Brewer's (2003) "Software for Analysis of Mixture Models using Discretisation" (available from http://www.bioss.ac.uk/~markb/mixtures). I computed ΔAIC as the difference of the AICc for the fitted mixture model of two normal distributions from the AICc for the fitted single normal distribution of each sample. $\Delta AIC > 10$ represented strong and $10 \geq \Delta AIC > 4$ represented moderate support for fit to a bimodal distribution (mixture of two normal distributions). I interpreted $4 \geq \Delta AIC \geq -4$ as equivalent support for either a unimodal or bimodal distribution, and $-4 > \Delta AIC \geq -10$ as moderate and $-10 > \Delta AIC$ as strong support for fit to a unimodal distribution (Burnham and Anderson 2004).

Where available, I used preliminary data on age distributions estimated from otoliths extracted from separate samples from a subset of my 22 populations which were frozen on dry ice and preserved at -80°C until preparation (Table 1.2) to determine whether populations with unimodal size distributions represented age 1, age 2+, or a mixture of ages 1 and 2+ specimens (Table 1.3). Otolith data suggested that most freshwater populations with bimodal size distributions consisted of age 1 (smaller size mode) and 2+ (larger size mode) individuals. In populations where I did not have otolith data to confirm my interpretation of the size modes, I inferred age from size data alone based on size-at-age data from known-age populations. I then compared the mean of "age 1" individuals among populations where possible. Where sample distributions were unimodal and known or assumed to be age 1, I computed the arithmetic mean of all individuals from the sample. Where bimodal, the first mode was often known or assumed to be age 1 individuals, and the mean and variance of the first mode was estimated by a Bayesian procedure using Brewer's (2003) "Software for Analysis of Mixture Models using Discretisation." The mixture analysis software was also used to obtain an estimate of the proportion of individuals in the sample that fell into the different age modes (*p*). These proportions were used to separate bimodal samples into their respective ages, and individuals thus assigned to the first, presumably age 1 mode, were used in ANOVA analyses (SAS Institute, Inc. 2009) with presumed age 1 fish from unimodal samples to determine the significance of divergence in size across populations within age 1. A similar procedure was used to examine size divergence across populations within age 2+.

Finally, I generated matrices according to Francis *et al.* (1986) and performed Mantel tests (Mantel 1967; BIOMstat 4.11) to test for correlations between pairwise differences in mean standard length among populations (Bayesian estimates for bimodal samples and arithmetic means of unimodal samples) and (a) pairwise straight-line distances among populations, (b) pairwise straight-line distances among populations within drainages, with an arbitrarily large distance assigned to distances between populations from different drainages, and (c) pairwise differences among populations in each of their straight-line distances to the sea.

1.3: Results

Significant interactions between sexes and populations in preliminary ANOVA analyses made detection of size divergence across populations difficult, so I analyzed sexes separately within each age category, for a total of four tests of size differences among populations. (However, population size differences were significant in preliminary two-way ANOVAs, for both age 1 and 2+, with sex and population as fixed effects.) Differences in standard length across populations were significant for both males and females within age classes (1 and 2+;

Table 1.4, Figures 1.2 and 1.3). Analyses with *ln*-transformed standard lengths did not differ qualitatively from results presented here.

Thus, I found significant size divergence among Cook Inlet, AK, populations of Threespine Stickleback. Over all sexes and ages, mean standard lengths differed among populations by a factor of 2.39 (between 31.30 and 74.66 mm; Fig. 1.3), which is similar to the 2-fold difference in body size I expected based on estimates for size differences between anadromous and lake stickleback (Baker *et al.* 2008). However, some of this variation in body size may be due to differences in age structure across populations (Maccoll 2009), and stickleback females are typically larger than males (Baker 1994). Standard lengths for presumed age 1 females and males ranged from 31.30 to 61.38 mm (approximately 2-fold difference) and 31.51 to 46.82 mm (approximately 1.5-fold difference), respectively, across populations according to Bayesian estimates for bimodal, and arithmetic means for unimodal, distributions (Fig. 1.3). For age 2+ females and males, standard lengths ranged from 41.89 to 74.66 mm (approximately 1.8-fold difference) and 40.62 to 61.06 mm (approximately 1.5-fold difference), respectively, across populations (Fig. 1.3). Thus, within age categories and sexes, I still found significant size divergence among populations (Table 1.4). Unfortunately, since many populations consisted largely of only one or the other age class, I could not determine the magnitude of the effect of age structure differences on the variation in size. Also, due to a significant interaction between the effects of population and sex, I could not determine the magnitude of the effect of sex on size differences. However, within age and sex categories, population accounted for between 70 and 86% of the variation in body size (Table 1.4). Within this small geographic region, I did not expect large differences in climate. Thus, the size differences across this region suggest either that local conditions play a large role in the evolution or plasticity of body size in stickleback populations (see also Moodie and Reimchen 1976), and that these local conditions must vary considerably across lakes, or that historical processes could explain size divergence.

Francis *et al.* (1986) examined size and other morphological trait variation across some lake populations in Cook Inlet (only four of which matched the lakes I chose for this study on the basis of size divergence). They found that size was a large factor that discriminated population groups, suggesting large size divergence among populations. They found a significant correlation of general size with interpopulation distances and geographic distance from the sea, suggesting that "isolation by distance, gene flow within drainages, or recent common ancestry of adjacent populations" contributed to the variation rather than local ecological factors, or that local conditions must be correlated with interpopulation distances and distance to the sea. Unlike Francis et al. (1986), where effects of neither sex nor age structure on size were accounted for, I ran separate correlation analyses for age 1 females, age 1 males, age 2+ females, and age 2+ males and found no significant correlations between standard length differences among populations and pairwise population distances, drainage affiliation, or distance to the sea (Mantel tests; all $n > 105$, $P > 0.05$). Thus, the degree of size divergence among populations suggests that, despite recent common ancestry and potential gene flow within drainages, considerable adaptation or plastic responses to local conditions have contributed.

1.4: Discussion

The ecological factors affecting body size within Threespine Stickleback populations are currently unknown, but are of considerable interest. Trout predation was thought to explain the evolution of gigantism in some stickleback populations, where mean adult body length is between 80-115 mm (McPhail 1977, Moodie 1972b, Reimchen 1990). While there is some support for the predation hypothesis (Moodie and Reimchen 1976; Reimchen 1990), levels of predation are difficult to quantify (Bell 1984) and have so far only been found to coincide with larger maximum body size across stickleback populations in the Queen Charlotte Islands, British Columbia, Canada (Moodie and Reimchen 1976). Size-assortative mating has been implicated as the basis for reproductive isolation between sympatric benthic-limnetic species pairs, stream-lake pairs, and anadromous-freshwater pairs of sticklebacks (e.g., Borland 1986, Rowland 1989a, McPhail 1994, Nagel and Schluter 1998, Rundle and Schluter 1998, Ishikawa and Mori 2000, McKinnon *et al.* 2004, Maccoll 2009, and Matthews *et al.* 2010). Female and male sticklebacks have each been shown to prefer larger-bodied mates within and sometimes across morphotypes between allopatric populations (Moodie 1982; Rowland 1982, 1989a, 1989b). Thus, intersexual selection may explain the evolution of body size differences among stickleback populations (Wootton 1972, Moodie 1982, Rowland 1982). Finally, female fecundity is correlated with body size, and selection for clutch size in habitats with variable resource availability may determine a correlated response in the evolution of body size differences (Hagen 1967, Moodie 1972a, Wootton 1973, McPhail 1977, Craig and FitzGerald 1982, Crivelli and Britton 1987, Rowland 1989a).

Alternatively, size divergence may reflect plastic responses to local habitat conditions. Variation across habitats in lake size (e.g., Moodie and Reimchen 1976) and population density (e.g., Hatfield 1997), temperature (e.g., Crivelli and Britton 1987), resource availability (e.g., Allen and Wootton 1982, Wootton 1973), abundance of predators and competitors (e.g., Moodie 1972b, Moodie and Reimchen 1976, Reimchen 1990), and amount of available shelter (e.g., Moodie 1972a) have the potential to trigger differential plastic responses in life history traits across populations as opposed to adaptive responses. Although the effects of genetic drift and phenotypic plasticity cannot be ruled out, the relatively large sizes of stickleback populations (e.g., Reimchen 1990), large and temporally stable intrapopulation variation in body size (Wootton 1984, Table 1.5), and the expected high heritabilities of growth rates and size/age at maturity (e.g., McPhail 1977; Snyder 1991a, b; McKinnon and Rundle 2002) suggest a larger role for natural selection.

Anadromous Threespine Stickleback are thought to be larger-bodied than freshwater stickleback populations (e.g., Baker 1994). However, data from Yershov and Sukhotin (2015) and Rollins *et al.* (2017) show that most anadromous fish returning to spawn are older than previously thought, with the majority maturing at 2 and 3. Due to the near absence of breeding age 1 anadromous fish (Yershov and Sukhotin 2015, Rollins et al. 2017), many age 2 and 3 anadromous fish were previously thought to be age 1 and 2, respectively, on the basis of size frequency distributions, since the two size modes were assumed to represent age 1 and 2 fish instead of age 2 and 3 fish (e.g., reviews in Baker 1994, Baker *et al.* 2008). Thus, the smaller size mode, which was thought to represent age 1 anadromous fish (but is actually mostly comprised of age 2 fish; Table 1.3), were previously compared to the smaller size mode of freshwater populations, which truly does represent age 1 fish in most cases (Table 1.3). Here, I show that Rabbit Slough anadromous Threespine Stickleback that are presumably age 2 (represented in the smaller size mode; Table 1.3, Rollins et al. 2017) are not significantly larger in size than most age 2 freshwater fish (represented in the larger size mode; Fig. 1.2 and 1.3), although the size of age 2 Rabbit Slough fish used here was likely an underestimate of mean size for this age class since many in the larger size mode were probably also age 2 (Rollins *et al.* 2017). Thus, anadromous stickleback may not grow faster than freshwater populations. However, these conclusions are tentative until confirmed using bony structures to determine age, since age overlap between size modes in size-frequency distributions can be considerable (Rollins *et al.* 2017 and preliminary data).

Accounting for age structure differences among populations is crucial when comparing growth rates across populations, defined in this study as differences in relative size attained by ages 1 or 2. Others have found that ages based on structures such as otoliths, scales, or spines correlate well with estimates from size-frequency distributions (Greenbank and Nelson 1959, Hagen and Gilbertson 1972, Hay 1974). While I remain skeptical given the confusion that has arisen from misinterpretation of size-frequency distributions (e.g., Baker 1994) and the considerable overlap in size between age classes found elsewhere (Rollins *et al.* 2017), I used my best estimates from a combination of size-frequency distributions and preliminary otolith data to determine age modes for the present study. I found significant size and growth rate divergence among populations of Threespine Stickleback within age cohorts and sexes. I presumed that this size and growth divergence represents adaptation or plastic responses to local ecological conditions because I found no correlation with interpopulation distances, drainage affiliation, or distance to the sea. My data also suggested substantial age structure and possibly life span differences across populations in Cook Inlet (Fig. 1.1).

1.5: Tables and Figures

Table 1.1. Dates of collection (all in June), location, and sample sizes for each population of *G. aculeatus* measured in this study. The first 21 rows describe lake (L.) stickleback populations and the last row describes an anadromous stickleback population. MatSu = Matanuska-Susitna Valley and Kenai = Kenai Peninsula.

Population (Abbreviation)	Collection Day/Year	Geographic Region	Drainage	Latitude (°N)	Longitude (°W)	*n* (females)	*n* (males)	*n* (other)
Bear Paw L. (BP)	21/2012	MatSu	Fish Creek	61.616	149.755	181	214	24
Beverley L. (BV)	02/2012	MatSu	Fish Creek	61.614	149.573	75	31	28
Cheri L. (CH)	04/2012	MatSu	Fish Creek	61.601	149.736	183	59	0
Frog L. (FG)	21/2013	MatSu	Fish Creek	61.614	149.723	191	46	31
Herkimer L. (HK)	04/2012	MatSu	Fish Creek	61.572	149.698	371	480	11
Kalmbach L. (KB)	02/2012	MatSu	Fish Creek	61.608	149.573	66	129	19
Lazy L. (LZ)	04/2012	MatSu	Fish Creek	61.572	149.805	297	127	87
Morvro L. (MO)	02/2012	MatSu	Fish Creek	61.606	149.782	323	168	24
Jack L. (JK)	06/2012	MatSu	Little Susitna R.	61.728	150.032	523	385	0
East Sunshine L. (ES)	11/2012	MatSu	Susitna R.	62.181	150.038	132	190	0
South Friend L. (SF)	11/2012	MatSu	Susitna R.	62.138	150.051	81	51	0
Willow L. (WW)	07/2012	MatSu	Susitna R.	61.744	150.059	104	15	0
Barley L. (BL)	05 and 15/2012	MatSu	Knik Arm	61.361	150.084	409	232	0
Carpenter L. (CP)	05 and 15/2012	MatSu	Knik Arm	61.434	150.047	116	39	90
Mud L. (MD)	03/2012	MatSu	Knik R.	61.565	148.947	318	146	0
Neklason L. (NL)	07/2012	MatSu	Palmer Slough	61.629	149.270	177	122	31
Kidney L. (KN)	14/2012	Kenai	Cook Inlet	60.665	151.267	53	57	0
Kingsley L. (KL)	14/2012	Kenai	Cook Inlet	60.285	151.347	39	14	0
Headquarters L. (HQ)	14/2012	Kenai	Kasilof R.	60.462	151.024	33	75	10
Mosquito L. (MQ)	16/2012	Kenai	Kenai R.	60.633	150.819	109	60	14
Picnic L. (PN)	16/2012	Kenai	Kenai R.	60.548	150.272	159	143	0
Rabbit Slough (RS)	10/2013	Anadromous	N/A	61.536	149.253	80	52	0

Table 1.2. Otolith age data used to interpret age cohorts suggested by size modes of size-frequency distributions for *G. aculeatus* (population abbreviations and descriptions in Table 1.1).

Population	Female Age				Male Age			
	1	2	3	4	1	2	3	Total *n*
FG	7	28	0	0	20	13	1	69
HK	14	6	0	0	30	2	0	52
MO	62	4	0	0	25	3	0	94
JK	0	0	1	0	6	2	0	9
ES	1	2	1	0	0	1	3	8
SF	0	2	0	0	0	5	0	7
WW	1	5	0	0	2	1	0	9
BL	1	41	1	0	2	34	0	79
CP	1	10	0	0	1	1	0	13
MD	29	17	3	0	17	7	1	74
KN	1	3	1	0	8	38	3	74
KL	0	6	1	1	0	2	0	10

Table 1.3. Modality of size-frequency distributions determined using methods in Brewer (2003; see text); the proportions (p_1) of fish found in the first, or smaller-bodied, size class when distributions were bimodal; and the interpretation of size modes in size-frequency distributions from model fitting and otolith data (Table 1.2; Rollins *et al.* 2017 for RS) across populations of *G. aculeatus* (population abbreviations and descriptions in Table 1.1).

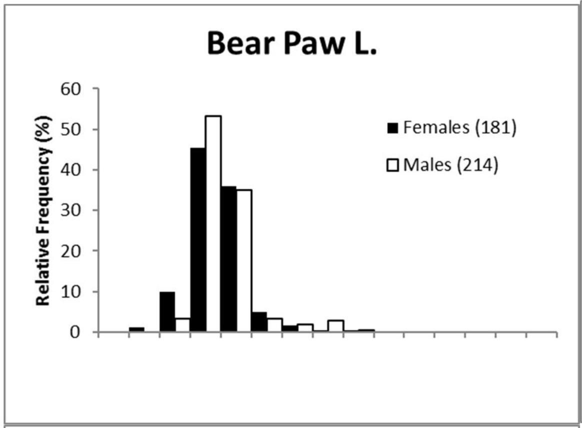
Sample	Sex	AICc-1	AICc-2	ΔAIC	Modality (Support)	p_1 (SD)	Ages Known	Interpretation of Modes
BP	F	237	204	32.94	bimodal (strong)	0.9693 (0.0134)		1, 2+
	M	281	145	135.95	bimodal (strong)	0.9404 (0.0162)		1, 2+
BV	F	177	158	18.89	bimodal (strong)	0.2736 (0.0569)		1, 2+
	M	68	44	23.42	bimodal (strong)	0.7577 (0.0736)		1, 2+
CH	F	383	369	13.61	bimodal (strong)	0.1767 (0.0574)	*	1, 2+
	M	150	150	0.14	equivalent	0.5566 (0.1595)	*	1, 2+
FG	F	509	483	25.70	bimodal (strong)	0.1368 (0.0334)	*	1, 2+
	M	96	94	2.40	equivalent	0.4448 (0.1340)	*	1, 2+
HK	F	792	778	13.99	bimodal (strong)	0.9043 (0.0422)	*	1, 2+
	M	763	787	-23.74	unimodal (strong)		*	1
KB	F	206	189	17.85	bimodal (strong)	0.2368 (0.0618)		1, 2+
	M	373	357	16.56	bimodal (strong)	0.6617 (0.0574)		1, 2+
LZ	F	465	360	104.47	bimodal (strong)	0.2464 (0.0261)		1, 2+
	M	241	220	20.82	bimodal (strong)	0.3022 (0.0476)		1, 2+
MO	F	495	468	26.78	bimodal (strong)	0.9824 (0.0114)	*	1, 2+
	M	221	219	2.38	equivalent		*	1, 2+
JK	F	854	696	158.44	bimodal (strong)	0.8822 (0.0159)	*	1, 2+
	M	526	492	34.63	bimodal (strong)	0.9605 (0.0130)	*	1, 2+
ES	F	277	286	-9.63	unimodal (moderate)		*	mix 1/2+
	M	397	394	2.54	equivalent		*	mix 1/2+
SF	F	78	83	-4.90	unimodal (moderate)			mix 1/2+
	M	77	81	-3.64	equivalent			mix 1/2+
WW	F	186	191	-4.49	unimodal (moderate)		*	mix 1/2+
BL	F	1012	990	21.58	bimodal (strong)	0.3751 (0.0381)	*	1, 2+
	M	373	383	-9.84	unimodal (moderate)		*	2+
CP	F	322	298	24.76	bimodal (strong)	0.2864 (0.0451)	*	1, 2+
	M	85	88	-3.52	equivalent	0.3293 (0.2244)	*	1, 2+
MD	F	732	599	133.48	bimodal (strong)	0.5063 (0.0289)	*	1, 2+
	M	281	281	-0.17	equivalent	0.6598 (0.1784)	*	1, 2+
NL	F	393	324	69.37	bimodal (strong)	0.3612 (0.0369)		1, 2+
	M	224	203	21.60	bimodal (strong)	0.7629 (0.0464)		1, 2+
KN	F	121	91	29.99	bimodal (strong)	0.9454 (0.0304)	*	1, 2+
	M	104	108	-3.65	equivalent	0.5135 (0.2298)	*	1, 2+
KL	F	79	85	-5.62	unimodal (moderate)		*	2+
HQ	F	74	79	-4.94	unimodal (moderate)			mix 1/2+
	M	127	132	-4.30	unimodal (moderate)			mix 1/2+
MQ	F	263	243	19.91	bimodal (strong)	0.8191 (0.0447)		1, 2+
	M	138	143	-5.33	unimodal (moderate)			1
PN	F	371	302	69.33	bimodal (strong)	0.1535 (0.0297)		1, 2+
	M	340	289	50.35	bimodal (strong)	0.2452 (0.0387)		1, 2+
RS	F	71	36	34.90	bimodal (strong)	0.0489 (0.0237)	*	2, mix 2/3+
	M	35	11	24.22	bimodal (strong)	0.0688 (0.0355)	*	2, mix 2/3+

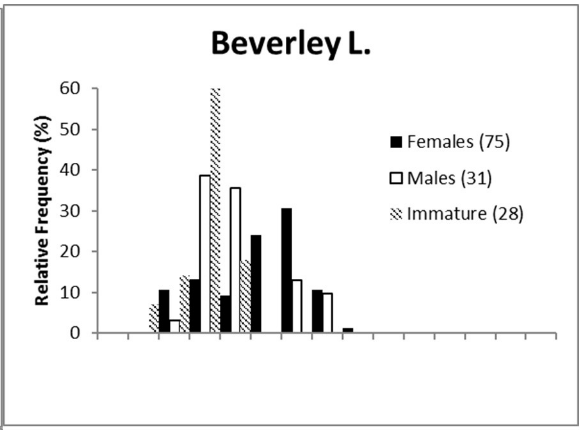
Table 1.4. Results of four separate ANOVA tests for differences in standard length across *G. aculeatus* populations within sex and age classes. *df* = degrees of freedom; α = 0.05.

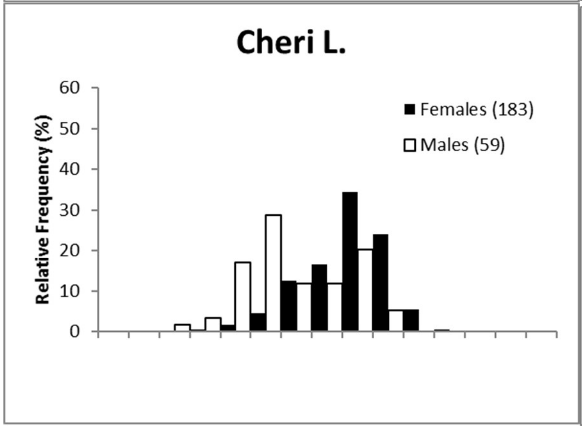
Sex	Age	df_{among}	df_{within}	*F*-stat	*P*-value	R^2
Female	1	15	2014	401	<0.0001	0.749
Male	1	15	1951	426	<0.0001	0.766
Female	2+	17	1553	561	<0.0001	0.860
Male	2+	13	447	79	<0.0001	0.697

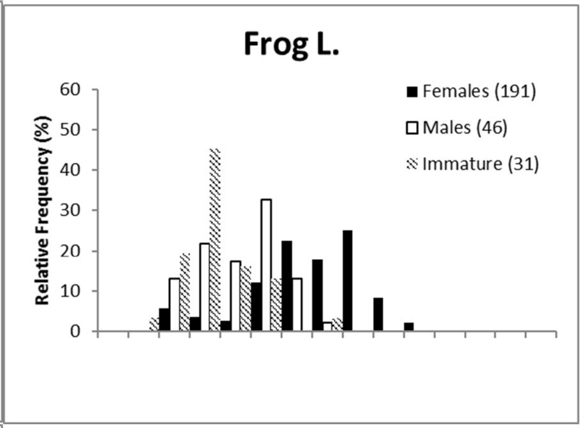
Table 1.5. Literature review results which reveal temporal stability in mean standard lengths (SL; mm) for five populations sampled in this study. Mean SL (± standard deviation) of older samples from (1) BP, FG, and ES; (2) LZ; and (3) RS populations were taken from Francis *et al.* (1986), Baker *et al.* (1998), and Rollins *et al.* (2017), respectively. Single samples (from a single year) were collected sometime between May and August of 1982 or 1983 in Francis *et al.* (1986) and 1983 – 1992 in Baker *et al.* (1998). In Rollins *et al.* (2017), standard length data from 12 separate samples taken over a 22 year interval between 1992 – 2014 were averaged. The difference in mean SL between older samples and samples from this study (collected in 2012 or 2013) are provided along with the estimated range of time (years) between collections used to estimate mean SL.

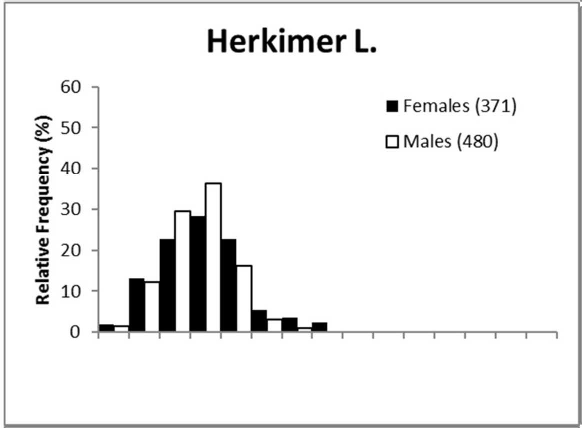
	RS	FG	ES	BP	LZ
$\overline{SL}$ (*SD*) this study	67.67 (4.17)	51.14 (10.41)	47.23 (7.35)	39.74 (4.84)	37.85 (6.13)
$\overline{SL}$ (*SD*) previous study	68.26 (4.57)	48.39 (8.44)	47.03 (7.84)	42.00 (7.26)	44.2 (5.9)
Difference in $\overline{SL}$ between studies	-0.59	2.75	0.20	-2.26	-6.35
Time interval between collection dates	-1 to 21	29 to 30	29 to 30	29 to 30	20 to 29

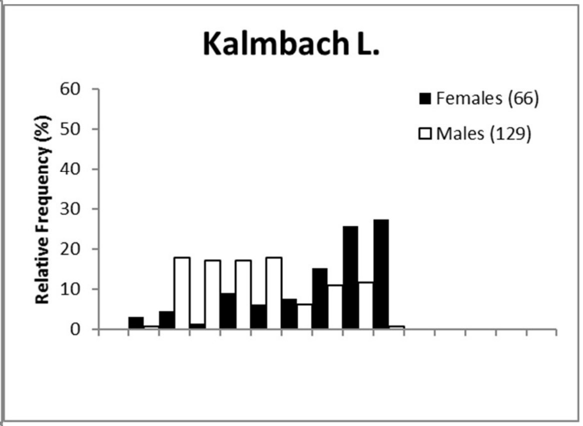

Bear Paw L.
Relative Frequency (%)
0
10
20
30
40
50
60
■ Females (181)
□ Males (214)

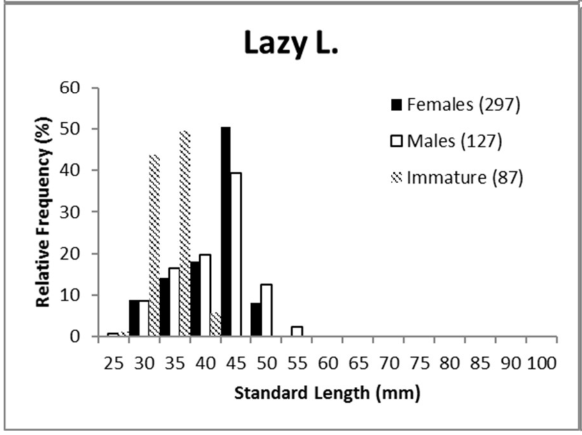

Beverley L.
Relative Frequency (%)
0
10
20
30
40
50
60
■ Females (75)
□ Males (31)
▨ Immature (28)

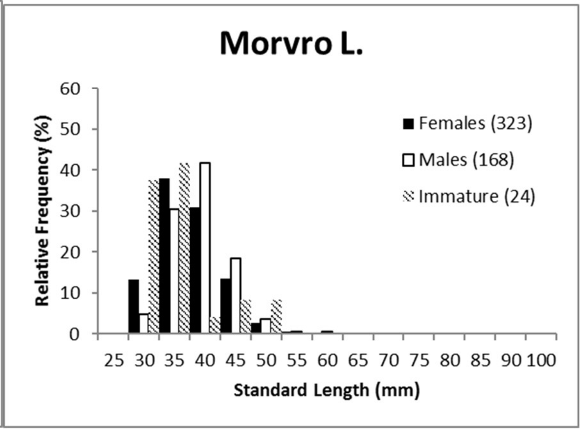

Cheri L.
Relative Frequency (%)
0
10
20
30
40
50
60
■ Females (183)
□ Males (59)

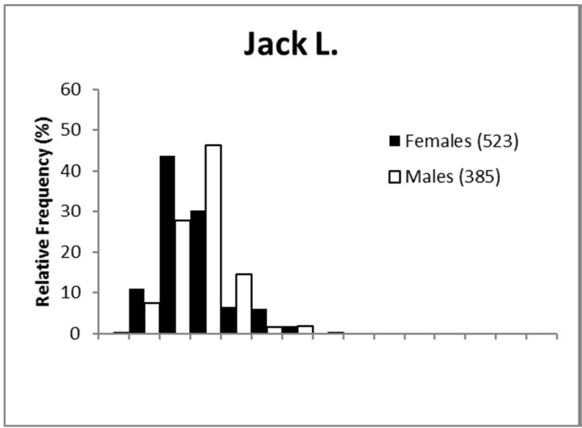

Frog L.
Relative Frequency (%)
0
10
20
30
40
50
60
■ Females (191)
□ Males (46)
▨ Immature (31)

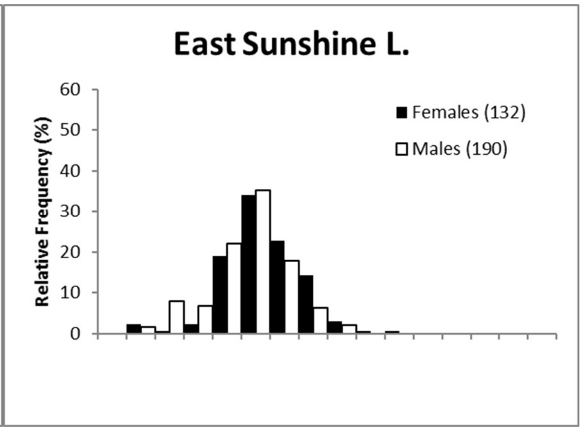

Herkimer L.
Relative Frequency (%)
0
10
20
30
40
50
60
■ Females (371)
□ Males (480)

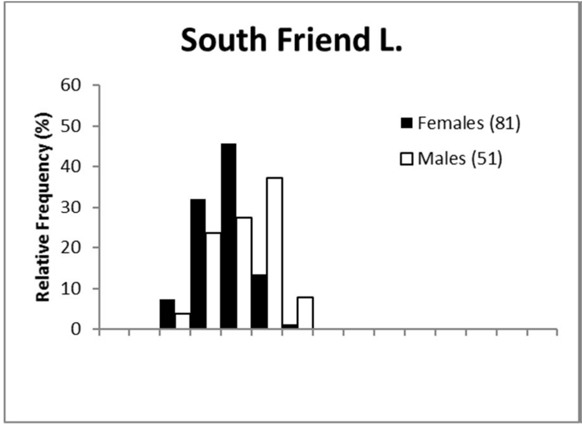

Kalmbach L.
Relative Frequency (%)
0
10
20
30
40
50
60
■ Females (66)
□ Males (129)

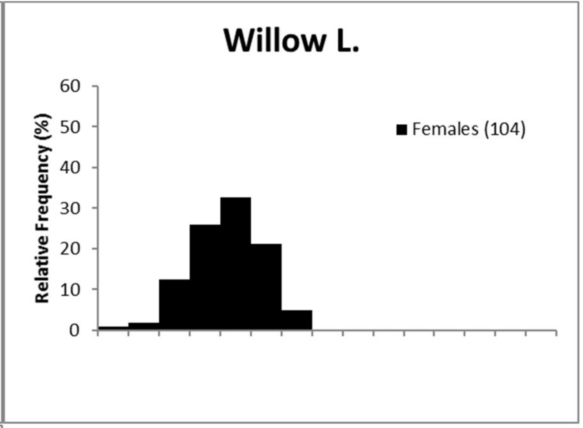

Lazy L.
Relative Frequency (%)
0
10
20
30
40
50
60
■ Females (297)
□ Males (127)
▨ Immature (87)
25 30 35 40 45 50 55 60 65 70 75 80 85 90 100
Standard Length (mm)

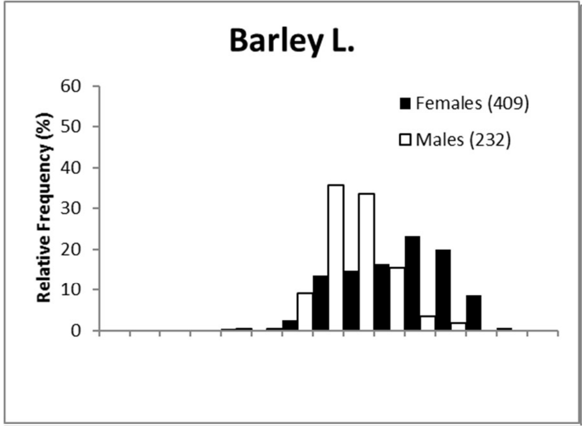

Morvro L.
Relative Frequency (%)
0
10
20
30
40
50
60
■ Females (323)
□ Males (168)
▨ Immature (24)
25 30 35 40 45 50 55 60 65 70 75 80 85 90 100
Standard Length (mm)

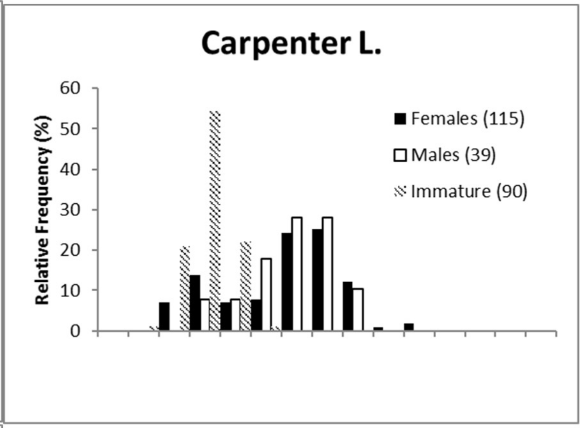

Jack L.
Relative Frequency (%)
0
10
20
30
40
50
60
■ Females (523)
☐ Males (385)

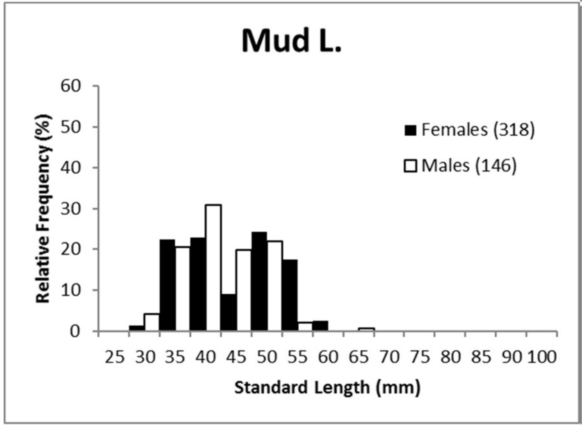

East Sunshine L.
Relative Frequency (%)
0
10
20
30
40
50
60
■ Females (132)
☐ Males (190)

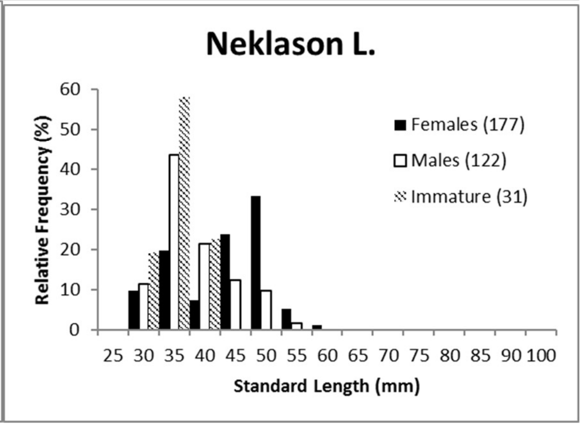

South Friend L.
Relative Frequency (%)
0
10
20
30
40
50
60
■ Females (81)
☐ Males (51)

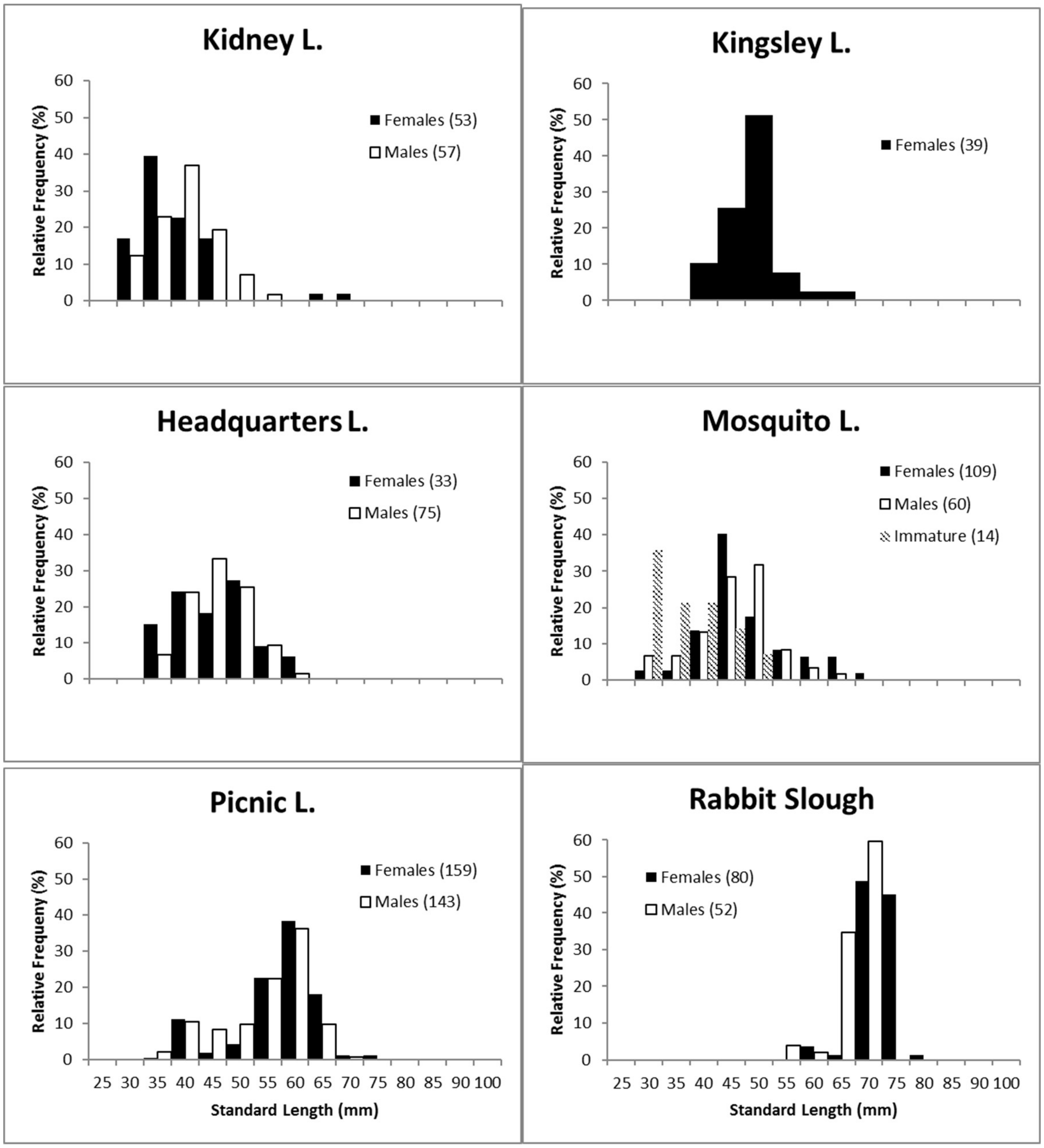

Willow L.
Relative Frequency (%)
0
10
20
30
40
50
60
■ Females (104)


Barley L.
Relative Frequency (%)
0
10
20
30
40
50
60
■ Females (409)
☐ Males (232)


Carpenter L.
Relative Frequency (%)
0
10
20
30
40
50
60
■ Females (115)
☐ Males (39)
▨ Immature (90)


Mud L.
Relative Frequency (%)
0
10
20
30
40
50
60
■ Females (318)
☐ Males (146)
25 30 35 40 45 50 55 60 65 70 75 80 85 90 100
Standard Length (mm)


Neklason L.
Relative Frequency (%)
0
10
20
30
40
50
60
■ Females (177)
☐ Males (122)
▨ Immature (31)
25 30 35 40 45 50 55 60 65 70 75 80 85 90 100
Standard Length (mm)



Kidney L.

Kingsley L.

Headquarters L.

Mosquito L.

Picnic L.

Rabbit Slough

Figure 1.1. Size-frequency distributions for individual populations of *G. aculeatus*. Y-axes are not consistent across plots, and bins are right-handed. Sample sizes for each sex are indicated in parentheses. Note: Raw data, and not distributions shown here with 5 mm size bins, were used for tests of fit to a single normal or a mixture of two normal distributions. The method used here to determine fit of data to alternative models explores many possible partitions of observations into two components and does not consider only one type of distribution with a set bin width (Brewer 2003).

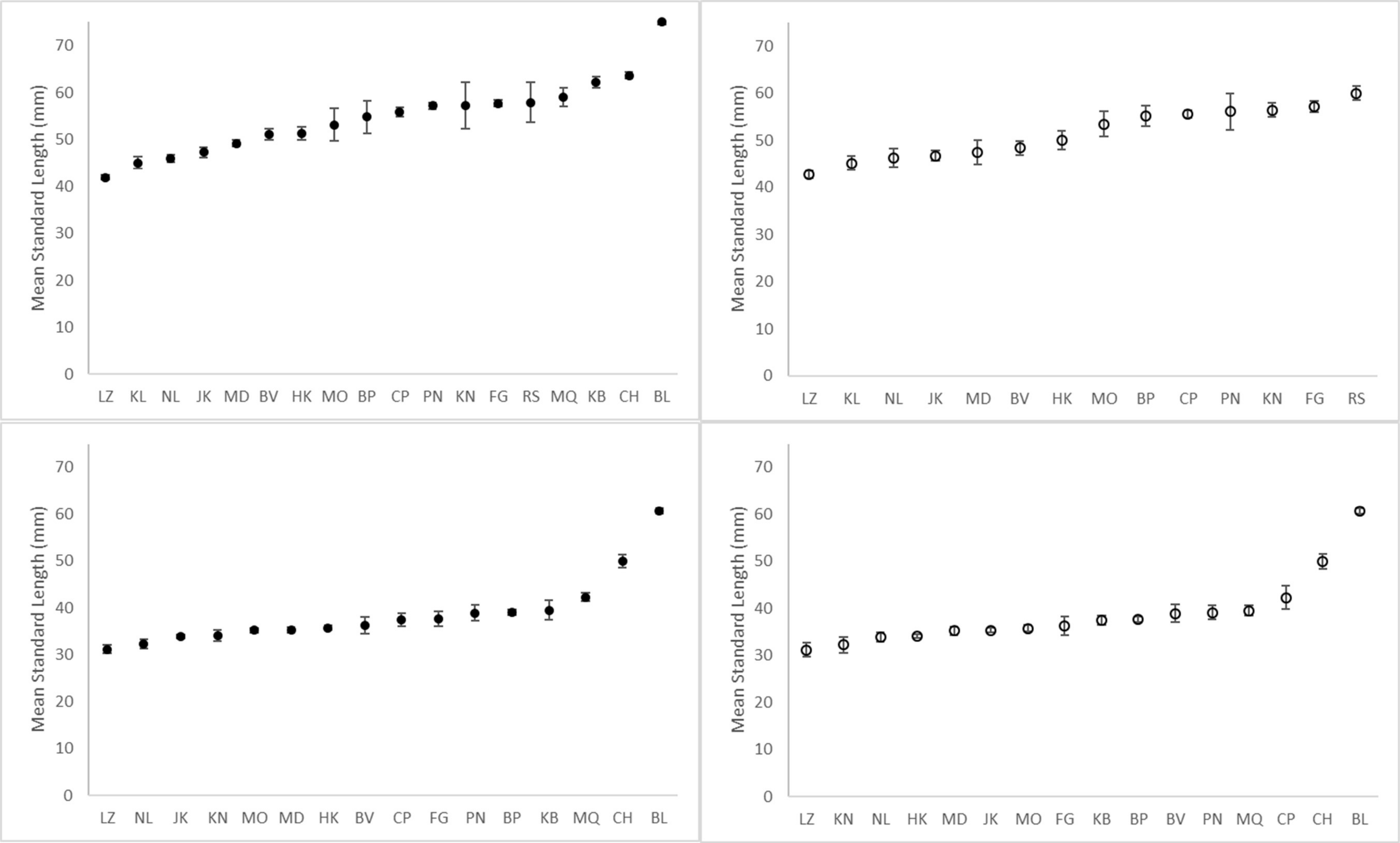


Figure 1.2. Mean standard length ± 95% Tukey-adjusted confidence intervals for age 2+ (top panels) females (closed circles) and males (open circles) and age 1 (bottom panels) females and males from multiple comparisons of populations used in four separate ANOVA analyses.

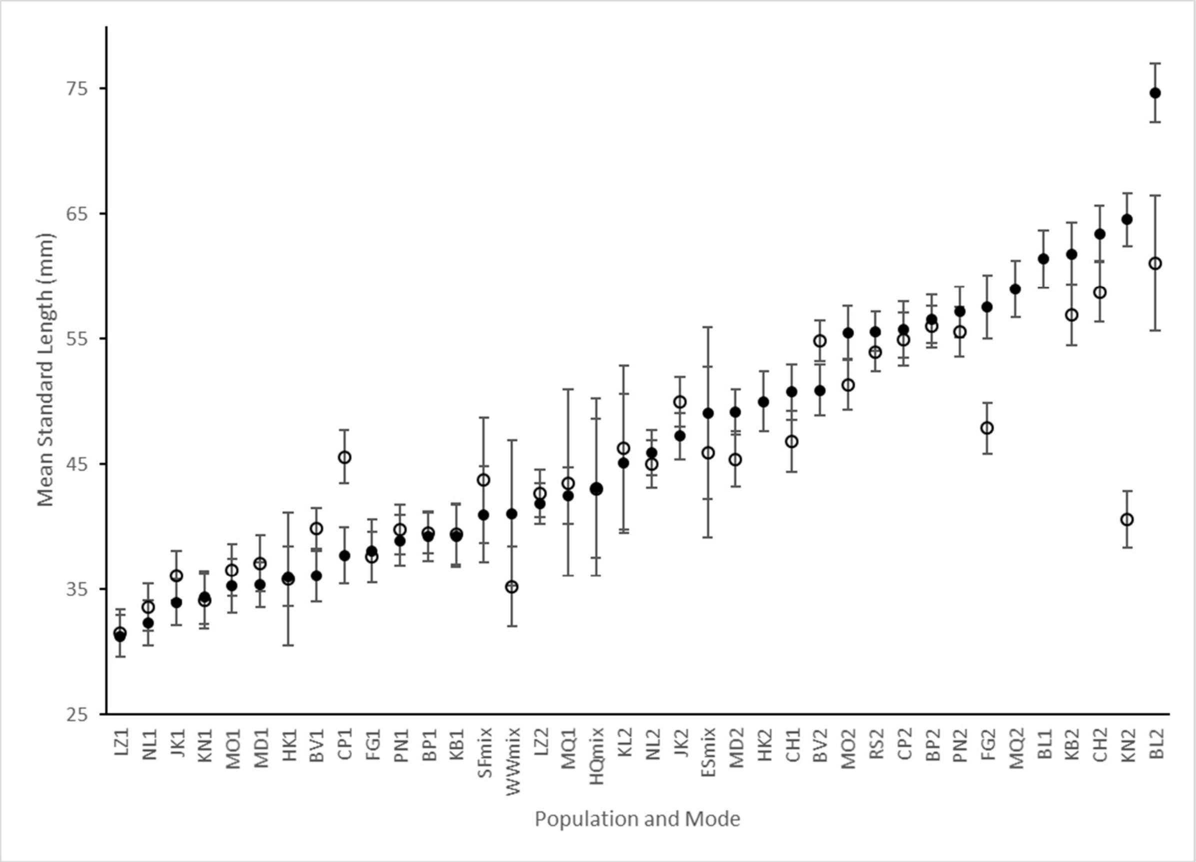


Figure 1.3. Bayesian estimates or arithmetic mean standard length ± standard deviation where bimodal or unimodal, respectively. Females and males are indicated by closed and open circles, respectively. Population abbreviations as in Table 1.1. 1 = presumed or confirmed age 1, 2 = presumed or confirmed age 2+, mix = presumed or confirmed overlapping/unimodal mixture of age 1 and 2+ fish.

Chapter 2: Divergence in Life Histories (General Body Size and Age Structure, Longevity, and Age and Size at Maturity) Among Populations of Threespine Stickleback in Cook Inlet, AK Lakes

2.1: Introduction

Body size differences often characterize the various morphs or species involved in adaptive radiations (Schluter 2000), and sometimes body size is either directly or indirectly responsible for maintaining divergence between morphs or species in adaptive radiations, including that of Threespine Stickleback (*Gasterosteus aculeatus*; e.g., Wootton 1972, Moodie 1982, Rowland 1982, Borland 1986, McPhail 1994, Nagel and Schluter 1998, Rundle and Schluter 1998, McKinnon *et al.* 2004, Ishikawa and Mori 2000, Maccoll 2009, and Matthews *et al.* 2010). However, it remains unclear (1) whether body size is the trait under selection or whether other aspects of the stickleback life history that are correlated with body size or that cause differences in body size are the target of selection, (2) if body size is the target of selection, how other aspects of the stickleback life history which are correlated with or may cause differences in body size are affected by selection for body size, and (3) whether body size along with other aspects of the stickleback life history evolve in concert as a suite of co-adapted traits. Expected trade-offs between components of a population or species life history should not only constrain life history evolution, but also lead to an expectation of changes in all the component traits of a life history if one or another has indeed changed in the context of divergent selection (Stearns 1992). Thus, where we see divergence in body size, one of the following must be the case: (1) growth rates have diverged among morphs or species, perhaps as a consequence of partitioning available resources, or (2) selection on a different aspect of life history, like age or size at maturity, may instead lead to greater expected longevity, with concomitant increases in body size because individuals simply have more time to grow (an expectation specifically for species with indeterminate growth; Stearns 1992).

The Threespine Stickleback radiation is characterized by colonization by anadromous fish of numerous freshwater habitats across the Holarctic after glacial retreat between 9.5 to 15 KYA (e.g., McPhail and Lindsey 1970, Bell and Foster 1994, Reger and Pinney 1996). Threespine Stickleback are known to exhibit size divergence, both between anadromous and freshwater ecotypes and among freshwater populations (e.g., Moodie and Reimchen 1976, McPhail 1977, Wootton 1984, Reimchen *et al.* 1985, Baker 1994, McKinnon and Rundle 2002). Differences in longevity and age at maturity may underlie or have evolved as a consequence of body size evolution in the stickleback adaptive radiation. While I cannot determine which life history trait has been the target of selection, it is likely that the various components of the Threespine Stickleback life history have evolved in concert since colonization of freshwater. In fact, body size may have evolved as a correlated response to selection on a different aspect of the life history (e.g., Bell 1984, Chapter 1).

In this study, I investigated divergence among freshwater populations in body size and other components of the stickleback life history that may have changed in concert with body size in the stickleback radiation: age at maturity, size at maturity, age structure, and longevity. Previous research (Chapter 1) suggests that (a) there are differences in age structure (and potentially longevity) between freshwater and anadromous sticklebacks and potentially among freshwater stickleback populations and (b) body size divergence among freshwater populations of Threespine Stickleback is significant within putative age categories, suggesting growth rate differences have also evolved. These conclusions, however, were based on size-at-age data where age was determined based on size-frequency distributions with some support from otolith data in which to validate interpretations of size modes within the distributions. Since such methodology can lead to misinterpretations, and hence, false conclusions (Jones and Hynes 1950; Yershov and Sukhotin 2015; Rollins *et al.* 2017), this study used otoliths to determine age of all specimens and asked (a) is there divergence in age structure, and (b) do age structure differences across populations explain interpopulation size divergence? I also determined whether age and size at maturity of stickleback females differ among populations. I predicted that all components of the species life history must have evolved in concert with any changes due to local adaptation. Thus, I predicted significant interpopulation differences in age structure and longevity, adult body size and growth rates, and age and size at maturity.

Size-assortative mating may be responsible for maintaining niche partitioning when oceanic and freshwater sticklebacks were sympatric (i.e., when freshwater habitats were open to the sea, with the potential for gene flow with anadromous ancestral populations), as we see in currently sympatric scenarios (e.g., Borland 1986, Ishikawa and Mori 2000, McKinnon *et al.* 2004). This may explain evolutionary divergence in body size between marine and freshwater sticklebacks, where marine forms are known to be often larger-bodied than freshwater sticklebacks (Baker 1994, McPhail 1994). In addition, it is common among anadromous fish species that those individuals which colonize freshwater, and either become landlocked or lose the inclination to migrate out to sea, experience dwarfism (reviewed in Roff 1992). The cause for this directional change is often thought to be initially plastic (Roff 1992) and may be due to density-dependent growth in a smaller, less-suitable habitat. On a longer time-scale, heritable differences in body size between such ecotypes may be an evolutionary response to limited resources or reduction in extrinsic mortality due to lack of native predators in the freshwater environment (Palkovacs 2003). However, neither of the above hypotheses, in concert or alone, explains why some freshwater populations are as large or larger than anadromous sticklebacks (e.g., Moodie 1972a, Moodie and Reimchen 1976, Bell 1984), or why large differences in body size exist among freshwater populations colonized by relatively phenotypically-uniform oceanic ancestors (e.g., Moodie and Reimchen 1976, Reimchen *et al.* 1985, Baker 1994, Wootton 1994, McKinnon and Rundle 2002, Chapter 1). These observations and others led Rollins (Chapter 1) to conclude that body size divergence among freshwater threespine stickleback populations may be due to plastic responses or adaptation to small-scale, local habitat conditions which themselves must vary considerably. In this study, I also investigated the role of (a) historical processes like gene flow and time since divergence from oceanic stickleback ancestors and (b) lake-specific ecological factors like lake size, resource availability, and presence of predators on interpopulation growth differences. To evaluate whether historical processes like isolation by distance, gene flow within drainages, and descent from a common oceanic ancestor have played a role in growth differences, I tested for correlations between interpopulation differences in growth rate (age-specific size) and (a) population pairwise geographic distances, (b) drainage affiliation, and (c) distance to the sea, respectively. I predicted positive correlations if historical processes have contributed to interpopulation growth variation. I predicted positive correlations between lake-specific environmental variables and growth rates (age-specific size) if plastic responses or local adaptation to ecological factors have contributed to interpopulation growth variation.

2.2: Methods

I collected Threespine Stickleback using unbaited, fine-mesh (1/8” or ¼”) minnow traps set near shore from 26 lakes in the Matanuska-Susitna Valley and Kenai Peninsula of the Cook Inlet watershed, from one lake in the Alaskan Peninsula in the Bristol Bay watershed, and from three streams or bays in southern Alaska with anadromous fish (Table 2.1). The 26 lakes in the Cook Inlet region were chosen to span the range of body sizes found across the region based on preliminary (1990) collections. The lake sample (JJ) from the Bristol Bay watershed was provided by E. Bowles. All samples (except JJ) were frozen on dry ice after capture and stored at -80°C until processing.

I measured standard length (SL; distance from the tip of the rostrum to the last vertebra) of fish using digital calipers (±0.01 mm), weighed them on a digital balance (±0.01 or 0.0001 g), and determined sex by gonadal inspection. Female fish were classified as sexually “mature” according to McPhail (1977). Males’ maturity status could not be determined (McPhail 1977). Gonads were removed and weighed, and somatic wet weight (SWW) was computed by subtracting gonad weight from total wet weight. I determined fish age using sagittal otoliths (Fig. 2.1) extracted and prepared according to Rollins *et al.* (2014). Briefly, otoliths were cleaned after extraction to remove excess tissue, glued to glass slides, and sanded with fine sanding sheets if necessary to reveal annular growth rings. J.L.R. (10 years’ experience; 8 with stickleback) counted annuli according to Jones and Hynes (1950) at 200x magnification, and I assigned confidence scores for reads and excluded uninterpretable samples according to Rollins *et al.* (2014). Sample sizes of aged specimens are given in Fig. 2.2. A subsample of otoliths was read on four separate occasions across the time interval across which all otolith samples were processed (4.5 years). Precision on otolith counts was 100%.

I used a log-linear model to determine if age structure differences across populations and between sexes were significant (BIOMstat 4.11, Exeter Software, Setauket, NY; http://www.exetersoftware.com/). I report the results of marginal *G*-tests for row by column independence for the effect of population within sexes and for the effect of sex within populations. I used a *G*-test for row by column independence to determine if sex ratios differed across populations (BIOMstat 4.11). For sex ratio analyses, I added counts from formalin-fixed samples collected in the same year (or 2013 in some instances) used in the previous chapter to the frozen samples used in this study for age structure analyses (Table 2.1).

To determine if size divergence is present after accounting for the effect of age structure and sex differences across populations, I attempted three-way ANOVAs with age, population, and sex as fixed effects for both SL and SWW. However, most populations consisted almost entirely of either age 1 or 2 fish, precluding analysis with most of the data. Thus, I instead attempted two ANOVAs (age 1 and age 2 fish) each for SL and SWW with population and sex as fixed effects. Since interactions between population and sex were significant (age 1 SL: $P_{[5,210]} < 0.001$; age 2 SL: $P_{[8,239]} < 0.001$; age 1 SWW: $P_{[4,202]} < 0.001$; age 2 SWW: $P_{[7,222]} = 0.046$), I performed and report results for six separate ANOVAs testing for size divergence among populations within age and sex categories (age 1 females, age 2 females, age 3 females, age 1 males, age 2 males, and age 3 males) using both SL and SWW as dependent variables (for a total of 12 analyses). SL was *ln*-transformed and SWW was converted to mg and *ln*-transformed for analysis. I report values back-transformed to their original units. All ANOVAs were performed in SAS (SAS Institute, Inc. 2009).

To determine whether historical (isolation by distance, common ancestry, or gene flow) and ecological factors (lake-specific abiotic and biotic conditions) may affect size divergence, I repeated analyses described in Francis *et al.* (1986). For historical factors, I considered the effect of geographic distance between populations, drainage affiliation, and distance to the sea on size differences. I computed overland pairwise distances among populations and overland distances between each population and the sea using Google Earth (Google, Inc. 2017). I used Mantel tests (Mantel 1967; BIOMstat 4.11) for correlations of matrices of (a) pairwise distances among populations (Appendix Table S2.1) and (b) pairwise distances among populations within the same drainage, and an arbitrarily large distance assigned between populations not in the same drainage (Appendix Table S2.2), with pairwise differences in mean SL and SWW among populations within age and sex categories (separate analyses for age 1 and 2 females and males). I also tested for significance of correlations between distance to the sea (Appendix Table S2.3) and mean SL and SWW across populations within age and sex categories (product-moment correlation coefficients; BIOMstat 4.11). I also tested for correlations (product-moment correlation coefficients; BIOMstat 4.11) for mean SL and SWW with the following lake-specific ecological variables: lake surface area, presence/absence of predators, conductivity, pH, and concentrations of calcium, magnesium, chlorophyll A, total phosphorus, ammonia, and nitrate. Lake surface areas were estimated using KML Tools software (available at https://extension.unh.edu/kmlTools/) to compute the area of overlaid polygons on lake images in Google Earth (Google, Inc. 2017; Appendix Table S2.4). Unpublished data for other environmental variables (listed above) were provided by M.A. Bell and estimated according to Bourgeois *et al.* (1994). I used a Bonferroni adjustment to account for multiple comparisons. For all product-moment correlations, SL and SWW data were transformed as above for analysis.

Not all fish used in above analyses were sexually mature. Thus, I report descriptive statistics for female age-at-maturity, minimum size-at-maturity, and mean size-at-maturity for populations of Threespine Stickleback. I performed additional ANOVAs for population differences in size-at-maturity (SL and SWW; without accounting for age structure) using the subset of female stickleback that were classified as sexually mature. Only females were used for these analyses since I could not determine the maturity status of males from gonadal inspection. SL and SWW were transformed as above for analyses, and results reported in original units.

2.3: Results

Age structure differences among populations within sexes were significant ($G = 298.11$, $df = 18$, $P < 0.001$; Fig. 2.2). Age structure also differed between the sexes within populations ($G = 57.91$, $df = 10$, $P < 0.001$); females had larger frequencies of age 2+ relative to age 1 fish than males (Fig. 2.2). Sex ratios also differed significantly across populations ($G = 622.13$, $df = 29$, $P < 0.001$; Fig. 2.3).

Both SL and SWW differed significantly across populations for age 1, 2, and 3 females and males (Tables 2.2-2.6; Fig. 2.4 and 2.5; Appendix Fig. S2.1 and S2.2). SL and SWW also differed between sexes (and populations) in preliminary two-way ANOVAs within age categories, but significant population × sex interactions precluded interpretation of main effects, and the 12 separate analyses within sexes and ages precludes estimation of the effect of either sex or age on size differences. However, I note that sexual dimorphism for body size, which is reported frequently for stickleback studies, was present in this study for many populations within age categories (Tables 2.7 and 2.8).

Correlations between matrices of pairwise differences in mean SL and SWW across populations, within age and sex categories, and matrices of interpopulation distances or interpopulation distances within drainages, and arbitrarily large assigned interpopulation distances between populations not in the same drainage, were not statistically significant ($P > 0.05$ for all Mantel tests; Appendix Table S2.5). Population mean SL and SWW were not correlated with distance to the sea or any of the lake-specific ecological variables I tested ($P > 0.05$ for all but one correlation coefficient, which was not significant after Bonferroni adjustment for multiple comparisons; Appendix Table S2.6).

Populations differed significantly in female size-at-maturity ($F = 181.33$, $df_{among} = 7$, $df_{within} = 215$, $P < 0.001$, $R^2 = 0.855$; Table 2.9). Ages-at-maturity and both minimum and mean sizes-at-maturity are given in Table 2.10.

2.4: Discussion

Body size has presumably played an important role in speciation and divergent selection in the Threespine Stickleback adaptive radiation (e.g., Borland 1986, McPhail 1994, Schluter 1993, Nagel and Schluter 1998, Rundle and Schluter 1998, McKinnon *et al.* 2004, Ishikawa and Mori 2000, Maccoll 2009, and Matthews *et al.* 2010). I asked whether less conspicuous aspects of the life history of Threespine Stickleback have diverged in concert with body size among freshwater populations in the stickleback radiation. I collected Threespine Stickleback from numerous lakes in the Cook Inlet region, AK, USA, which were thought to represent the range of body sizes encountered in that region. I determined body size, age, sex, and female maturity of individuals from each lake to quantify differences in longevity, age-specific body size (growth rates), sex ratio, age at maturity, and size at maturity.

I detected significant differences in age structure among populations (Fig. 2.2). For seven out of 10 populations where sample sizes were sufficient to determine relative proportions of age 1 to age 2 fish, age 2 fish were more prevalent than age 1 fish (Fig. 2.2). Contrary to observations, I expected a higher relative frequency of younger fish since survivorship should be monotonically declining functions of age. I offer two explanations for my observations of greater numbers of age 2 relative to 1 fish. First, I may have collected fish in a year following either a depauperate spring reproductive season or a particularly harsh winter that differentially affected the younger fish, leaving only older and larger survivors. Second, my sample collections may be biased with respect to age or size. I used minnow traps placed very near shore and collected during the breeding season. Stickleback males may tend to nest near shore, where shelter among the benthos may be more prevalent, and are territorial (Kynard 1978), and thus tend to chase away younger, non-breeding or less competitive individuals (Rowland 1989a). I would thus expect a greater proportion of breeding-age fish in my collections if traps were placed on the breeding grounds, which may be avoided by younger, non-breeding individuals. The observation of age 2-biased catches for stickleback seen here are not unique to this study or catch year (e.g., Greenbank and Nelson 1959), lending support for the breeding ground explanation over the first hypothesis of a bad year for young fish. Thus, my samples appear to be biased towards disproportionate inclusion of breeding stickleback, and I thus cautiously interpret results herein as regards age structure. Trap bias may also have affected sex ratio estimates. Sex ratio in 20 of 30 stickleback populations were female-biased. Female-biased sex ratios are also noted elsewhere for stickleback catches (e.g., Coad and Power 1973 and citations therein) and may reflect reluctance for territorial males to enter the same traps or to travel outside their own territories during the breeding season. Since trapping during the spawning season and near-shore using minnow traps or beach seines is a common method in stickleback studies (e.g., Aneer 1973, Francis et al. 1986, Baker et al. 1998), some stickleback studies may be affected by this sampling bias. Therefore, stickleback researchers should consider alternative means of achieving samples if unbiased sampling of populations are necessary or desirable.

Regardless of the biased catches, it is apparent that stickleback in my freshwater populations have a 2 year lifespan. However, some populations consist predominantly of age 1 individuals and others age 2 (Fig. 2.2). I found a few age 3 and 4 fish in some of the populations, but these were comparatively rare (usually < 7% of each sample where they were detected). Anadromous stickleback were predominantly ages 3 and 2, with a few age 1 and 4 individuals (this study and Rollins *et al.* 2017). Annual and two-year lifespans based on otolith analysis are noted elsewhere for freshwater Threespine Stickleback (Jones and Hynes 1950 and citations therein, Greenbank and Nelson 1959, Mann 1971, Coad and Power 1973, Allen and Wootton 1982), and two- and three- year lifespans for anadromous stickleback (Aneer 1973, Yershov and Sukhotin 2015, Rollins *et al.* 2017).

I also found significant divergence in age- and sex- specific body size across populations (Tables 2.2-2.6, Fig. 2.4 and 2.5), which represented differences in rates of growth and confirmed the results of Rollins (Chapter 1) from size-frequency distributions across additional populations in this region. Mean lengths (SL) at age 1 ranged from 31.77-52.82 mm for females and 34.11-46.51 mm for males, respectively, across six freshwater populations (Table 2.7, Fig. 2.4). Mean SL at age 2 ranged from 38.06-70.05 mm for females and 38.86-63.75 mm for males across eight freshwater populations (Table 2.7, Fig. 2.4). Results for SWW mirrored those for SL (Table 2.8, Fig. 2.5). Estimates for size-at-age reported by others for freshwater populations of Pacific- and Atlantic- clade stickleback (reviewed in Wootton 1984) fell within the range of sizes

I found across a larger number of lakes. Barley Lake stickleback in this study were on average between 63-70 mm SL at age 2, and a single female age 3 Barley Lake stickleback was 84.14 mm SL. This size approached the minimum value in the range of mean sizes reported for "gigantic" sticklebacks (between 80 and 115 mm; Moodie 1972a, b; Moodie and Reimchen 1976; Bell 1984; Reimchen 1990), which can live up to 8 years instead of only 2 or 3 (Reimchen 1992).

Collections in multiple years will be necessary to establish the repeatability of life history divergence among populations. However, some evidence suggests that differences in life histories across populations are likely to be maintained to some extent across years. Preliminary collections of stickleback populations which suggested these studies were from a large sample of lakes across the Cook Inlet region taken in the summer of 1990. I did not have sufficient access to these samples to obtain measurements for comparison, but observations suggest that relative size differences among these populations are very similar to relative differences in size measured for the collections taken in 2012 in this study and for formalin-fixed collections from 2012 in Chapter 1. Also, M.A. Bell *et al.* have estimated size-frequency distributions for a few lake populations in the region (e.g., Bear Paw Lake) over the last 30 years, and collections across numerous populations (e.g., Frog L., Herkimer L., Morvro L., Willow L., Mud L., and Rabbit Slough; see Rollins et al. 2017 for Rabbit Slough) suggest that variation in mean size across years falls within a narrow to moderate range within a population, but more importantly, relative differences in mean size among populations have been maintained across many years (also see Table 1.5 in Chapter 1). In addition, M.A. Bell *et al.* have sampled at various points around the perimeter of Loberg Lake in the Cook Inlet region since about 1992. Morphological data from different points around the lake suggest that there is no real signature of spatial heterogeneity in the population. However, Loberg Lake is a very small and shallow lake, and all samples were taken near-shore using minnow traps.

I also confirmed (Chapter 1) lack of any significant correlations between growth rates (age- and size- specific length and weight) and geographical proximity among or within drainages and distance to the sea (Appendix Table S2.5 and S2.6), ruling out strictly historical explanations for size divergence among populations, like the potential for interpopulation gene flow, isolation by distance, or time since divergence from an anadromous common ancestor. I did not find any correlations between growth and lake-specific ecological variables, including lake surface area, presence/absence of predators, ion concentrations, or proxies for primary productivity (Appendix Table S2.6).

Like many others (e.g., Greenbank and Nelson 1959, van Mullem and van der Vlugt 1964, Aneer 1973, Coad and Power 1973), I found female-biased sexual dimorphism for body size in my populations of Threespine Stickleback (Tables 2.7 and 2.8). However, this was not a universal phenomenon. In a few lakes, males were larger than or of similar size to females. Also, where age was considered, most others report significant size differences between the sexes only for older, presumably sexually mature fish (e.g., Greenbank and Nelson 1959, Coad and Power 1973). The degree of size dimorphism at age 1 in some of my populations was similar in magnitude and direction to that at age 2 (Tables 2.7 and 2.8), suggesting real differences in growth rate between the sexes even in younger individuals. Trends for sexual dimorphism for growth in Forest Lake (FT) are unique. In FT, males are much larger than females at age 1, but the reverse is true at age 2. This may be an artifact of small sample size for age 1 individuals from this lake. Alternatively, it may represent differences in breeding age for males and females. For many populations, the ratio of age 1:2 females was lower than that for males (Fig. 2.2), suggesting that females generally live longer or reproduce later than males. If either is the case, FT males may predominantly breed at age 1 while 100% of mature females in FT were age 2 in my sample (Table 2.10). Thus, males may grow faster than females in the first year of life in FT and in a few other populations to reach a size to induce sexual maturation, for territorial defense, for attractiveness to females, or to prepare for the energetic demands of parental care sooner in life, while females grow faster later in life than males in FT (between ages 1 and 2) to reach maturity by age 2. Greater energy expenditure in production of eggs may explain why a higher proportion of females bred at age 2 relative to males in most lakes from this study, if indeed the age structure differences between the sexes were due to biased catch on the breeding grounds. Rollins *et al.* (2017) found fewer young (age 1 and 2) than old (3+) fish in female relative to male anadromous stickleback, where the catches are known to consist of only those returning to breed. Larger energy expenditure may require delayed maturation if larger body size is required to induce the process in females. In addition to or instead of a larger minimum size threshold for maturity in females, the direct and positive relationship between body size and clutch size may also select for later age and, hence, larger size at maturity in females (Hagen 1967, Moodie 1972a, Wootton 1973, McPhail 1977, Craig and FitzGerald 1982, Crivelli and Britton 1987, Rowland 1989b).

Values for female size-at-maturity (Table 2.10) were consistent with those reported in McPhail (1977) for 25 lakes in the Queen Charlotte Islands, BC. Both age and size at maturity were highly variable among populations (Table 2.10), suggesting plastic responses or local adaptation of life histories to different environmental conditions. While I found no significant correlations of size differences with lake-specific environmental variables, differences in size at maturity of stickleback populations persist when raised in a common environment (McPhail

1977) and have high heritabilities (Snyder 1991a, b), suggesting the potential for adaptive response to selection pressures of the local environment. Age at maturity for most females occurred at age 1 in MO and HK lakes and at age 2 in most other lakes. Mud Lake (MD) fish matured at either 1 or 2+, and anadromous fish from RS matured predominantly at 3+ (Table 2.10). Jones and Hynes (1950) suggested that fish in the Birket Lake of northwestern England, which live to be up to 3 years old, began breeding at age 1 and were potentially iteroparous. In my populations, most females matured at either 1 or 2 (with the exception of Mud Lake; Table 2.10), suggesting that stickleback in my populations are probably (though not strictly) semelparous, with very few breeding in two or more consecutive years. This is supported by numerous authors which find that breeding sticklebacks often die *en masse* after spawning, whether the usual generation time be one year or two (e.g., Greenbank and Nelson 1959, van Mullem and van der Vlugt 1964, Mann 1971). Differences among populations in age and size at maturity may be due to differences in age-specific survival rates. Early maturation or maturation at smaller sizes are common in populations that experience high extrinsic mortality, especially high or highly variable mortality among juveniles relative to adults (Stearns 1977). Conversely, high or highly variable adult mortality rates select for later maturation and longer lifespan (Stearns 1977). Quantification of age-specific rates of survival among populations is needed to determine if this factor has been responsible for divergence in size and age at maturity, and better quantification of underlying factors that may contribute to variation in extrinsic mortality, like levels of predation, could also shed light on the role of predation vs. competition and resource partitioning on the evolution of life histories in the Threespine Stickleback adaptive radiation.

2.5: Tables and Figures

Table 2.1. Dates of collection (all in June), location, and sample sizes for each population of *G. aculeatus* measured in this study. The first 27 rows describe lake (L.) stickleback populations and the last three rows describe an anadromous stickleback population. MatSu = Matanuska-Susitna Valley and Kenai = Kenai Peninsula.* = formalin sample only (used for sex ratio analysis); *n* = total sample size between formalin and frozen samples (used for sex ratio analysis).

Population (Abbreviation)	Collection Day/Year	Geographic Region	Drainage	Latitude (°N)	Longitude (°W)	*n*
Bear Paw L. (BP)	02, 21*/2012	MatSu	Fish Creek	61.616	149.755	395
Beverley L. (BV)	02/2012	MatSu	Fish Creek	61.614	149.573	106
Cheri L. (CH)	04/2012	MatSu	Fish Creek	61.601	149.736	242
Frog L. (FG)	02/2012, 21*/2013	MatSu	Fish Creek	61.614	149.723	307
Herkimer L. (HK)	04/2012	MatSu	Fish Creek	61.572	149.698	908
Kalmbach L. (KB)	02/2012	MatSu	Fish Creek	61.608	149.573	195
Lazy L. (LZ)	04/2012	MatSu	Fish Creek	61.572	149.805	478
Morvro L. (MO)	02/2012	MatSu	Fish Creek	61.606	149.782	588
Seymour L. (SM)	03/2012	MatSu	Fish Creek	61.614	149.669	70
Visnaw L. (VW)	03/2012	MatSu	Fish Creek	61.614	149.681	86
Jack L. (JK)	06/2012	MatSu	Little Susitna R.	61.728	150.032	964
East Sunshine L. (ES)	11/2012	MatSu	Susitna R.	62.181	150.038	342
South Friend L. (FR)	11/2012	MatSu	Susitna R.	62.138	150.051	141
Willow L. (WW)	07/2012	MatSu	Susitna R.	61.744	150.059	176
Barley L. (BL)	04, 05*, 15*/2012	MatSu	Knik Arm	61.361	150.084	734
Carpenter L. (CP)	05, 15*/2012	MatSu	Knik Arm	61.434	150.047	208
Mud L. (MD)	03*, 07/2012	MatSu	Knik R.	61.565	148.947	540
Loberg L. (LO)	13/2012	MatSu	Palmer Slough	61.559	149.255	36
Neklason L. (NL)	07/2012	MatSu	Palmer Slough	61.629	149.270	337
East Bottleneck L. (EB)	14/2012	Kenai	Cook Inlet	60.283	151.362	36
Kidney L. (KN)	14/2012	Kenai	Cook Inlet	60.665	151.267	166
Kingsley L. (KL)	14/2012	Kenai	Cook Inlet	60.285	151.347	136
Headquarters L. (HQ)	14/2012	Kenai	Kasilof R.	60.462	151.024	134
Forest L. (FT)	16/2012	Kenai	Kenai R.	60.666	150.820	58
Mosquito L. (MQ)	16/2012	Kenai	Kenai R.	60.633	150.819	184
Picnic L. (PN)	16/2012	Kenai	Kenai R.	60.548	150.272	312
JoJo L. (JJ)	Unknown	Alaska Pen.	Resurrection Bay	58.615	155.219	32
Ingram Creek (IC)	2012	Anadromous	Resurrection Bay	unknown	unknown	25
Resurrection Bay (RB)	2012	Oceanic	N/A	unknown	unknown	56
Rabbit Slough (RS)	07/2012, 10*/2013	Anadromous	Cook Inlet	61.536	149.253	184

Table 2.2. Results of separate ANOVA tests for differences in standard length and somatic wet weight across *G. aculeatus* populations within sex and age classes. *df* = degrees of freedom; α = 0.05. See Figures 2.3 and 2.4 for differences in population mean standard length and somatic wet weight.

Sex	Age	df_{among}	df_{within}	*F*-stat	*P*-value	R^2
			Standard Length (mm)			
Female	1	5	114	9.41	<0.001	0.292
Male	1	7	108	16.19	<0.001	0.512
Female	2	12	136	47.16	<0.001	0.806
Male	2	11	145	46.79	<0.001	0.780
Female	3	1	23	68.65	<0.001	0.749
Male	3	3	21	52.88	<0.001	0.883
			Somatic Wet Weight (g)			
Female	1	4	108	11.69	<0.001	0.302
Male	1	7	108	7.34	<0.001	0.322
Female	2	11	125	16.32	<0.001	0.589
Male	2	10	139	34.29	<0.001	0.712
Female	3	1	23	46.39	<0.001	0.669
Male	3	2	19	43.16	<0.001	0.820

Table 2.3. *P*-values for Tukey-adjusted pairwise comparisons for female (above the diagonal) and male (below the diagonal) stickleback among populations for SL of age 1 fish. Bolded *P*-values are significant at $\alpha \leq 0.05$. Populations KN and JK had sample sizes large enough to include in the male analysis only. X = does not apply due to population omission from analysis for one or the other sex due to low sample size. See Table 2.1 for population abbreviations and Fig. 2.4 for population mean standard lengths.

	KN	JK	FT	MO	MD	LO	HK	FG
KN	–	x	x	x	x	x	x	x
JK	0.960	–	x	x	x	x	x	x
FT	0.062	0.653	–	0.762	0.384	0.457	0.179	**<0.001**
MO	1.000	0.981	**0.029**	–	0.531	0.857	0.165	**<0.001**
MD	0.867	0.221	**<0.001**	0.273	–	1.000	0.916	**<0.001**
LO	0.984	1.000	0.873	0.995	0.534	–	1.000	**0.011**
HK	1.000	0.989	**0.034**	1.000	0.167	0.997	–	**0.002**
FG	**<0.001**	**0.002**	0.429	**<0.001**	**<0.001**	0.064	**<0.001**	–

Table 2.4. *P*-values for Tukey-adjusted pairwise comparisons for female (above the diagonal) and male (below the diagonal) stickleback among populations for SWW of age 1 fish. Bolded *P*-values are significant at α ≤ 0.05. Populations KN, JK, and LO had sample sizes large enough to include in the male analysis only. X = does not apply due to population omission from analysis for one or the other sex due to low sample size. See Table 2.1 for population abbreviations and Fig. 2.5 for population mean somatic wet weights.

	KN	JK	FT	MO	MD	LO	HK	FG
KN	−	x	x	x	x	x	x	x
JK	1.000	−	x	x	x	x	x	x
FT	0.176	0.337	−	0.642	**0.048**	x	**0.023**	**0.001**
MO	0.998	0.999	0.308	−	**0.002**	x	**0.002**	**<0.001**
MD	1.000	1.000	**0.040**	0.848	−	x	0.940	0.054
LO	0.884	0.943	0.999	0.986	0.757	−	x	x
HK	0.974	0.997	0.342	1.000	0.744	0.991	−	0.288
FG	**0.001**	**0.005**	0.927	**<0.001**	**<0.001**	0.776	**<0.001**	−

Table 2.5. *P*-values for Tukey-adjusted pairwise comparisons for female (above the diagonal) and male (below the diagonal) stickleback among populations for SL of age 2 fish. Bolded *P*-values are significant at α ≤ 0.05. Populations ES, FR, and EB had sample sizes large enough to include in the male analysis only, and populations HK, WW, KL, and CP were included in the female analysis only. X = does not apply due to population omission from analysis for one or the other sex due to low sample size. See Table 2.1 for population abbreviations and Fig. 2.4 for population mean standard lengths.

	ES	JJ	MO	VW	HK	WW	MD	KN	FR	KL	CP	FG	FT	EB	RS	BL
ES	−	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
JJ	1.000	−	1.000	0.770	**0.027**	**0.003**	**<0.001**	**0.004**	x	**<0.001**	**<0.001**	**<0.001**	**<0.001**	x	**<0.001**	**<0.001**
MO	0.998	0.998	−	0.941	0.281	0.072	**0.001**	**0.043**	x	**<0.001**	**<0.001**	**<0.001**	**<0.001**	x	**<0.001**	**<0.001**
VW	**0.011**	**0.002**	0.179	−	1.000	0.978	0.546	0.867	x	0.198	**0.008**	**<0.001**	**<0.001**	x	**<0.001**	**<0.001**
HK	x	x	x	x	−	1.000	0.820	0.988	x	0.358	**0.007**	**<0.001**	**<0.001**	x	**<0.001**	**<0.001**
WW	x	x	x	x	x	−	1.000	1.000	x	0.902	0.164	**<0.001**	**<0.001**	x	**<0.001**	**<0.001**
MD	1.000	1.000	1.000	**0.004**	x	x	−	1.000	x	0.990	0.155	**<0.001**	**<0.001**	x	**<0.001**	**<0.001**
KN	0.651	0.219	1.000	0.089	x	x	0.459	−	x	1.000	0.868	**0.011**	**0.024**	x	**0.010**	**<0.001**
FR	0.855	0.719	1.000	0.332	x	x	0.876	1.000	−	x	x	x	x	x	x	x
KL	x	x	x	x	x	x	x	x	x	−	0.995	**0.008**	**0.034**	x	**0.021**	**<0.001**
CP	x	x	x	x	x	x	x	x	x	x	−	0.076	0.249	x	0.123	**<0.001**
FG	**<0.001**	**<0.001**	**<0.001**	0.983	x	x	**<0.001**	**<0.001**	**<0.001**	x	x	−	1.000	x	0.981	**0.018**
FT	**<0.001**	**<0.001**	**<0.001**	0.885	x	x	**<0.001**	**<0.001**	**<0.001**	x	x	1.000	−	x	0.990	0.286
EB	**<0.001**	**<0.001**	**<0.001**	0.328	x	x	**<0.001**	**<0.001**	**<0.001**	x	x	0.633	0.796	−	x	x
RS	**<0.001**	**<0.001**	**<0.001**	0.122	x	x	**<0.001**	**<0.001**	**<0.001**	x	x	0.317	0.441	0.977	−	1.000
BL	**<0.001**	**<0.001**	**<0.001**	0.116	x	x	**<0.001**	**<0.001**	**<0.001**	x	x	0.096	0.086	1.000	0.996	−

Table 2.6. *P*-values for Tukey-adjusted pairwise comparisons for female (above the diagonal) and male (below the diagonal) stickleback among populations for SWW of age 2 fish. Bolded *P*-values are significant at α ≤ 0.05. Populations ES, FR, and EB had sample sizes large enough to include in the male analysis only, and populations HK, WW, KL, and CP were included in the female analysis only. X = does not apply due to population omission from analysis for one or the other sex due to low sample size. See Table 2.1 for population abbreviations and Fig. 2.5 for population mean somatic wet weights.

	ES	MO	VW	HK	WW	MD	KN	FR	KL	CP	FG	FT	EB	RS	BL
ES	−	x	x	x	x	x	x	x	x	x	x	x	x	x	x
MO	1.000	−	0.747	0.780	0.579	**0.006**	0.694	x	0.589	**0.003**	**<0.001**	**<0.001**	x	**<0.001**	**<0.001**
VW	**0.028**	**0.033**	−	1.000	1.000	0.989	1.000	x	1.000	0.920	0.087	0.127	x	**0.039**	**0.001**
HK	x	x	x	−	1.000	0.608	1.000	x	1.000	0.354	**<0.001**	**0.002**	x	**0.002**	**<0.001**
WW	x	x	x	x	−	0.941	1.000	x	1.000	0.759	**0.007**	**0.021**	x	**0.010**	**<0.001**
MD	1.000	1.000	**0.033**	x	x	−	0.995	x	0.849	1.000	**0.014**	0.098	x	0.061	**<0.001**
KN	0.997	0.999	**0.010**	x	x	1.000	−	x	1.000	0.947	0.114	0.160	x	**0.049**	**0.001**
FR	0.986	0.991	0.200	x	x	1.000	1.000	−	x	x	x	x	x	x	x
KL	x	x	x	x	x	x	x	x	−	0.598	**0.001**	**0.006**	x	**0.005**	**<0.001**
CP	x	x	x	x	x	x	x	x	x	−	0.433	0.630	x	0.250	**<0.001**
FG	**0.002**	**0.003**	1.000	x	x	**<0.001**	**<0.001**	**0.021**	x	x	−	1.000	x	0.948	**0.037**
FT	**<0.001**	**<0.001**	1.000	x	x	**<0.001**	**<0.001**	**<0.001**	x	x	0.999	−	x	0.997	0.442
EB	**<0.001**	**<0.001**	1.000	x	x	**<0.001**	**<0.001**	**<0.001**	x	x	0.926	0.999	−	x	x
RS	**<0.001**	**<0.001**	0.478	x	x	**<0.001**	**<0.001**	**<0.001**	x	x	0.094	0.200	0.529	−	1.000
BL	**<0.001**	**<0.001**	0.513	x	x	**<0.001**	**<0.001**	**<0.001**	x	x	**0.001**	**0.001**	0.194	0.999	−

Table 2.7. Mean (coefficient of variation) female and male SL for populations where n ≥ 3 for both sexes within an age category, the deviations between mean SL for females and males, and the percent deviation of female from male mean SL across populations (abbreviations as in Table 2.1).

Population	$\overline{SL}_f$ (CV)	$\overline{SL}_m$ (CV)	$\overline{SL}_f - \overline{SL}_m$	$\left(\frac{\overline{SL}_f - \overline{SL}_m}{\overline{SL}_m}\right) \times 100$
Age 1				
FT	31.77 (9.31)	42.39 (13.47)	-10.61	-25.04
MO	35.86 (11.66)	36.76 (9.94)	-0.91	-2.47
MD	38.02 (22.14)	34.11 (15.50)	3.91	11.47
LO	38.92 (14.06)	38.54 (2.65)	0.37	0.96
HK	39.96 (9.12)	36.94 (8.08)	3.01	8.16
FG	52.82 (37.41)	46.51 (8.44)	6.31	13.56
Age 2				
JJ	38.06 (8.00)	38.86 (7.06)	-0.80	-2.05
MO	38.32 (23.72)	41.68 (33.77)	-3.36	-8.06
VW	43.28 (0.84)	52.89 (0.70)	-9.61	-18.17
MD	50.07 (10.22)	39.53 (27.76)	10.54	26.66
KN	50.15 (21.10)	43.63 (9.85)	6.51	14.93
FG	63.81 (8.86)	57.48 (8.21)	6.33	11.02
FT	63.84 (10.35)	58.67 (7.92)	5.17	8.81
RS	69.38 (2.41)	68.03 (0.49)	1.35	1.99
BL	70.05 (10.97)	63.75 (8.96)	6.29	9.87
Age 3				
RS	72.09 (6.45)	67.69 (4.00)	4.40	6.51

Table 2.8. Mean (coefficient of variation, %) female and male SWW for populations where n ≥ 3 for both sexes within an age category, the deviations between mean SWW for females and males, and the percent deviation of female from male mean SL across populations (abbreviations as in Table 2.1).

Population	$\overline{SWW_f}$ (CV)	$\overline{SWW_m}$ (CV)	$\overline{SWW_f} - \overline{SWW_m}$	$\left(\frac{\overline{SWW}_f - \overline{SWW}_m}{\overline{SWW}_m}\right) \times 100$
		Age 1		
FT	0.31 (56.08)	0.84 (61.39)	-0.53	-63.10
MO	0.47 (52.88)	0.60 (34.57)	-0.13	-21.67
MD	0.75 (87.18)	0.52 (61.15)	0.23	44.23
HK	0.86 (50.64)	0.61 (30.54)	0.25	40.98
FG	1.39 (176.87)	1.00 (26.75)	0.39	39.00
		Age 2		
MO	0.75 (50.43)	0.83 (55.30)	-0.08	-9.64
VW	1.29 (16.14)	2.07 (9.33)	-0.78	-37.68
MD	1.71 (43.47)	0.95 (10.83)	0.76	80.00
KN	1.33 (82.01)	0.98 (30.39)	0.35	35.71
FG	2.65 (36.32)	1.99 (32.51)	0.66	33.17
FT	2.69 (23.91)	2.19 (24.15)	0.50	22.83
RS	3.70 (9.19)	3.77 (9.96)	-0.07	-1.86
BL	3.64 (37.52)	3.19 (35.28)	0.45	14.11
		Age 3		
RS	4.18 (22.92)	3.94 (11.12)	0.24	6.09

Table 2.9. *P*-values for Tukey-adjusted pairwise comparisons among populations for size-at-maturity in female Threespine Stickleback. Bolded *P*-values are significant at α ≤ 0.05. See Table 2.1 for population abbreviations and Table 2.10 for population mean standard lengths.

	MO	HK	MD	CP	FG	FT	BL	RS
MO	−	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
HK		−	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
MD			−	**0.014**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
CP				−	**0.012**	0.067	**<0.001**	**<0.001**
FG					−	1.000	**0.004**	**0.002**
FT						−	0.186	0.090
BL							−	0.998
RS								−

Table 2.10. Percentage of mature age 1, 2, or 3; minimum size at maturity (“min SL”); and mean size at maturity ($\overline{SL}$ ± 95% Tukey-adjusted confidence intervals) of females among populations of Threespine Stickleback. LL CI = lower limit of confidence interval; UL CI = upper limit of confidence interval.

Population	*n*	1 (%)	2 (%)	3 (%)	min *SL*	$\overline{SL}$	LL CI	UL CI
MO	51	94.12	5.88	0	30.28	36.98	35.89	38.10
HK	19	68.42	31.58	0	36.19	42.15	40.13	44.26
MD	29	37.93	55.17	6.90	32.18	48.74	46.84	50.71
CP	11	9.09	90.91	0	46.23	55.70	52.22	59.40
FG	33	15.15	84.85	0	47.81	63.61	61.28	66.02
FT	11	0	100.00	0	54.36	63.84	59.86	68.09
BL	43	2.33	95.35	2.33	54.43	70.06	67.82	72.38
RS	26	3.85	11.54	84.62	58.46	71.33	68.40	74.38

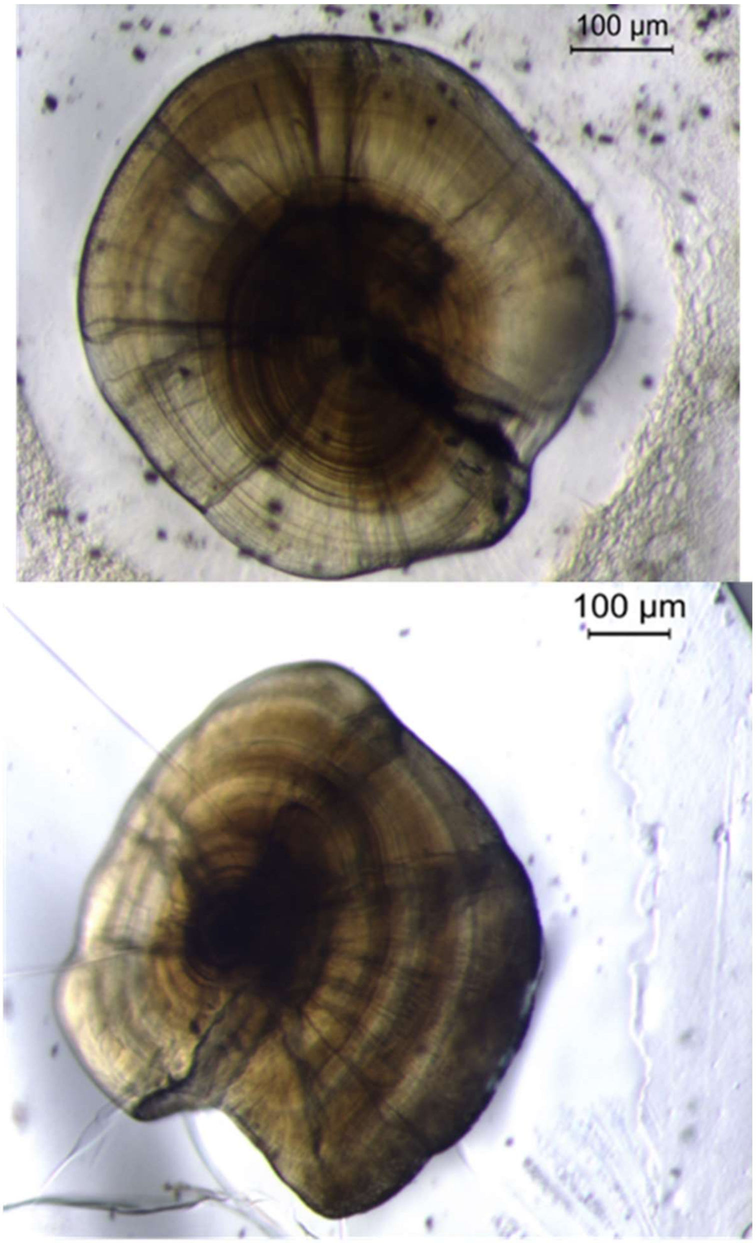


Figure 2.1. Sagittal otoliths from age 2 (top) and 4 (bottom) Threespine Stickleback (from RS). Photographs courtesy of Antonio Lanzirotti.

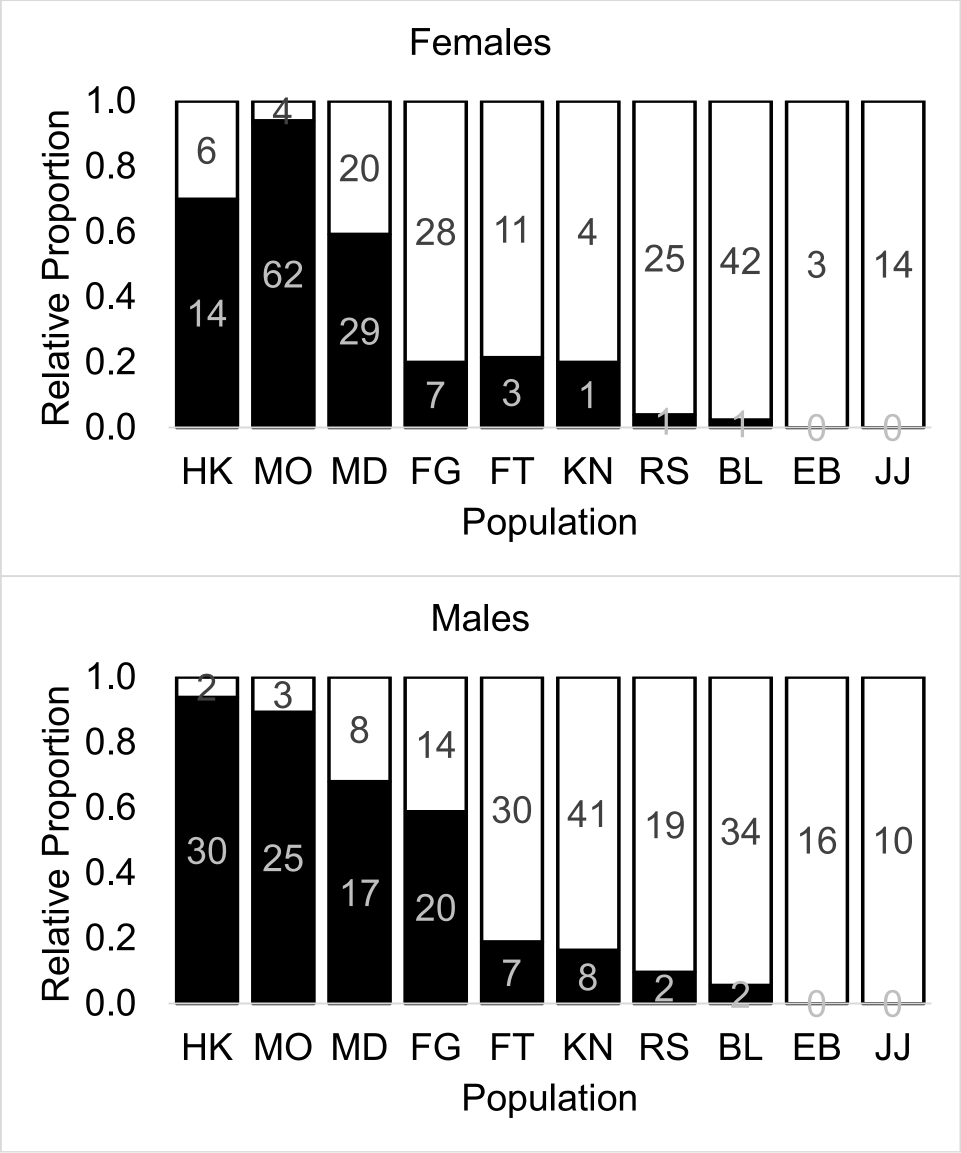


Figure 2.2. Age structure of female and male Threespine Stickleback populations. Population abbreviations are given in Table 2.1. Numbers within bars represent sample sizes for age 1 (black) and 2+ (white) fish.

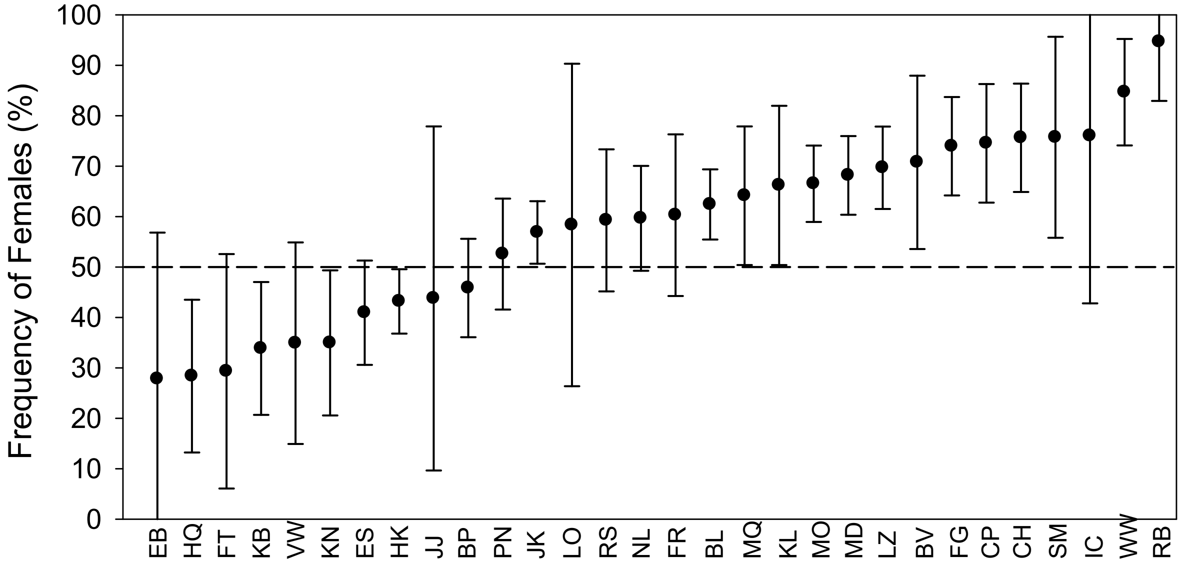


Figure 2.3. Percentage of females (±95 % Bonferroni-adjusted Wald confidence interval) in each sample among populations of Threespine Stickleback. Population abbreviations are given in Table 2.1. Dashed horizontal line represents 1:1 ratio of females to males. Confidence intervals were adjusted according to the number of pairwise comparisons across populations and do not represent statistical tests of difference from this 1:1 line.

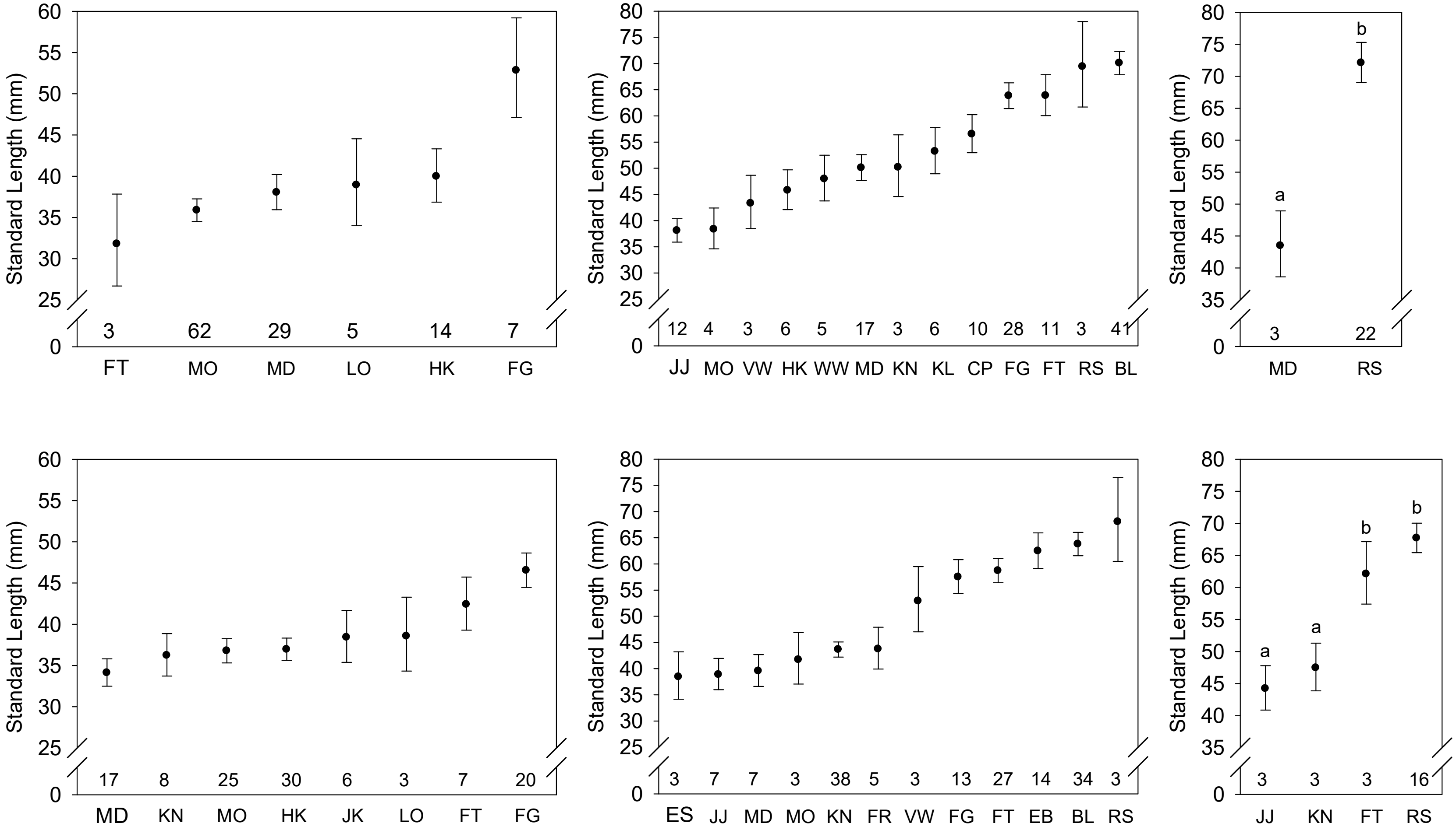


Figure 2.4. Mean SL (±95% Tukey-adjusted confidence intervals) among populations of female (top panels) and male (bottom panels) Threespine Stickleback at age 1 (leftmost panels), 2 (middle panels), and 3 (rightmost panels). Population abbreviations are given in Table 2.1. *P*-values for pairwise comparisons of ages 1 and 2 fish are given in Tables 2.3 and 2.5. Significance of pairwise comparisons of age 3 fish are indicated on the plots by letters above points. Numbers above the x-axis are sample sizes for each population in the ANOVA.

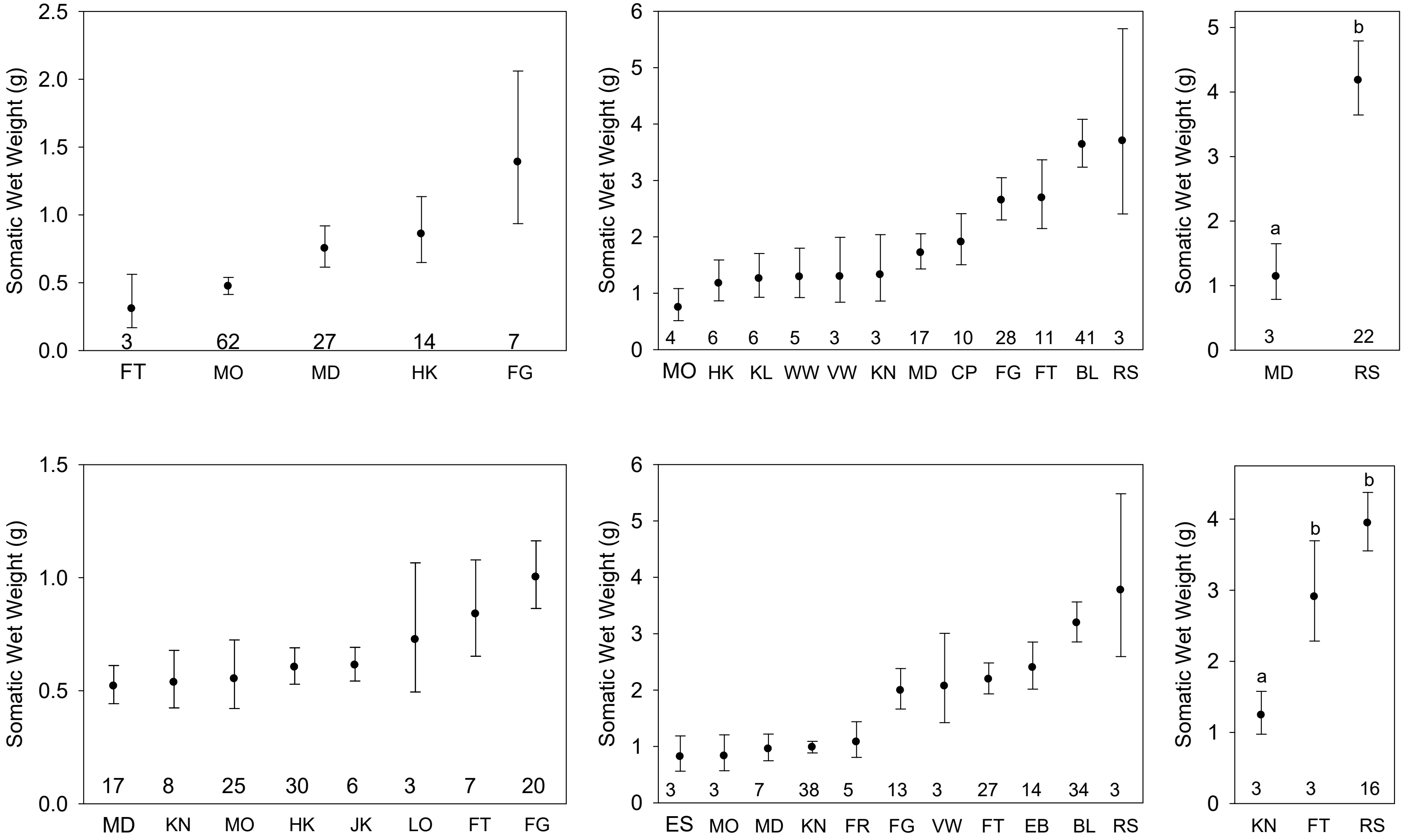


Figure 2.5. Mean SWW (±95% Tukey-adjusted confidence intervals) among populations of female (top panels) and male (bottom panels) Threespine Stickleback at age 1 (leftmost panels), 2 (middle panels), and 3 (rightmost panels). Population abbreviations are given in Table 2.1. *P*-values for pairwise comparisons of ages 1 and 2 fish are given in Tables 2.4 and 2.6. Significance of pairwise comparisons of age 3 fish are indicated on the plots by letters above points. Numbers above the x-axis are sample sizes for each population in the ANOVA.

Chapter 3: Association of Growth Phenotypes with Candidate Growth Gene Markers and Signatures of Selection for Growth Genes in Populations of Field-Caught Threespine Stickleback

3.1: Introduction

Body size, growth rates, and other life history traits are known to be polygenic, with many genes of small effect encoding multiple proteins involved in the various pathways of development and growth (Falconer and Mackay 1996). Current whole-genomic analyses may not allow enough statistical power to detect the many small-effect loci that contribute to such a polygenic phenotype. It is also difficult to eliminate environmental effects on growth in the laboratory (Schreck and Moyle 1990), and these may obscure genetic effects in traditional quantitative trait locus (QTL) analyses. However, research on growth enhancement in agricultural and aquacultural species, as well as studies on human disease and growth deficiencies, have amassed a large list of candidate genes involved in growth pathways, many of which are common to all vertebrates (Duan 1997, Perez-Sanchez *et al.* 2002).

Genes of the somatotropic axis have received considerable attention in studies of vertebrate growth genetics. Growth hormone (GH; a peptide hormone) and insulin-like growth factors (IGFs) are key proteins of the somatotropic axis, so it is also known as the GH-IGF growth pathway. This pathway is involved in regulation of post-natal growth and development as well as cell proliferation throughout life (Waters *et al.* 2006, Rodriguez *et al.* 2007). GH controls the indeterminate growth of skeletal muscle in fish both directly and indirectly. Through its interactions with IGF1, GH is involved with growth by hyperplasia, amino acid uptake, protein synthesis, DNA synthesis, RNA synthesis, lipolysis, and transcription (reviewed in Mommsen

2001). GH also regulates proteoglycan synthesis, sulfate uptake, and growth of support structures. GH indirectly controls behaviors like dominance and appetite, intestinal actions like glucose and amino acid transport, and overall nitrogen retention (reviewed in Mommsen 2001). The somatotropic axis is tangentially involved in various aspects of reproduction and the immune response (e.g., Liu *et al.* 2001, Waters *et al.* 2006, Rodriguez *et al.* 2007).

Gene structure and protein function of candidates involved in the somatropic pathway have been studied in considerable detail in vertebrates, and several studies that quantify their phenotypic effects in the mouse and human have been published (e.g., Godfrey *et al.* 1993, Liu *et al.* 1993, Duquesnoy *et al.* 1998, Moutoussamy *et al.* 1998, Netchine *et al.* 1998, Aguiar-Oliveira *et al.* 1999, Baessler *et al.* 2005, Flores-Morales *et al.* 2006, Waters *et al.* 2006). Zebrafish have been used to study the functions of some of these candidate genes in fish (Ulloa *et al.* 2011), and knockout and gene silencing studies targeting these genes already exist (Acosta *et al.* 2005). Somatotropic genes as well as many in the transforming growth factor superfamily are known to play important roles in fish skeletal growth (reviewed in De-Santis and Jerry 2007, Ulloa *et al.* 2011). The transforming growth factor superfamily includes myostatins (MSTN), myogenic regulatory factors (MRFs), follistatin, and others (Ulloa *et al.* 2011).

Polymorphisms within somatotropic genes are associated with growth differences in species artificially selected for enhanced muscular growth, including cattle, pigs, chickens, and fishes (e.g., Larsen *et al.* 1995, Casas-Carillo *et al.* 1997, Feng *et al.* 1997, Kuhnlein *et al.* 1997, Kim *et al.* 2000, De-Santis and Jerry 2007, Thomas *et al.* 2007, Garrett *et al.* 2008, Sherman *et al.* 2008). Despite the growing number of studies on the gene structure of these growth candidates in fish species (reviewed in Perez-Sanchez *et al.* 2002, De-Santis and Jerry 2007, and Ulloa *et al.* 2011), there are still few studies that indicate an association between allelic variation of growth genes with growth rate or size in fishes (De-Santis and Jerry 2007). Though my review is by no means exhaustive, only polymorphisms in *GH* (e.g., Gross and Nilsson 1999, Kang *et al.* 2002, Martyniuk *et al.* 2003, Tao and Boulding 2003, Almuly *et al.* 2005) and *IGFs* (e.g., Li *et al.* 2009, Hu *et al.* 2013) have been studied for association with growth rate or body size in more than a few fish species.

There are few studies that associate putative growth gene polymorphisms with growth phenotypes in natural vertebrate populations (non-cultured species), addressing the evolution of growth traits in natural populations. Exceptions include Chaoui *et al.* (2012), which revealed that short microsatellite alleles within the promoter regions of *GH* and *prolactin* (*PRL*) are found at higher frequency in lagoonal than in open-sea habitats in a natural population of gilthead seabream (*Sparus aurata*). These short alleles enhance *GH* and *PRL* expression levels in fish species (tilapia *Oreochromis niloticus:* Streelman and Kocher 2002, gilthead seabream: Almuly *et al.* 2008), which may cause increased growth rates (i.e., as in Kaneko *et al.* 2011 for torafugu, *Takifugu rubripes*). Quere *et al.* (2010) found genetic differentiation at the *GH* gene that was between three- and ten- times higher than at anonymous simple sequence repeats (SSRs) among wild populations of sea bass (*Dicentrarchus labrax*), but did not associate these polymorphisms to growth differences among or within populations.

Threespine Stickleback (*Gasterosteus aculeatus*) offers a rare opportunity to study the evolutionary genetics of growth in natural populations. Stickleback have colonized and adapted to numerous freshwater habitats throughout northern temperate and boreal regions from common oceanic ancestors since the most recent glacial retreat, giving us natural replicates of divergent evolution (Ortí *et al.* 1994, Bell 1995). Among the most conspicuous differences between oceanic and freshwater sticklebacks is body size. Oceanic stickleback tend to lack apparent variation for this and other traits (e.g., Bell and Foster 1994, Walker and Bell 2000), but tend to be larger than many freshwater sticklebacks (e.g., McKinnon *et al.* 2004, Chapter 2), though a few freshwater stickleback populations have evolved large body size relative to their oceanic ancestors (e.g., Moodie 1972, Moodie and Reimchen 1976, Bell 1984, Reimchen 1991, Deagle *et al.* 2012, Reimchen *et al.* 2013). Both the *insulin-like growth factor binding protein 2* (*IGFBP2*) and the *signal transducer and activator of transcription 5* (*STAT5*) genes are found within loci implicated in a genome-wide scan for regions divergent between marine and freshwater stickleback populations (Jones *et al.* 2012), suggesting that these and other somatotropic genes may be responsible for body size divergence in stickleback. In addition, *GH*, *myogenic differentiation 1* (*MYOD1*), *neuropeptide Y precursor* (*NPYP*), and *fibroblast growth factor 2* (*FGF2*) candidate growth genes were all found to be under directional selection in Scandinavian oceanic and freshwater stickleback populations (Shimada *et al.* 2011). *MYOD1* and *NPYP* were also found to be under directional selection in a global analysis of stickleback freshwater adaptation (DeFaveri *et al.* 2011). The stickleback model system can be used to address which candidate growth genes and alleles of those genes are involved in body size divergence between oceanic and freshwater sticklebacks and among freshwater populations. With this naturally replicated system, it is possible to assess the degree of genome-level convergence in the evolution of body size and growth rate.

Natural replicate populations of stickleback also provide an opportunity to address fundamental questions about genetic architecture of an important quantitative trait. Many evolutionary models assume that many loci of equal and small effect contribute to quantitative traits (Lynch and Walsh 1998), which may preclude detection of effects in genome-wide scans for QTL. Thus, this study instead used a candidate gene approach to address the following questions:

1. Are stickleback growth traits associated with variation at candidate growth genes?
2. What is the magnitude of polymorphism within and divergence among stickleback populations for candidate growth genes?
3. Are candidate growth genes under selection in stickleback populations?
4. Are the same alleles at the same genes associated with convergent evolution for small or large body size in stickleback populations?

3.2: Methods

3.2.1: Collections

Threespine Stickleback were collected in June of 2012 using fine-mesh (1/8 or ¼”), unbaited minnow traps from seven lakes and one slough in the Cook Inlet region of Alaska, USA (Table 3.1). The lake populations represent the extreme large- and small- bodied freshwater fish sampled from a larger subset of lakes (Chapter 2). I chose one relatively large- and one relatively small- bodied (two for Fish Creek) freshwater population from within each of three separate drainages or from two nearby drainages (Table 3.1). The Rabbit Slough population is anadromous, and fish were captured *en route* to spawn in freshwater. The Rabbit Slough population is thought to resemble, both phenotypically and genetically, the putative ancestral colonizer of the freshwater lakes in the region (e.g., Bell 1976, 1994). All samples were frozen immediately on dry ice after capture and euthanasia with MS-222 and stored at -80°C until processing.

3.2.2: Phenotyping and DNA extraction

I determined body length, weight, age, and sex of each individual used for genotyping. Standard length (SL), the distance from the tip of the rostrum to the end of the last vertebra, was measured using digital calipers. Both SL and frozen wet weights were measured prior to removal of the caudal peduncle and fin for DNA extraction. DNA was extracted from 15-25 mg of caudal peduncle and fin tissue with DNeasy® Blood & Tissue Kits (Qiagen™, USA; spin columns), following the manufacturer's protocol.

Age was estimated from sagittal otoliths ("earstones"), which are accretionary structures in the fish inner ear. Otoliths contain both daily and annular growth rings – a series of alternating, concentric light and dark bands projecting outwards from a central nucleus. Annular growth rings were counted to determine the age of the fish according to Jones and Hynes (1950). Otolith extraction and preparation protocols, in addition to separate analyses of phenotypic differences in size, age, and other life history parameters of the populations used here, can be found in Chapter 2.

Since males and females differ in mean size and growth rate (Chapter 2 and citations therein), especially following the onset of sexual maturation, sex was determined by gonadal inspection. Gonads were extracted and weighed to the nearest 0.01 or 0.0001 g, and gonadal wet weights were subtracted from total wet weight of each specimen to obtain estimates of somatic wet weights (SWW).

3.2.3: Candidate genes and genetic markers

Candidate genes for growth were identified using the literature search described above. I used the Ensembl genome browser to locate many of these genes within the stickleback genome (Table 3.2; Flicek *et al.* 2013). *GH*, *MYOD1*, *NPYP*, *FGF2*, *IGFBP2*, and *STAT5* were of particular interest. *GH* is one of the most well-characterized genes in the somatotropic axis, is known to have polymorphisms associated with growth differences in several fish species, and is under directional selection in stickleback populations (Shimada *et al.* 2011). *MYOD1*, *NPYP*, and *FGF2* are not in the somatotropic axis, but were found to be under directional selection in stickleback populations (*MYOD1* and *NPYP*: DeFaveri *et al.* 2011; all three genes: Shimada *et al.* 2011). MYOD1 is involved in muscle cell differentiation and regeneration. NPYP is a precursor of neuropeptide Y, which is involved in regulation of food intake, stress response, circadian rhythms, and cardiovascular function. FGF2 is involved in development of limbs and the central nervous system and is responsible for proliferation of blood vessel cells (http://www.ncbi.nlm.nih.gov/gene/). *IGFBP2* and *STAT5* were implicated in the genome-wide scan for divergent loci between freshwater (mostly smaller-bodied) and oceanic (large-bodied) sticklebacks (Jones *et al.* 2012).

Genome-wide single nucleotide polymorphisms (SNPs) were discovered in a 20-genome re-sequencing study by Jones *et al.* (2012) and are available online at www.sticklebrowser.stanford.edu. Additional SNPs were chosen based on additional stickleback re-sequenced genomes from 23 populations in the Cook Inlet region provided by D.M. Kingsley *et al.* To determine an appropriate number of SNPs to place within each of the candidate genes, I obtained local recombination rates by interpolation of plots in Hohenlohe *et al.* (2012) and estimated extent of linkage disequilibrium (LD) by computation of pairwise D’ between SNPs in exons from the genotype data available at www.sticklebrowser.stanford.edu. SNPs were considered appropriate for genotyping if the minor allele was present in at least 20% of the populations from Cook Inlet. Based on LD estimates and gene lengths, I chose a subset of SNPs at each gene spaced approximately equidistant across the gene region, including flanking regions up to 10 kb from each locus or until another functional gene was encountered (Table 3.3).

3.2.4: Genotyping

Specimens were genotyped at SNP markers by Boston Children's Hospital SNP Genotyping Core (and assistance by GenomeQuebec) using Sequenom MassARRAY technology (Gabriel *et al.* 2009). Briefly, each SNP was tagged in polymerase chain reactions (PCR) with a locus-specific primer. An extension reaction added an allele-specific nucleotide that, when separated by MALDI-TOF mass spectrometry, allowed for identification of the alleles present at each locus for each sample by weight. Locus-specific primers allowed for simultaneous genotyping at various SNP loci for each sample. I genotyped at a total of 93 loci distributed into four separate SNP pools. Primers were designed by Boston Children's Hospital SNP Genotyping Core (and GenomeQuebec) and were tested for specificity against the entire stickleback genome prior to genotyping to avoid non-specific binding, especially to paralogs present in the genome. SNP pools and primers are listed in Appendix Table S3.1.

3.2.5: Analyses

For each of the 93 SNPs within candidate genes and for each of the eight populations, I performed a separate general linear model (GLM) with SL [ln(x) –transformed] as dependent variable, and with SNP genotype, age, and sex as fixed effects where applicable (SAS®; SAS Institute Inc. 2009). Only SL was used as my measure of fish body size because (a) analyses were within-population and accounted for sex and age differences, and were therefore independent of body shape differences that may exist among populations, sexes, or ages; (b) SL and SWW were highly correlated (Appendix Fig. S3.1); and (c) SL estimates were more accurate and precise than SWW estimates. Single-SNP analyses are simpler, have similar statistical power as haplotype-based tests (Long and Langley 1999), and have been used in candidate gene scans for associations (e.g., Tao and Boulding 2003, Eckert *et al.* 2009). False discovery rate (FDR) analysis was used to find comparison-wise significance thresholds to account for multiple comparisons using a maximum acceptable FDR (q) of 0.05 (Pike 2011). P-values for the fixed effect of SNP genotype were used in the FDR to determine which comparisons were significant. Any comparisons for which a significant genotype × age, genotype × sex, age × sex, or genotype × age × sex interaction were found at an unadjusted $\alpha = 0.05$ were discarded and replaced instead by separate tests of the effects of SNP genotype within age or sex categories, or within all levels of each if necessary. In many instances, genotype data were insufficient (small sample sizes or not enough genotypic variation within ages, sexes, and populations) to perform analyses at some SNPs within certain populations. All performed tests are reported in Appendix Tables S3.2-3.9. I performed posthoc sensitivity analyses using G-Power (v. 3.1.9.2, Franz Faul, University of Kiel, Kiel, Germany) to determine the range of effect sizes I could detect in my analyses given observed sample sizes (highest and lowest for each population) and FDR significance thresholds (highest = 0.05 and lowest observed for each population) and assuming power = 0.80. Since many SNPs in analyses were linked, FDR analysis may be overly conservative due to non-independence of comparisons. Therefore, I also determined significance using the simpleM method of Gao et al. (2008). Briefly, principal component analysis (PCA) using SNP genotype data was used to determine the effective number of independent tests that were run in single SNP-wise analyses. The number of principle components that explain 99.5% of the variation in the data were considered to be the effective number of independent tests, and this value was used to compute a comparison-wise corrected Bonferonni significance threshold as $\alpha_C = \alpha_E / M_{eff}$, where α_E was the experiment-wise significance threshold (here, 0.05) and M_{eff} was the effective number of independent tests. Here, separate PCAs were performed for each population (R v. 3.3.3). Imputation of missing data was necessary in some cases to perform PCAs, and imputation was performed using the linkim package in R (v. 3.3.3) according to Xu et al. (2015). Since imputation of missing data should increase overall variation in the data over that which was truly used in SNP association analyses, results for M_{eff} may be conservative. However, where over 10% of samples were missing SNP genotypes, whole SNPs were dropped from the analysis to avoid using too much inferred data. Thus, deletion of some information may have decreased overall variation in the data, resulting in less conservative estimates for M_{eff} and hopefully compensated for the effect of imputation.

To test for genotype-phenotype associations across populations, I tested for significance of Pearson product-moment correlation coefficients between population mean SWW [ln(*x*)-transformed] and population SNP allele frequencies [arcsin(*x*)-transformed] separately for all SNP loci. SWW was used instead of SL because SWW is independent of body shape differences across populations, sexes, and ages. FDR and simpleM analyses were used to account for multiple comparisons. Since single-locus analyses suffer from lack of power and non-independence across comparisons due to linkage, I also performed PCA on a matrix of population-level allele frequencies [arcsin(*x*)-transformed] at SNPs within each gene or within linkage groups when genes were close enough to be considered a linked set (R v. 3.3.3). I then tested for significance of Pearson product-moment correlation coefficients between population mean SWW [ln(*x*)-transformed] and the first principal component (PC) from each PCA analysis. I accounted for multiple comparisons in this analysis using a Bonferonni threshold of $P \leq 0.003$. I note that associations or the lack thereof may instead reflect population structure (drift effects) and may not reflect real association between genotypes and phenotypes due to local adaptation to different life histories.

I tested for among-genotype associations to determine whether any variants within or across genes were linked. Tests for LD were performed between pairs of markers arranged in 2 × 2 contingency tables using Fisher's exact tests with FDR correction for multiple testing (GENEPOP®; Rousset 2008). Observed heterozygosities (H_O), within-population gene diversity (H_S) estimates, overall gene diversity estimates (H_T'), the amount of gene diversity among populations (D_{ST}'), deviation from Hardy-Weinberg (HWE) proportions in the total population (F_{IT}), the average deviation from HWE proportions in subpopulations (F_{IS}), and degree of population differentiation (F_{ST} over all loci and populations, locus-specific $F_{ST,}$ and population pair-wise F_{ST} estimates) were computed in Fstat (Goudet 2002) based on Nei and Weir and Cockerham estimators. Population-specific F_{IS} were computed in Arlequin (Excoffier and Lischer 2010).

I tested for correlations between population pairwise F_{ST} and (a) geographic distance (pairwise overland distances among all populations), (b) drainage affiliation (pairwise overland distances among populations within drainages, with arbitrarily large distances assigned to populations from different drainages), and (c) distance to the sea (population pairwise differences between the distance of each population to the sea) using Mantel tests (Mantel 1967;

BIOMstat 4.11), with a Bonferroni correction to account for multiple comparisons (Francis *et al.* 1986). Geographic distance and pairwise differences in population distances from the sea can be found in Chapter 2. The drainage affiliation matrix used here reflects chosen large- and small-bodied population pairs within drainages or from nearby drainages (Appendix Table S3.10).

Analysis of molecular variance (standard) was performed in Arlequin (Excoffier and Lischer 2010) to test for genetic divergence among drainages, populations, and individuals. The eight populations were grouped by drainage, with the three Fish Creek drainage populations in one group (one large-bodied, FG, and two small-bodied, HK and MO, populations), BL (large-bodied) and MD (small-bodied) together in a second Knik drainage group, FT (large-bodied) and KN (small-bodied) together in a third Kenai drainage group, and the anadromous RS population (large-bodied) on its own as a fourth group. To determine which SNPs have undergone recent natural selection, I used Arlequin (Excoffier and Lischer 2010) to detect F_{ST} outliers (100 groups and 50000 runs) using these same drainage groupings. I ran a second F_{ST} outlier analysis excluding the anadromous RS population to differentiate loci under selection between anadromous and freshwater populations from those under selection among freshwater populations alone. F_{ST} outlier results should be interpreted with some caution in this study since all SNPs were chosen within or nearby functional genes or an identified QTL for size variation (LG17QTL; Greenwood *et al.* 2011). I did not choose a set of loci to set a neutral baseline F_{ST}. However, this bias may be conservative for loci under positive (directional) selection since I would expect a higher baseline F_{ST} in my analyses and more conservative thresholds for identification of F_{ST} outliers.

3.3: Results

3.3.1: Within population genotype-phenotype associations

Genotypes at 30 SNPs within 14 genes were associated with growth (size- and age-specific SL) at the unadjusted comparison-wise $\alpha \leq 0.05$ (Table 3.4). After accounting for multiple comparisons using the simpleM method, only six SNP loci showed significant association between genotypes and SL, and only within Mud Lake and for age 1 individuals (usually for females only; Table 3.5; Appendix Tables S3.2-S3.9; 89 usable loci). For these six SNPs, mean SL differed between genotype categories by between 6.21 and 16.7 mm.

Sensitivity analyses indicated that mean differences of about 1.3 to 76.3 mm SL were detectable, with a mean detectable effect size of around 13.5 mm across all analyses. Thus, only biologically moderate (1.3 to 4 mm) to large or (for some populations) impossible differences in mean size (4 to 62.2 mm) for a Threespine Stickleback were detectable in these analyses (Appendix Table S3.11). Thus, much larger sample sizes within populations or many fewer SNPs were necessary to detect differences in many cases.

3.3.2: Among population allele frequency-phenotype associations

A single SNP in the *STAT5* gene (XI: 5763059) showed significant correlation between mean SWW and allele frequencies across populations (Appendix Table S3.12) after accounting for multiple comparisons using the simpleM procedure, and only two loci had significant correlations without accounting for multiple comparisons, in single-locus analyses (91 usable loci). Interestingly, the *STAT5* SNP was also one of those with genotype significantly associated with growth within Mud Lake (Table 3.5). I had power to detect effects larger than about 0.77 to

0.95 SD from the expectation of r = 0 (no correlation) in these analyses. I found patterns of geographic covariance in SNP allele frequencies within linkage groups (Fig. 3.1). That is, allele frequencies at SNPs on the same chromosome tend to covary across populations for SNPs on chromosomes XIX, XVIII, and somewhat for XI. This suggests that, at least for those three chromosomes, linkage between SNPs within a linkage group do mean that the pattern in allele frequencies across populations is very similar for each SNP marker in the linkage group. Thus, for associations between SNP allele frequencies and mean SWW across populations, SNPs within genes in LGs XIX, XVIII, and XI show a similar pattern and therefore do not represent independent tests of association.

I did not find significant correlations between population mean SWW and PC1 of PCA analyses on population-level allele frequencies within linkage sets after accounting for multiple comparisons, although allele frequencies and SWW were significantly correlated at unadjusted α (0.05) for a paralog of the *MYOD1* gene (Appendix Table S3.13). However, since covariance in allele frequencies between SNPs among populations were quite low across most LGs (except XIX, XVIII, and XI; Fig. 3.1), pooling single-marker data across LGs may actually have diminished power to detect associations between allele frequency variation and SWW across populations even though I used the axis explaining the most variation in the allele frequency data within a linked set of markers (PC1). Moreover, I also did not find a significant correlation between population mean SWW and PC1 of a PCA on population-level allele frequencies across all linkage groups (that is, with all markers included). PC1 in this analysis accounted for 31% of the variation in allele frequency data, and the association between mean SWW and PC1 was moderate ($r = 0.386$, $df = 6$, $P = 0.344$).

3.3.3: Linkage disequilibrium among SNPs, gene diversity and deviations from HWE, genetic population differentiation, and correlations between genetic and geographic distance

I found significant LD within 2 to 22 kb of autosomes and within 14 to 758 kb on LG XIX (the sex-determining chromosome; Table 3.6, Appendix Table S3.14; 91 usable loci). Estimators of gene diversity and F_{ST} across all loci and populations are given in Table 3.7 (91 usable loci), along with population-specific F_{IS}. Population-specific F_{IS} were not significantly different from 0. (See Appendix Table S3.15 for locus-specific F_{IS} within populations and Appendix Table S3.16 for locus-specific F_{ST}.) Population pairwise F_{ST} (Table 3.8) were not significantly correlated with overland geographic distance, drainage affiliation, or population pairwise differences in distance to the sea (Table 3.9). Locus-specific F_{ST} were higher than average within the *STAT5*, *GH2*, *LG17QTL*, and *MYOD1.1* genes, indicating that these may be genes under positive selection which may contribute to growth divergence among stickleback populations; however, SNPs detected to have significant genotype-phenotype associations within Mud Lake or to have significant allele frequency – mean SWW correlations among populations did not have higher-than-average (or abnormally low) F_{ST} except in two cases within the *STAT5* gene (Appendix Table S3.16).

3.4.4: Loci under selection and analysis of molecular variance

AMOVA showed significant genetic differentiation among populations within drainages, but not among drainages or within populations (Table 3.10; 55 usable loci). An AMOVA excluding the RS anadromous population produced similar results (not shown). To visualize the genetic separation among populations, I performed a PCA on the SNP genotype data arranged in a 578 specimen by 88 (usable) SNP matrix. Missing genotypes were imputed using the linkim package in R (v. 3.3.3). The PCA confirmed population-level genetic differentiation (Fig. 3.2). Mud Lake, the only lake population still open to the sea, was genetically similar to the anadromous Rabbit Slough population. Populations did not appear to separate according to drainage on either PC1, PC2, or PC3, which accounted for 13.3%, 9.1%, and 8.4% of the variation in the data, respectively. I detected 22 (25.58%) and 23 (26.74%) F_{ST} outlier loci across all populations and for freshwater populations only, respectively (Fig. 3.3; Table 3.11; 86 usable loci). F_{ST} outlier loci did not have larger or smaller major allele frequencies relative to loci not detected as F_{ST} outliers (*t*-test on arcsine-transformed data; *t-stat* = 0.474, *df* = 67, *P* = 0.637; Appendix Table S3.16).

3.4: Discussion

This study used a candidate gene approach to determine whether specific genes in well-known pathways are associated with an important quantitative trait, growth rate, in natural populations of Threespine Stickleback. I found significant associations between genotypes at candidate growth genes and age-specific body size (SL; growth rates) at six SNP loci within four genes: *MYOD1.2*, *STAT5*, *IGF1r.1,* and *IGF2r* (Table 3.5). All significant associations were found only in one of eight populations tested (Mud Lake), and effects were only significant for age 1 fish, usually only age 1 females. Mean differences between genotype categories in these analyses was between 6.21 and 16.7 mm SL. While these are large mean differences, they were only found within a single population of eight, and only within a single age and, usually, sex category. This may mean the difference is spurious, however the difference was detected despite low power (Appendix Table S3.11). If the differences are real, then these loci may explain a large proportion of genetic variance in age-specific body size (or growth rate) in at least Mud Lake stickleback. Interestingly, Mud Lake is the only freshwater population used in this study that is open to the sea and receives anadromous stickleback during the spawning season. While there is considerable avoidance of introgression between freshwater residents of Mud Lake and the anadromous spawning population (Karve *et al.* 2008, Bell *et al.* 2010), the potential for gene flow is considerably greater than for the other lake populations used in this study. *STAT5* was detected in a 20-genome screen as a locus significantly divergent between marine and freshwater stickleback populations on a global scale (Jones *et al.* 2012), and the difference in size between Mud Lake individuals with alternative alleles at SNPs located in and around *STAT5* indicated that possible introgression with anadromous stickleback may have caused not only a greater frequency of the marine allele at *STAT5* within Mud Lake compared to the other lakes in this study, but also contributed substantially to intrapopulation and (potentially) interpopulation body size divergence in Threespine Stickleback. Since size-assortative mating is crucial to the maintenance of niche partitioning and divergence between sympatric species pairs of Threespine Stickleback on marine-freshwater, benthic-limnetic, and lake-stream confluences (e.g., Borland 1986, Rowland 1989, McPhail 1994, Nagel and Schluter 1998, Rundle and Schluter 1998, Ishikawa and Mori 2000, McKinnon *et al.* 2004, Maccoll 2009, and Matthews *et al.* 2010), *STAT5* may have played a large role in the Threespine Stickleback adaptive radiation. These data also suggested similar roles for *MYOD1.2*, *IGF1r.1,* and *IGF2r*, although further studies are necessary to confirm these hypotheses. Evidence for potential introgression of Mud Lake individuals with anadromous fish also comes from the fact that, for at least two of the SNPs found to have significant genotype-phenotype associations within Mud Lake (XI: 5763059 and XIX: 16842698), the allele most prevalent among individuals with fast growth rates (large age-specific body size) was also present at high frequency among anadromous Rabbit Slough fish. In a PCA on SNP genotypes across loci for all individuals in the study, Mud Lake was genetically close to Rabbit Slough (Fig. 3.2), also suggesting some introgression. Introgression with another population may cause smaller-than-expected F_{IS} (due to an increase in heterozygotes over the number expected given no introgression) within Mud Lake, and the observed F_{IS} over all loci for Mud Lake was indeed negative, although it did not appear to differ from that of other populations and was not significantly different from zero (Table 3.7). For loci with significant genotype-phenotype associations within Mud Lake, locus-specific F_{IS} were also negative, indicating a possible signature of increased heterozygosity due to introgression with anadromous fish at those loci (Appendix Table S3.15). But again, these negative locus-specific F_{IS} were not apparently different from values for all other populations (Appendix Table S3.15).

My power to detect significant effects was low due to inclusion of many comparisons and small sample sizes within population, age, and sex categories. I estimated that mean differences in the effect of genotypes on SL between 1.3 and 76.3 mm were detectable across my analyses, suggesting low power to detect biologically important effects in many instances (Appendix Table S3.11). The mean SL of stickleback populations in this study were between 35 and 69 mm (Table 3.1), so mean differences greater than the average effect size across the range of analyses of 13.5 mm would represent a larger biological size difference than I would predict exists within most population, age, and sex categories. Thus, my lack of detection does not preclude the presence of real genotype-phenotype associations. Much larger sample sizes (or many fewer loci) are needed to obtain appropriate levels of power to detect these associations after accounting for sex and age effects on size. Large differences in size within age and sex categories were present in Mud Lake possibly due to introgression with anadromous stickleback, which are present in Mud Lake during their spawning season (Karve *et al.* 2008). I detected only a single significant correlation between population mean SWW and population-level allele frequencies for a SNP in the *STAT5* gene (Appendix Table S3.12). Thus, I did not detect many loci which may contribute to size divergence across populations or determine whether loci that cause differential local adaptation for body size are the same across populations (convergent evolution). A larger number of populations or smaller number of SNP loci may have increased power of these analyses. However, traditional QTL studies may be better suited to address these questions since population structure may confound interpretation of results from genotype-phenotype associations across populations if such associations were found.

I found significant LD within regions of 2 to 22 kb surrounding my SNPs (Table 3.6), and significant LD between my candidate gene loci from different chromosomes as well (all genes except *GH2*; Appendix Table S3.14). The Threespine Stickleback radiation has been represented traditionally as a bottlebrush phylogeny (or a raceme), with the core of the brush representing oceanic populations that remain relatively unchanged through time and numerous freshwater colonizations resulting in star phylogenies that form the bristles of the bottlebrush, with negligible gene flow from freshwater to oceanic populations (Bell and Foster 1994, Hohenlohe *et al.* 2012, Bell and Aguirre 2013). Recent studies, however, provide evidence of bidirectional gene flow, sufficient to maintain alleles adaptive in freshwater (and maladaptive in the ocean) at low frequencies in oceanic populations (Colosimo *et al.* 2005, Hohenlohe *et al.* 2012). Once oceanic sticklebacks colonize new freshwater populations, the alleles are "recycled," or brought to fixation again by natural selection (Colosimo *et al.* 2005, Schluter and Conte 2009, Hohenlohe *et al.* 2012, Bell and Aguirre 2013). Many of these alleles are genetically linked (present in regions with low recombination), and this is thought to facilitate adaptation to freshwater (Hohenlohe *et al.* 2012, Bell and Aguirre 2013). Results from Bell and Aguirre (2013) suggest that many of these alleles are partially recessive, which permits them to persist (in the heterozygous state) despite purifying selection in the ocean, but allows also for selection to favor not only the rare partially recessive homozygotes, but heterozygotes with phenotypes that may have a slight fitness advantage, upon initial colonization of freshwater. Some of the candidate growth genes I used in this study show significant LD despite the lack of physical linkage, suggesting that body size, along with a suite of other apparently linked traits, may have undergone convergent natural selection in similar freshwater environments after repeated, independent colonization events from standing genetic variation present at low frequencies in the anadromous Threespine Stickleback genome.

Estimates for heterozygosity (Nei's H_S) and F_{ST} across loci and populations (Tables 3.7 and 3.8) were similar to those found for Threespine Stickleback in other studies (heterozygosity: Lind and Grahn 2011, reviewed in Konijnendijk *et al.* 2015; F_{ST}: Borg 2011, Lind and Grahn 2011, Jones *et al.* 2012, Kaeuffer *et al.* 2012, Klepaker *et al.* 2012, Roesti *et al.* 2012, Konijnendijk *et al.* 2015). If loci have undergone recent selection in response to local conditions or if stabilizing selection is occurring at these loci, then I would not expect a genetic signature of population structure. I did not find any significant correlations between geographic distance, drainage affiliation, or population pairwise differences in distance to the sea with genetic distance (population pairwise F_{ST}) among populations (Table 3.9), indicating no signature of isolation by distance (IBD) across the Cook Inlet region for my candidate growth gene loci. Genetic variation among populations was significant in an AMOVA, but I found no indication of significant genetic variation among drainages or among individuals within populations (Table 3.10). Thus, these functional genes may have undergone directional selection among populations explaining the population-level genetic divergence, or have since been under stabilizing selection to conserve gene function. Sets of markers including neutral loci have found signatures of population structure and IBD for Threespine Stickleback (e.g., Lind and Grahn 2011). Unfortunately, this study did not include neutral markers to test for such a signature across these populations.

Despite (1) lack of neutral markers to set a baseline for F_{ST} in my samples, (2) the fact that my sample of SNP markers was not chosen at random across the genome due to placement of SNPs in functional loci, and (3) the presence of pseudoreplication within loci due to multiple SNPs used within each, I did try to detect selection at my loci using an F_{ST} outlier approach. Due to these biases, however, my results should be interpreted with caution. I found a total of 31 F_{ST} outlier loci, eight of which were detected only in the analysis including all populations, nine of which were detected only in the analysis that excluded the anadromous RS population, and 14 of which were detected as outliers in both analyses (Table 3.11, Fig. 3.3). F_{ST} outliers were detected within all candidate growth genes except *MYOD1.2* and *IGFBP2.2*, both paralogs of the presumably fully-functional *MYOD1.1* and *IGFBP2.1*. *IGFBP2.1*, *IGF1*, and *GHr1* had SNPs which were detected as F_{ST} outliers only in the analysis including the anadromous RS population, indicating that these genes have been under presumably divergent natural selection between anadromous and freshwater populations and convergent selection among freshwater populations. However, only *IGFBP2.1* was found to be under positive selection, while *IGF1* and *GHr1* were under stabilizing selection. *STAT5*, *GH2*, *MYOD1.1*, and *LG17QTL* were detected as genes under positive selection in both analyses including and excluding RS, while *GHr2*, *IGF1r.1*, *FGF2*, and *NPYP* were detected as genes under stabilizing selection in both analyses. *IGF1r.2* was detected as an F_{ST} outlier under stabilizing selection when RS was considered, but a different SNP marker within the gene was detected as an outlier indicating positive selection among freshwater populations with RS excluded. *IGF2* and its receptor, *IGF2r*, both had SNPs detected as F_{ST} outliers under positive selection when RS was included, but under stabilizing selection among freshwater populations. In the case of *IGF2*, the same SNP was under positive selection when RS was included, but under stabilizing selection when only freshwater populations were considered. *PIT1* had 2 SNPs detected as F_{ST} outliers, one of which was under stabilizing and the other under positive selection. Compared to other Threespine Stickleback studies (reviewed in Konijnendijk *et al.* 2015), I found a higher proportion of F_{ST} outliers in my sample of loci; however, this was likely due to pseudoreplication and lack of incorporation of presumably neutral loci. However, my detection of F_{ST} outliers under positive selection at F_{ST} above around 0.6 for heterozygosities below 0.5 were consistent with thresholds found in other studies that used neutral markers to obtain baseline F_{ST} (e.g., Borg 2011, Lind and Grahn 2011). Loci detected to be under stabilizing selection should be interpreted with much more caution since a neutral baseline would surely shift this threshold for detection down, which would make some of the low-F_{ST} outliers detected here no longer outliers. Thus, outliers detected as under stabilizing selection in this study are questionable, while those under positive selection likely represent real signatures of selection.

Shimada *et al.* (2011) and DeFaveri *et al.* (2011) also detected signatures of directional selection in *GH2*, *MYOD1*, and *FGF2* candidate growth genes in stickleback populations. Unlike Shimada *et al.* (2011) and DeFaveri *et al.* (2011), *NPYP* and *FGF2* were not found to be under directional selection in the Cook Inlet stickleback populations I studied. This study also confirmed the results of Jones *et al.* (2012), which found that *IGFBP2* and *STAT5* genes were divergent between marine and freshwater stickleback populations; *IGFBP2* was under positive selection between marine and freshwater populations, and *STAT5* was under positive selection among all populations of Cook Inlet stickleback. This study also implicated *IGF2* and *IGF2r* in marine-freshwater size divergence. Finally, I found that the previously-identified QTL on linkage group 17 associated with juvenile size differences in Threespine Stickleback (Greenwood *et al.* 2011) was under positive selection among Cook Inlet stickleback populations divergent for body size, and the SNP marker discovered here as an F_{ST} outlier may help hone the search region within this QTL for fine mapping to uncover the specific gene or regulatory region responsible for growth differences.

To conclude, this study detected significant associations between growth and genotypes at six SNP markers within four candidate growth genes for one population with large differences in size within age and sex categories (Table 3.5). With larger sample sizes, I may have been able to detect further loci associated with growth differences within populations. This study found that a large proportion of candidate growth genes have been under selection (Table 3.11, Fig. 3.3). I found significant population differentiation (Tables 3.8 and 3.9), but found only a single significant correlation between mean SWW and allele frequencies across populations. I did not find significant relationships between geographic distance, drainage affiliation, or distance to the sea with genetic distance (Table 3.9), suggesting these functional loci were not affected by drift processes reflecting a pattern of IBD or gene flow between freshwater and anadromous stickleback populations.

3.5: Tables and Figures

Table 3.1. Dates of collection (all in June 2012), location, sample sizes (*n*), and mean SL and SWW (± standard deviation = SD) for each population of *G. aculeatus* measured in this study. The first seven rows describe lake (L.) stickleback populations and the last row describes an anadromous stickleback population. MatSu = Matanuska-Susitna Valley and Kenai = Kenai Peninsula.

Population (Abbreviation)	Collection Day	Geographic Region	Drainage	Latitude (°N)	Longitude (°W)	*n*	Mean SL (SD)	Mean SWW (SD)
Frog L. (FG)	02	MatSu	Fish Creek	61.614	149.723	87	50.72 (12.74)	1.32 (1.11)
Herkimer L. (HK)	04	MatSu	Fish Creek	61.572	149.698	53	39.09 (4.95)	0.75 (0.45)
Morvro L. (MO)	02	MatSu	Fish Creek	61.606	149.782	115	35.19 (4.95)	0.47 (0.47)
Barley L. (BL)	04	MatSu	Knik Arm	61.361	150.084	84	66.57 (8.30)	3.34 (1.17)
Mud L. (MD)	07	MatSu	Knik R.	61.565	148.947	83	38.45 (9.02)	0.78 (0.71)
Forest L. (FT)	16	Kenai	Kenai R.	60.666	150.820	58	54.06 (10.74)	1.71 (0.88)
Kidney L. (KN)	14	Kenai	Cook Inlet	60.665	151.267	67	41.18 (7.71)	0.82 (0.79)
Rabbit Slough (RS)	07	Anadromous	N/A	61.536	149.253	52	69.34 (5.23)	3.93 (0.80)

Table 3.2. Gene names, location in the Threespine Stickleback genome, and number of single nucleotide polymorphisms (SNPs) placed within the upstream flank (UF), introns, exons, and downstream flank (DF) of each candidate growth gene in this study. The mean and range distance (bp) between SNPs within a gene are also given, along with gene length (bp). See Table 3.3 for full gene names and functions.

Gene	Genome Location	# in UF	# in Introns	# in Exons	# in DF	# SNPs total	Mean Spacing	Spacing Range b/w SNPs	Gene Length
GH2	XI:16069731-16072008	1	2	0	0	3	3280	1418-5142	2277
GHr1	XIII:5675029-5680240	2	1	2	1	6	2522	1115-4121	5211
GHr2	XIV:10388550-10398180	1	3	1	1	6	2196	2072-2382	9630
PIT1	XVI:13150514-13155698	0	1	0	1	2	2753	2753	5184
IGF1	IV:32098461-32108128	2	4	0	0	6	2910	2062-4107	9667
IGF2	XIX:13287163-13291582	1	1	0	1	3	2748	2414-3081	4419
IGF1r.1	XIX:16907878-16962832	1	6	1	0	8	7555	4351-13805	54954
IGF1r.2	II:4540717-4572472	1	5	2	0	8	4503	2008-7263	31755
IGF2r	XVIII:3138884-3167789	1	7	2	0	10	3306	2421-6283	28905
IGFBP2.1	I:21589378-21596657	2	1	1	0	3	3645	2383-4605	7279
IGFBP2.2	XVI:5906187-5912186	5	3	0	1	9	7815	1140-23820	5999
STAT5	XI:5771316-5781666	2	2	2	1	7	3015	1032-6434	10350
MYOD1.1	XIX:9370025-9372397	3	1	0	1	5	3413	1459-7727	2372
MYOD1.2	II:21930786-21932621	2	0	1	0	3	2581	1485-3676	1835
FGF2	IV:3331137-3335024	2	3	0	1	6	1056	998-1119	3887
NPYP	X:9526273-9527519	3	1	1	0	5	2119	1091-3879	1246
LG17QTL	XVII:4909843-9697366	n/a	n/a	n/a	n/a	3	3057016	568513-2488503	6125100

Table 3.3. Full names, acronyms, functional descriptions, and reason for selection of genes used in this study.

Acronym	*Gene Name*	Function	Reason for Selection
GH2	*Growth hormone 2*	Stimulates synthesis of IGF1 and IGF2	Under directional selection in stickleback populations
GHr1	*Growth hormone receptor 1*	Stimulates IGF synthesis, activates MAP kinases to regulate growth/differentiation	Polymorphisms associated with growth in several species
GHr2	*Growth hormone receptor 2*	Stimulates IGF synthesis, activates MAP kinases to regulate growth/differentiation	Polymorphisms associated with growth in several species
PIT1	*Pituitary specific transcription factor 1*	Control cell differentiation into somatotrophs in the pituitary, synthesize GH	Polymorphisms associated with growth in several species
IGF1	*Insulin-like growth factor 1*	Promotion of cell proliferation, inhibition of cell death	Polymorphisms associated with growth in several species
IGF2	*Insulin-like growth factor 2*	Promotion of cell proliferation, inhibition of cell death, growth factor for early development	Polymorphisms associated with growth in several species
IGF1r	*Insulin-like growth factor 1 receptor*	Promotion of cell proliferation, inhibition of cell death	Polymorphisms associated with growth in several species
IGF2r	*Insulin-like growth factor 2 receptor*	Promotion of cell proliferation, inhibition of cell death, growth factor for early development	Polymorphisms associated with growth in several species
IGFBP2	*Insulin-like growth factor binding protein 2*	Promotion of cell proliferation, inhibition of cell death	Divergent between marine and freshwater stickleback
STAT5	*Signal transducer and activator of transcription 5*	Stimulates expression of proteins in the liver, including IGF1	Divergent between marine and freshwater stickleback
MYOD1	*Myogenic differentiation 1*	Involved in muscle cell differentiation and regeneration	Under directional selection in stickleback populations
FGF2	*Fibroblast growth factor 2*	Limb and CNS development, proliferation of blood vessel cells	Under directional selection in stickleback populations
NPYP	*Neuropeptide Y precursor*	Regulation food intake, stress response, circadian rhythms, and cardiovasc. funct.	Under directional selection in stickleback populations
LG17QTL	QTL associated with size in stickleback crosses	N/A	QTL associated with size in stickleback crosses

Table 3.4. Number of populations (out of eight) for which SNP genotypes were associated with growth (age- and sex- specific SL) at the unadjusted comparison-wise significance threshold of $P \leq 0.05$ and adjusted (for multiple comparisons) comparison-wise significance thresholds determined using the simpleM and FDR methods (see text). LG = linkage group. Gene abbreviations are defined in Table 3.2 and population abbreviations in Table 3.1. Effect size ranges are also given for each SNP.

Gene	LG	SNP	# at unadjusted $P \leq 0.05$	# with simpleM	# with FDR	Popul-tions	Range η_p^2
IGFBP2.1	I	21598355	1	0	0	MD	0.223
IGF1r.2	II	4541659	1	0	0	FG	0.112
		4547932	1	0	0	RS	0.136
		4566452	1	0	0	MO	0.045
MYOD1.2	II	21932462	2	1	1	FG, MD*	0.116-0.657
FGF2	IV	3329457	1	0	0	MD	0.297
		3334738	2	0	0	BL, HK	0.056-0.263
IGF1	IV	32097027	1	0	0	HK	0.228
		32103306	1	0	0	MD	0.307
NPYP	X	9528514	1	0	0	MD	0.667
STAT5	XI	5763059	1	1	1	MD	0.479
		5773228	1	1	0	MD	0.174-0.265
		5775144	2	1	1	FT, MD*	0.132-0.543
		5779439	2	0	0	FG, MD	0.179-0.304
		5780656	2	0	0	FT, MD	0.161-0.270
		5781688	2	0	0	HK, MD	0.102-0.566
GH2	XI	16076821	1	0	0	FG	0.197
GHr1	XIII	5673292	1	0	0	HK	0.286
		5681784	2	0	0	FG, HK	0.083-0.765
		5683842	2	0	0	FT, MD	0.125-0.152
GHr2	XIV	10396321	1	0	0	FG	0.592
LG17QTL	XVII	10618107	1	0	0	MD	0.163
IGF2r	XVIII	3143344	1	0	0	FG	0.173
		3149894	1	0	0	KN	0.533
		3156177	1	1	0	MD	0.273
		3162328	1	0	0	FG	0.170
MYOD1.1	XIX	9369810	1	0	0	FG	0.248
IGF1r.1	XIX	16924542	1	0	0	MD	0.250
		16942698	1	1	1	MD	0.451
		16959478	1	0	0	FG	0.071

Table 3.5. List of single nucleotide polymorphism (SNP) loci for which mean SL ($\overline{SL}$) differed significantly between genotype categories (using the simpleM approach to account for multiple comparisons). Due to low minor allele frequencies, heterozygotes and homozygous individuals for the minor allele were often pooled for analysis. Only analyses for Mud Lake age 1 females or age 1 fish were significant after accounting for multiple comparisons for the SNPs listed. The first four listed were significant after adjustment using both FDR and simpleM (Mud Lake threshold $P < 0.0013$) procedures. For each locus, the $\overline{SL}$, unadjusted lower and upper limits of 95% CI for $\overline{SL}$, difference in $\overline{SL}$ between genotype categories, unadjusted P-value, significance (sign) threshold from false discovery rate (FDR) analysis used to correct for multiple comparisons, the adjusted P-value based on the FDR (q), and effect size are given. SNPs are within the *MYOD1.2*, *STAT5*, *STAT5*, *IGF1r.1*, *IGF2r*, and *STAT5* genes, respectively. See Table 3.3 for full gene names and functions.

SNP, Analysis	Genotype	$\overline{SL}$ (mm)	LL 95% CI	UL 95% CI	Mean difference	F	df_{num}	df_{den}	P	FDR sign thresholds	q	η_p^2
chrII_21932462,	CC	35.27	33.41	37.25	16.72	47.93	1	25	0.00000029	0.00044643	0.00003248	0.657
Mud Lake age 1 fem	CG and GG	52.00	46.96	57.57								
chrXI_5775144,	AA	35.72	33.50	38.09	16.46	28.52	1	24	0.00001760	0.00089286	0.00098560	0.543
Mud Lake age 1 fem	AG and GG	52.18	45.74	59.52								
chrXI_5763059,	CC	35.69	33.27	38.28	14.09	22.11	1	24	0.00008856	0.00133929	0.00330624	0.479
Mud Lake age 1 fem	CG and GG	49.77	43.79	56.57								
chrXIX_16842698,	TT	49.28	43.03	56.43	13.63	19.65	1	24	0.00020000	0.00178571	0.00560000	0.451
Mud Lake age 1 fem	TC and CC	35.64	33.36	38.08								
chrXVIII_3156177,	TT	37.08	34.43	39.92	14.55	9.42	1	25	0.00510000	0.00223214	0.11386667	0.273
Mud Lake age 1 fem	CT	51.62	41.85	63.62								
chrXI_5773228,	CC	34.64	32.59	36.82	6.21	8.40	1	40	0.00610000	0.00267857	0.11386667	0.174
Mud Lake age 1	CT	40.58	37.04	45.06								

Table 3.6. Approximate minimum and maximum estimates for extent of linkage disequilibrium (LD) observed within SNP regions for each linkage group (LG) used in this study. Estimates were derived from results of tests for LD among pairs of SNPs (see Appendix Table S3.14).

LG	Minimum	Maximum
I		2383
II	5161	15395
IV		4164
X	8477	
XI	6211	12085
XIII		10550
XIV	4368	6610
XVI	5179	21284
XVIII	3088	15717
XIX	13650	7577450

Table 3.7. Nei's observed heterozygosity (H_O), expected heterozygosity within populations (H_S), expected heterozygosity in the total population (H_T'), amount of gene diversity among populations (D_{ST}'), average deviation from HWE within populations (G_{IS}), and genetic variance among populations (G_{ST}') across all loci for all eight populations (including the anadromous RS; "All Pop") and for freshwater populations only (excluding RS; "FW Only"). Average deviation from HWE within each population (F_{IS}) is also given, along with P-values indicating significance of deviations from HWE. Weir and Cockerham F-statistics, including F_{IT} (deviation from HWE across all populations), F_{IS}, and F_{ST} (genetic variance among populations) and bootstrap 95% confidence intervals ("CI"; "LL" = lower limit of CI, "UL" = upper limit of CI) are given for all populations and for freshwater populations only.

Nei Estimators				Population	F_{IS}	*P*
	All Pop	FW Only		BL	-0.024	0.794
H_O	0.244	0.244		FG	0.023	0.210
H_S	0.254	0.251		FT	-0.197	1.000
H_T'	0.399	0.384		HK	-0.014	0.634
D_{ST}'	0.145	0.133		KN	-0.216	1.000
G_{IS}	0.039	0.029		MD	-0.038	0.917
G_{ST}'	0.363	0.346		MO	-0.057	0.989
				RS	-0.024	0.743
Weir and Cockerham Estimators						
	All Pop	LL 95% CI	UL 95% CI	FW Only	LL 95% CI	UL 95% CI
F_{IT}	0.385	0.328	0.445	0.368	0.305	0.431
F_{IS}	0.035	-0.013	0.089	0.028	-0.022	0.082
F_{ST}	0.363	0.323	0.404	0.350	0.309	0.391

Table 3.8. Population pairwise F_{ST}. See Table 3.1 for population abbreviations.

	FG	HK	MO	BL	MD	KN	RS	FT
FG	0							
HK	0.1387	0						
MO	0.2798	0.2482	0					
BL	0.3331	0.2936	0.3983	0				
MD	0.3271	0.2080	0.4278	0.3581	0			
KN	0.3646	0.3774	0.4517	0.4312	0.3943	0		
RS	0.3659	0.2925	0.4884	0.4952	0.2975	0.4817	0	
FT	0.3271	0.2555	0.3828	0.4067	0.3450	0.4263	0.4350	0

Table 3.9. Results of Mantel correlation analyses of population pairwise F_{ST} with matrices of pairwise interpopulation distances (GEO; Chapter 2, Appendix Table S2.1), drainage affiliation (STR; this study, Appendix Table S3.10), and a matrix of pairwise interpopulation differences in each population's distance to the sea (DIST, Chapter 2, Appendix Table S2.3). *P*-value = Mantel standardized test statistic one-tailed *P*; *P* -value* = *P*-value for 1000 random matrix permutations. *n* = 28 for all tests. Bonferroni-adjusted significance threshold = 0.017.

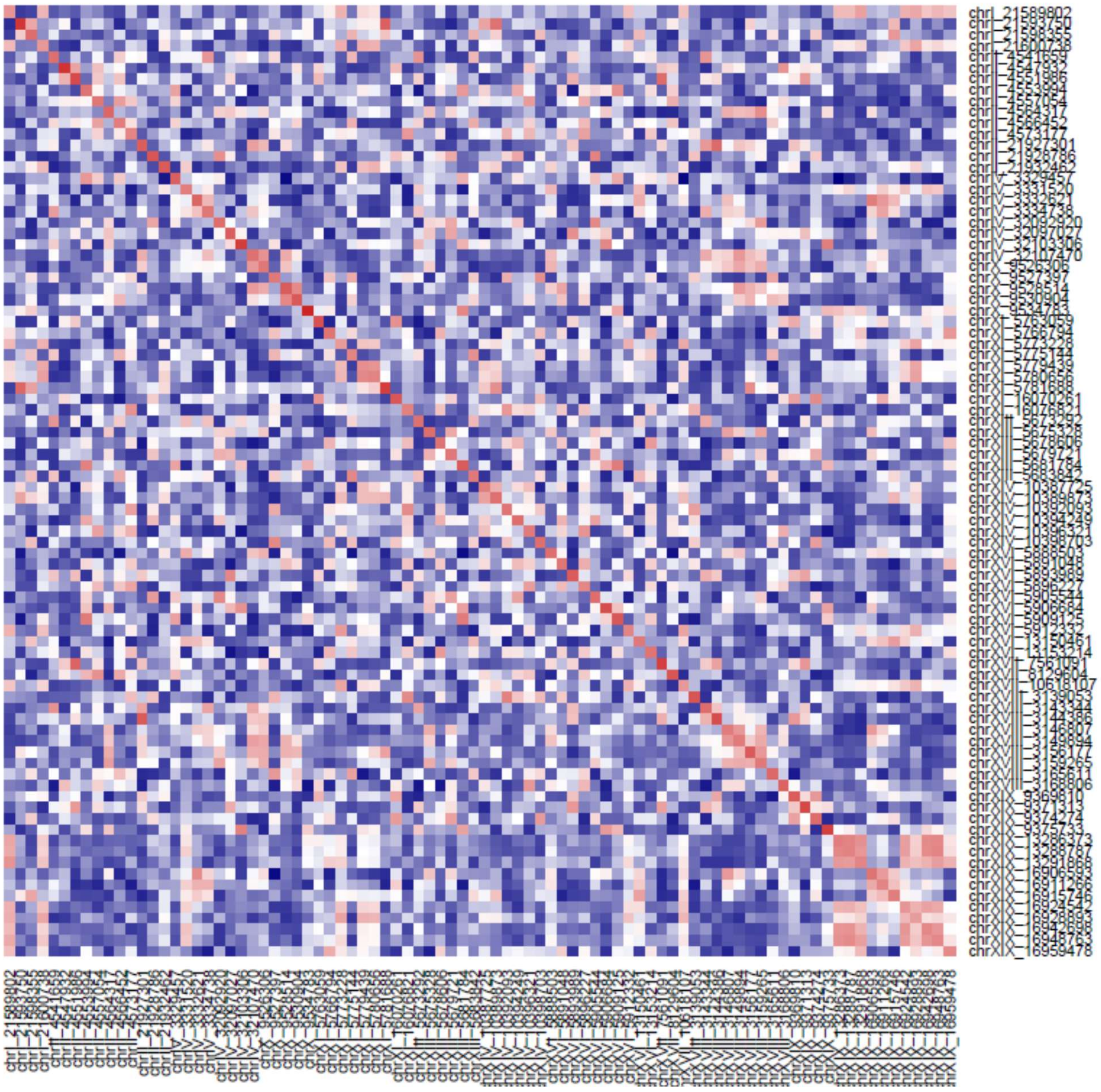
Correlation Analysis	*r*	*t*-stat	*P*-value	*P*-value*
F_{ST} v. GEO	0.394	1.50	0.067	0.049
F_{ST} v. STR	0.025	2.26	0.459	0.398
F_{ST} v. DIST	0.168	0.17	0.092	0.094

Table 3.10. Analysis of molecular variance showing genetic variation among 55 usable SNP markers in growth genes explained by variation among drainages, populations within drainages, and individuals within populations. Bolded *P*-values indicate significance at $\alpha \leq 0.05$.

Source of Variation	df	*% variation*	*P*
Drainages	3	7.26	0.074
Populations	4	28.53	**<0.001**
Individuals	595	-3.46	1.000
Error within individuals	603		

Table 3.11. List of loci with F_{ST} values in the lower ($\leq 5\%$) or upper ($\geq 95\%$) quartiles of the distribution of F_{ST} values found among 87 SNPs located within candidate growth genes. Some SNPs were discovered as F_{ST} outliers in an analysis with all eight populations used in this study (seven lake and one anadromous population), some as F_{ST} outliers in an analysis which excluded the anadromous RS population, and some as F_{ST} outliers in both analyses. *P*-values and modes of selection for all outliers are indicated on the left-hand side of the corresponding column if SNPs were outliers with RS included, on the right-hand side if outliers in the analysis with RS excluded, or in the middle if outliers in both analyses and classifications were the same in both analyses. If classifications for *P*-values or modes of selection were different for outliers detected in both analyses, both values are given, with that for the analysis with all populations ("ALL") included on the left side and for the analysis with only freshwater populations ("FW") on the right side.

<table>
<tr><th>Gene</th><th>SNP Location</th><th colspan="2">P ALL, FW</th><th colspan="2">Mode of Selection ALL, FW</th></tr>
<tr><td>IGFBP2.1</td><td>chrI_21589802</td><td>< 0.05</td><td></td><td>positive</td><td></td></tr>
<tr><td>IGF1r.2</td><td>chrII_4547932</td><td>< 0.05</td><td></td><td>stabilizing</td><td></td></tr>
<tr><td>IGF1r.2</td><td>chrII_4557054</td><td></td><td>< 0.05</td><td></td><td>positive</td></tr>
<tr><td>IGF1r.2</td><td>chrII_4564317</td><td></td><td>< 0.05</td><td></td><td>positive</td></tr>
<tr><td>FGF2</td><td>chrIV_3332621</td><td>< 0.01,</td><td>< 0.05</td><td colspan="2">stabilizing</td></tr>
<tr><td>IGF1</td><td>chrIV_32107470</td><td>< 0.05</td><td></td><td>stabilizing</td><td></td></tr>
<tr><td>NPYP</td><td>chrX_9527397</td><td>< 0.05</td><td></td><td>stabilizing</td><td></td></tr>
<tr><td>NPYP</td><td>chrX_9534783</td><td colspan="2">< 0.01</td><td colspan="2">stabilizing</td></tr>
<tr><td>STAT5</td><td>chrXI_5773228</td><td>< 0.01,</td><td>< 0.05</td><td colspan="2">positive</td></tr>
<tr><td>STAT5</td><td>chrXI_5779439</td><td colspan="2">< 0.01</td><td colspan="2">positive</td></tr>
<tr><td>STAT5</td><td>chrXI_5780656</td><td colspan="2">< 0.01</td><td colspan="2">positive</td></tr>
<tr><td>STAT5</td><td>chrXI_5781688</td><td colspan="2">< 0.05</td><td colspan="2">positive</td></tr>
<tr><td>GH2</td><td>chrXI_16070261</td><td></td><td>< 0.05</td><td></td><td>positive</td></tr>
<tr><td>GH2</td><td>chrXI_16076821</td><td colspan="2">< 0.05</td><td colspan="2">positive</td></tr>
<tr><td>GHr1</td><td>chrXIII_5678606</td><td>< 0.05</td><td></td><td>stabilizing</td><td></td></tr>
<tr><td>GHr2</td><td>chrXIV_10396321</td><td>< 0.05</td><td></td><td>stabilizing</td><td></td></tr>
<tr><td>GHr2</td><td>chrXIV_10398703</td><td>< 0.01,</td><td>< 0.05</td><td colspan="2">stabilizing</td></tr>
<tr><td>PIT1</td><td>chrXVI_13150461</td><td colspan="2">< 0.05</td><td colspan="2">positive</td></tr>
<tr><td>PIT1</td><td>chrXVI_13153214</td><td colspan="2">< 0.05</td><td colspan="2">stabilizing</td></tr>
<tr><td>LG17QTL</td><td>chrXVII_10618107</td><td colspan="2">< 0.01</td><td colspan="2">positive</td></tr>
<tr><td>IGF2r</td><td>chrXVIII_3159265</td><td colspan="2">< 0.05</td><td>positive,</td><td>stabilizing</td></tr>
<tr><td>MYOD1.1</td><td>chrXIX_9369810</td><td></td><td>< 0.05</td><td></td><td>positive</td></tr>
<tr><td>MYOD1.1</td><td>chrXIX_9371313</td><td colspan="2">< 0.01</td><td colspan="2">positive</td></tr>
<tr><td>MYOD1.1</td><td>chrXIX_9374274</td><td>< 0.05,</td><td>< 0.01</td><td colspan="2">positive</td></tr>
<tr><td>IGF2</td><td>chrXIX_13286373</td><td></td><td>< 0.01</td><td></td><td>stabilizing</td></tr>
<tr><td>IGF2</td><td>chrXIX_13288787</td><td></td><td>< 0.01</td><td></td><td>stabilizing</td></tr>
<tr><td>IGF2</td><td>chrXIX_13291868</td><td>< 0.01</td><td></td><td>positive</td><td></td></tr>
<tr><td>IGF1r.1</td><td>chrXIX_16915746</td><td>< 0.05</td><td></td><td>stabilizing</td><td></td></tr>
<tr><td>IGF1r.1</td><td>chrXIX_16924542</td><td></td><td>< 0.05</td><td></td><td>stabilizing</td></tr>
<tr><td>IGF1r.1</td><td>chrXIX_16942698</td><td></td><td>< 0.05</td><td></td><td>stabilizing</td></tr>
<tr><td>IGF1r.1</td><td>chrXIX_16948763</td><td></td><td>< 0.01</td><td></td><td>stabilizing</td></tr>
</table>



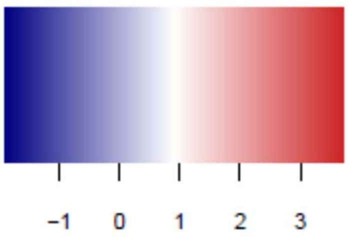


Figure 3.1. Heatmap showing the strength of correlations of allele frequencies between SNP loci across populations. Increasingly deep shades of red indicate correlation coefficients closer to 1 in magnitude (absolute value), and increasingly deep shades of blue indicate correlation coefficients closer to 0 in magnitude (absolute value). White indicates correlation coefficients close to 0.5 in magnitude (absolute value).

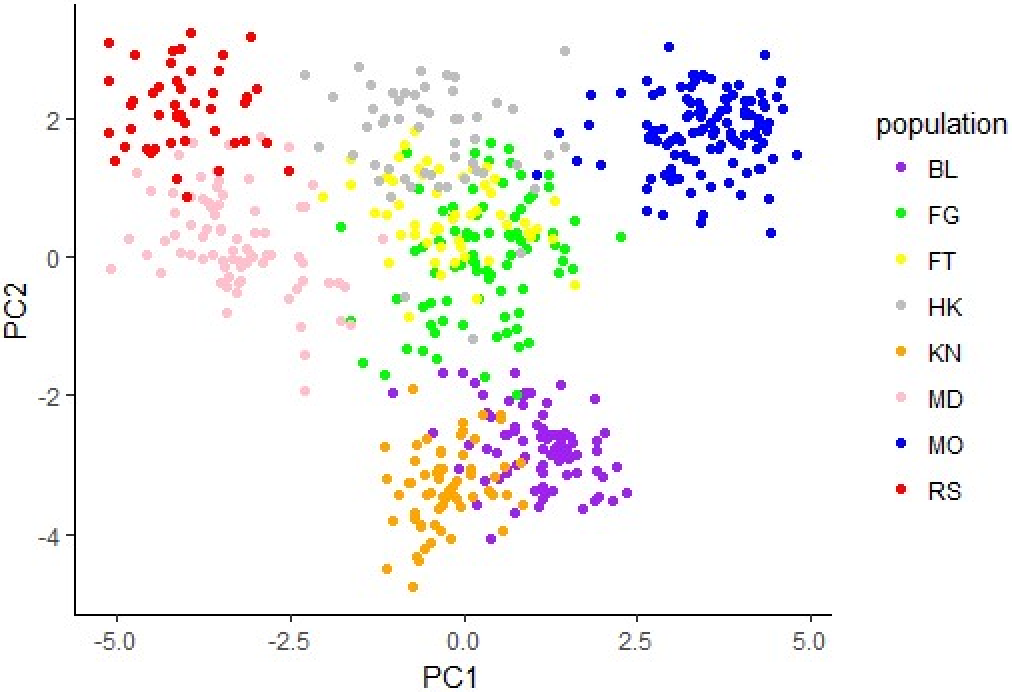


Figure 3.2. PCA plot showing genetic differentiation among populations (see Table 3.1 for definition of population abbreviations and population descriptions). Variation along principal component 1 (PC1; 13.3% of variation) and 2 (PC2; 9.1% of variation) are plotted together.

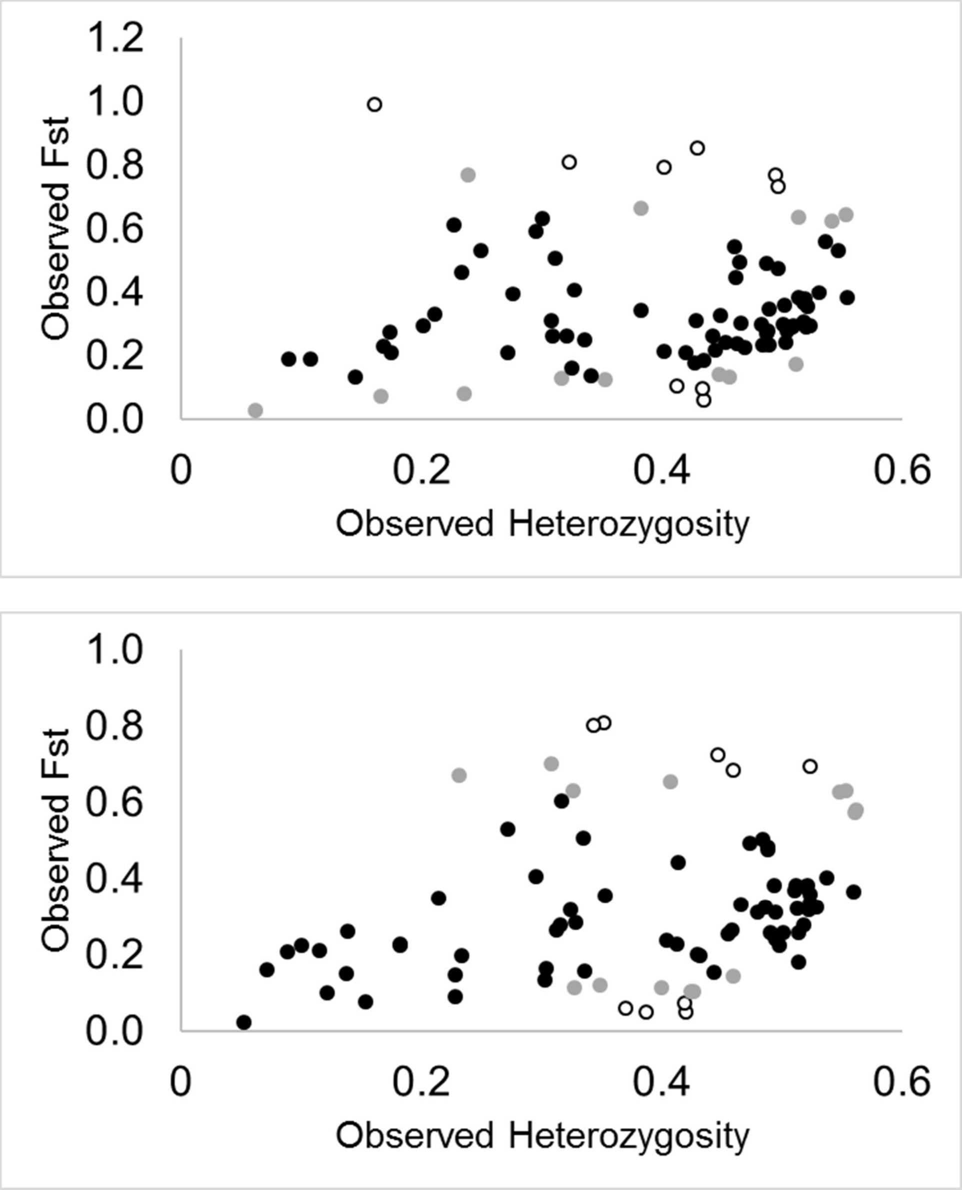


Figure 3.3. Results of F_{ST} outlier analysis among all populations (top) and among freshwater populations only (bottom) grouped by drainage. F_{ST} outliers are indicated as open (outside 1% and 99% quantiles) and gray points (outside 5% and 95% quantiles).

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Appendix

Chapter 2

Table S2.1. Pairwise distances among all populations (m) listed in Table 2.1 (SF = FR). Not all values in this matrix were used in correlation analyses with pairwise differences in mean SL and SWW since not all populations here were used in SL and SWW analyses.

	BP	BV	CH	FG	HK	KB	LZ	MO	JK	ES	SF	WW	BL	CP	MD	NL	KN	KL	HQ	MQ	PN	RS	FT	LO	JJ	VW	EB
BP		9729.29	1749.43	2038.09	5569.09	9792.98	5436.05	2015.11	19217.68	64835.69	60190.46	21648.69	33046.15	24642.23	43281.72	25926.51	133548.1	171014.7	146751	123181.9	121722	28203.47	120453.8	27123.98	451449.4	4207.13	171633.6
BV			8492.84	7736.19	8092.28	758.52	13438.48	11315.54	27332.48	67662.17	63383.57	29586.48	39212.59	31514.29	33602.1	16157.71	139887.2	176314.2	151825.8	128134.4	124425.9	19099.88	125604	17710.63	458373.3	5667.89	176940.8
CH				1244.79	3936.78	8415.9	5500.66	2853.82	20955.49	66181.58	61583.96	23370.1	32928.73	24684.66	41777.36	24666.17	133599.7	170852.1	146502.7	122912.7	121020.4	26551.17	120211.9	25551.68	451566.9	3256.13	171442
FG					4710.09	7767.52	6695.75	3738.48	20785.15	65317.74	60755.14	23181.59	34194.43	25922.11	41277.42	23882.9	134799.1	172112.5	147737	124165.1	122219.7	26248.01	121446.4	25131.92	452799	2222.36	172701.2
HK						7675.63	5974.75	5622.35	24633.57	70016.27	65445.85	27099.31	31283.42	23420.67	39774.24	23570.99	132012	168813.7	144339	120719.6	118081.3	24011.28	118099.7	23311.85	450356	5238.65	169430.1
KB							13219.22	11259.54	27652.43	68309.61	64029.28	29928.54	38689.31	31082.45	33520.45	16239.35	139346	175756.3	151256.3	127552.1	123827.9	18825.85	124980.4	17498.56	457832.1	5732.65	176341.9
LZ								3553.09	20846.59	68810.48	64054.82	23305.5	27630.05	19267.69	45739.17	29394.3	128179.7	165609	141322.7	117752	116549.4	29897.21	114998.2	29265.11	446076.6	8720.72	166201.3
MO									19107.31	65836.65	61160.22	21571.06	31148.4	22766.35	44578.27	27528.19	131586.2	169136.8	144836.3	121280.2	120053.4	29213.71	118561.1	28274.85	449391.6	5948.01	169725
JK										50482.84	45488.33	2467.91	40827.31	32677.72	60206.72	41739.2	135915.7	175501.7	151886.3	128932.7	131750.8	46421.86	125782.2	44953.68	450635.8	22217.23	176048.6
ES											5085.73	48531.58	91098.23	83115.08	89663.11	73540.85	181261.3	222253	199431.7	177210.4	181975.3	82884.29	173854.2	80469.12	489037.9	65353.01	222761.7
SF												43511.3	86167.51	78113.68	86513.34	69948.28	176272.4	217222.8	194384.4	172130.9	176921.2	79069.76	168757.2	76696.4	484519.1	60851.75	217715.2
WW													42596.74	34636.36	62257.05	43730.09	136878.8	176643	153173.9	130280.9	133535.5	48676.37	127112.6	47150.65	451065	24553.12	177190
BL														8574.94	64470.2	52644.61	100736.8	137997.6	113734.1	90222.66	90992.21	48456.09	87398.92	49167.94	419119.5	35919.95	138582.9
CP															59138.59	45816.42	108888	146444.1	122244.1	98772.99	99326.54	42862.47	95996.25	43186.67	427006.8	27723.17	147075.1
MD																18593.61	160051.9	192371.6	167736.7	144153	133411.5	16347	142355.6	16472.46	478728.4	39230.24	193068.7
NL																	152166.4	187031.9	162408	138649.3	131860.3	10431.09	136403.9	7844.39	471096.3	21724.52	187715.1
KN																		42157.45	25393.74	25139.13	55755.21	145813.2	23621.02	147213.3	318998.1	136613.3	42512.24
KL																			24670.57	48435.35	65794.62	179544.9	50740.3	181267.2	288078.4	173683.2	763.46
HQ																				23776.31	44421.08	154896.2	26308.07	156596.4	312216.4	149329.7	25350.21
MQ																					30794.1	131128.5	3990.07	132814.2	335219.7	125659.8	49119.63
PN																						122896.1	33321.06	125005.5	352746.2	123301.1	66564.79
RS																							129089.9	2583.69	464758.3	24437.63	180223.8
FT																								130735.1	336691.2	123017.4	51391.02
LO																									336710.6	23235.51	181939
JJ																										454710.6	287324.2
VW																											174313.2
EB																											

Table S2.2. Pairwise distances among all populations (m; Table 2.1; SF = FR) from within the same drainage, with pairwise distances between populations not from the same drainage assigned an arbitrarily large distance of 1000000 m. Not all values in this matrix were used in correlation analyses with pairwise differences in mean SL and SWW since not all populations here were used in SL and SWW analyses.

	BP	BV	CH	FG	HK	KB	LZ	MO	JK	ES	SF	WW	BL	CP	MD	NL	KN	KL	HQ	MQ	PN	RS	FT	LO	JJ	VW	EB
BP		9729.29	1749.43	2038.09	5569.09	9792.98	5436.05	2015.11	1000000	1000000	1000000	1000000	1000000	1000000	1000000	1000000	1000000	1000000	1000000	1000000	1000000	1000000	1000000	1000000	1000000	4207.13	1000000
BV			8492.84	7736.19	8092.28	758.52	13438.48	11315.54	1000000	1000000	1000000	1000000	1000000	1000000	1000000	1000000	1000000	1000000	1000000	1000000	1000000	1000000	1000000	1000000	1000000	5667.89	1000000
CH				1244.79	3936.78	8415.9	5500.66	2853.82	1000000	1000000	1000000	1000000	1000000	1000000	1000000	1000000	1000000	1000000	1000000	1000000	1000000	1000000	1000000	1000000	1000000	3256.13	1000000
FG					4710.09	7767.52	6695.75	3738.48	1000000	1000000	1000000	1000000	1000000	1000000	1000000	1000000	1000000	1000000	1000000	1000000	1000000	1000000	1000000	1000000	1000000	2222.36	1000000
HK						7675.63	5974.75	5622.35	1000000	1000000	1000000	1000000	1000000	1000000	1000000	1000000	1000000	1000000	1000000	1000000	1000000	1000000	1000000	1000000	1000000	5238.65	1000000
KB							13219.22	11259.54	1000000	1000000	1000000	1000000	1000000	1000000	1000000	1000000	1000000	1000000	1000000	1000000	1000000	1000000	1000000	1000000	1000000	5732.65	1000000
LZ								3553.09	1000000	1000000	1000000	1000000	1000000	1000000	1000000	1000000	1000000	1000000	1000000	1000000	1000000	1000000	1000000	1000000	1000000	8720.72	1000000
MO									1000000	1000000	1000000	1000000	1000000	1000000	1000000	1000000	1000000	1000000	1000000	1000000	1000000	1000000	1000000	1000000	1000000	5948.01	1000000
JK										1000000	1000000	1000000	1000000	1000000	1000000	1000000	1000000	1000000	1000000	1000000	1000000	1000000	1000000	1000000	1000000	1000000	1000000
ES											5085.73	48531.58	1000000	1000000	1000000	1000000	1000000	1000000	1000000	1000000	1000000	1000000	1000000	1000000	1000000	1000000	1000000
SF												43511.3	1000000	1000000	1000000	1000000	1000000	1000000	1000000	1000000	1000000	1000000	1000000	1000000	1000000	1000000	1000000
WW													1000000	1000000	1000000	1000000	1000000	1000000	1000000	1000000	1000000	1000000	1000000	1000000	1000000	1000000	1000000
BL														8574.94	1000000	1000000	1000000	1000000	1000000	1000000	1000000	1000000	1000000	1000000	1000000	1000000	1000000
CP															1000000	1000000	1000000	1000000	1000000	1000000	1000000	1000000	1000000	1000000	1000000	1000000	1000000
MD																1000000	1000000	1000000	1000000	1000000	1000000	1000000	1000000	1000000	1000000	1000000	1000000
NL																	1000000	1000000	1000000	1000000	1000000	1000000	1000000	7844.39	1000000	1000000	1000000
KN																		42157.45	1000000	1000000	1000000	1000000	1000000	1000000	1000000	1000000	42512.24
KL																			1000000	1000000	1000000	1000000	1000000	1000000	1000000	1000000	763.46
HQ																				1000000	1000000	1000000	1000000	1000000	1000000	1000000	1000000
MQ																					30794.1	1000000	3990.07	1000000	1000000	1000000	1000000
PN																						1000000	33321.06	1000000	1000000	1000000	1000000
RS																							1000000	1000000	1000000	1000000	1000000
FT																								1000000	1000000	1000000	1000000
LO																									1000000	1000000	1000000
JJ																										1000000	1000000
VW																											1000000
EB																											

Table S2.3. Distances of each population (Table 2.1) to the sea.

Population	Distance to sea (m)
BP	46188.21
BV	50117.09
CH	45715.93
FG	46988.33
HK	42979.75
KB	49657.55
LZ	40800.60
MO	44417.23
JK	56449.97
ES	106852.96
FR	101830.02
WW	61490.70
BL	15851.82
CP	23851.88
MD	0
NL	61498.95
KN	5893.91
KL	2876.79
HQ	15335.74
MQ	26681.70
PN	54281.10
RS	0
FT	27086.82
LO	56267.40
JJ	109433.21
VW	48441.77
EB	2127.27

Table S2.4. Lake surface areas determined using KML Tools software (available at https://extension.unh.edu/kmlTools/) to compute area of polygons drawn from Google Earth (Google, Inc. 2017) images of lakes.

Population	Surface area (m^2)
FG	250629.88
HK	49435.70
MO	386494.77
JK	404503.34
ES	89827.66
FR	188573.21
WW	822576.25
BL	167986.40
CP	532772.11
MD	248249.93
KN	301119.25
KL	151652.92
FT	464504.13
LO	44427.55
VW	663390.52
EB	94655.06

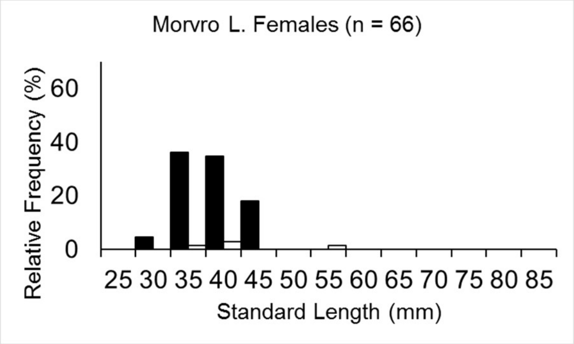
Table S2.5. Results of Mantel correlation analyses of pairwise differences among populations in mean size (SL and SWW) with matrices of pairwise interpopulation distances (GEO; Table S2.1) and drainage affiliation (STR; Table S2.2). * = P-value for 1000 random matrix permutations.

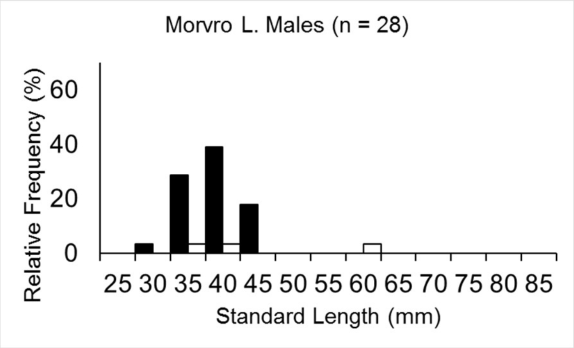
Age	Sex	Correlation Analysis	*r*	t-stat	P-value	P-value*
1	F	SL v. GEO	0.088	0.204	0.419	0.309
1	F	SL v. STR	-0.274	-0.786	0.216	0.220
2	F	SL v. GEO	0.070	0.458	0.323	0.333
2	F	SL v. STR	0.037	0.308	0.379	0.364
1	M	SL v. GEO	-0.125	-0.480	0.316	0.410
1	M	SL v. STR	-0.211	-0.889	0.187	0.202
2	M	SL v. GEO	-0.027	-0.226	0.411	0.480
2	M	SL v. STR	0.057	0.460	0.323	0.313
3	M	SL v. GEO	0.280	1.301	0.097	0.174
1	F	SWW v. GEO	0.026	0.061	0.476	0.359
1	F	SWW v. STR	-0.225	-0.574	0.283	0.337
2	F	SWW v. GEO	-0.189	-1.156	0.124	0.054
2	F	SWW v. STR	0.070	0.518	0.302	0.281
1	M	SWW v. GEO	-0.140	-0.575	0.283	0.339
1	M	SWW v. STR	-0.174	-0.765	0.222	0.253
2	M	SWW v. GEO	-0.119	-0.738	0.230	0.228
2	M	SWW v. STR	0.127	0.945	0.172	0.192

Table S2.6. Results of correlation analyses of mean size (SL and SWW) with distance to the sea (DIST) and lake-specific ecological variables (AMM = [ammonia], AREA = lake surface area, CALC = [calcium], CHLA = abundance of chlorophyll a, COND = conductivity, MG = [magnesium], NIT = nitrate, PH = pH, TP = total [phosphorus], PRED = presence/absence of predators). r = product moment correlation coefficient, df = degrees of freedom.

Age	Sex	Correlation Analysis	r	t-stat	df	P-value
1	F	SL v. DIST	0.324	0.684	4	0.532
1	F	SWW v. DIST	0.208	0.368	3	0.737
2	F	SL v. DIST	-0.091	-0.273	9	0.791
2	F	SWW v. DIST	0.023	0.068	9	0.947
1	M	SL v. DIST	0.450	1.233	6	0.264
1	M	SWW v. DIST	0.397	1.059	6	0.330
2	M	SL v. DIST	-0.297	-0.897	8	0.405
2	M	SWW v. DIST	-0.255	-0.747	8	0.476
1	F	SL v. AMM	-0.292	-0.611	4	0.574
1	F	SL v. AREA	-0.412	-0.904	4	0.417
1	F	SL v. CALC	0.036	0.071	4	0.947
1	F	SL v. CHLA	-0.290	-0.601	4	0.578
1	F	SL v. COND	-0.004	-0.008	4	0.994
1	F	SL v. MG	0.207	0.423	4	0.694
1	F	SL v. NIT	-0.247	-0.509	4	0.637
1	F	SL v. PH	0.197	0.402	4	0.708
1	F	SL v. TP	-0.149	-0.301	4	0.778
1	F	SWW v. AMM	-0.367	-0.684	3	0.543
1	F	SWW v. AREA	-0.719	-1.792	3	0.171
1	F	SWW v. CALC	0.332	0.609	3	0.585
1	F	SWW v. CHLA	-0.378	-0.707	3	0.531
1	F	SWW v. COND	0.302	0.550	3	0.621
1	F	SWW v. MG	0.807	2.368	3	0.099
1	F	SWW v. NIT	-0.106	-0.185	3	0.865
1	F	SWW v. PH	0.445	0.860	3	0.453
1	F	SWW v. TP	-0.056	-0.098	3	0.928
2	F	SL v. AMM	-0.629	-2.426	9	0.038
2	F	SL v. AREA	-0.243	-0.752	9	0.471
2	F	SL v. CALC	0.198	0.601	9	0.560
2	F	SL v. CHLA	-0.539	-1.920	9	0.087
2	F	SL v. COND	0.134	0.407	9	0.694
2	F	SL v. MG	0.264	0.820	9	0.434
2	F	SL v. NIT	-0.290	-0.908	9	0.388
2	F	SL v. PH	0.222	0.684	9	0.511
2	F	SL v. PRED	0.029	0.086	9	0.933
2	F	SL v. TP	-0.251	-0.778	9	0.457
2	F	SWW v. AMM	-0.583	-2.153	9	0.060
2	F	SWW v. AREA	-0.153	-0.464	9	0.654
2	F	SWW v. CALC	0.346	1.107	9	0.297
2	F	SWW v. CHLA	-0.585	-2.162	9	0.059
2	F	SWW v. COND	0.243	0.752	9	0.471
2	F	SWW v. MG	0.324	1.028	9	0.331
2	F	SWW v. NIT	-0.282	-0.882	9	0.401

2	F	SWW v. PH	0.393	1.282	9	0.232
2	F	SWW v. PRED	0.247	0.765	9	0.464
2	F	SWW v. TP	-0.261	-0.812	9	0.438
1	M	SL v. AMM	-0.226	-0.569	6	0.590
1	M	SL v. AREA	0.176	0.439	6	0.676
1	M	SL v. CALC	-0.417	-1.124	6	0.304
1	M	SL v. CHLA	-0.267	-0.677	6	0.523
1	M	SL v. COND	-0.281	-0.717	6	0.501
1	M	SL v. MG	0.152	0.378	6	0.719
1	M	SL v. NIT	-0.408	-1.096	6	0.315
1	M	SL v. PH	-0.139	-0.343	6	0.743
1	M	SL v. PRED	0.213	0.533	6	0.613
1	M	SL v. TP	-0.322	-0.832	6	0.437
1	M	SWW v. AMM	-0.406	-1.088	6	0.318
1	M	SWW v. AREA	-0.474	-1.320	6	0.235
1	M	SWW v. CALC	0.443	1.210	6	0.272
1	M	SWW v. CHLA	-0.506	-1.438	6	0.201
1	M	SWW v. COND	0.220	0.553	6	0.600
1	M	SWW v. MG	0.158	0.393	6	0.708
1	M	SWW v. NIT	0.075	0.184	6	0.860
1	M	SWW v. PH	0.533	1.541	6	0.174
1	M	SWW v. PRED	0.496	1.398	6	0.212
1	M	SWW v. TP	-0.121	-0.299	6	0.775
2	M	SL v. AMM	-0.334	-1.001	8	0.346
2	M	SL v. AREA	0.085	0.241	8	0.816
2	M	SL v. CALC	0.110	0.314	8	0.761
2	M	SL v. CHLA	-0.329	-0.984	8	0.354
2	M	SL v. COND	0.046	0.130	8	0.900
2	M	SL v. MG	0.236	0.687	8	0.512
2	M	SL v. NIT	-0.413	-1.283	8	0.235
2	M	SL v. PH	-0.059	-0.168	8	0.871
2	M	SL v. PRED	0.500	1.633	8	0.141
2	M	SL v. TP	0.005	0.015	8	0.989
2	M	SWW v. AMM	-0.398	-1.226	8	0.255
2	M	SWW v. AREA	0.126	0.359	8	0.729
2	M	SWW v. CALC	0.275	0.809	8	0.442
2	M	SWW v. CHLA	-0.420	-1.308	8	0.227
2	M	SWW v. COND	0.198	0.571	8	0.584
2	M	SWW v. MG	0.252	0.735	8	0.483
2	M	SWW v. NIT	-0.456	-1.448	8	0.186
2	M	SWW v. PH	0.102	0.289	8	0.780
2	M	SWW v. PRED	0.514	1.694	8	0.129
2	M	SWW v. TP	-0.039	-0.110	8	0.915


Morvro L. Females (n = 66)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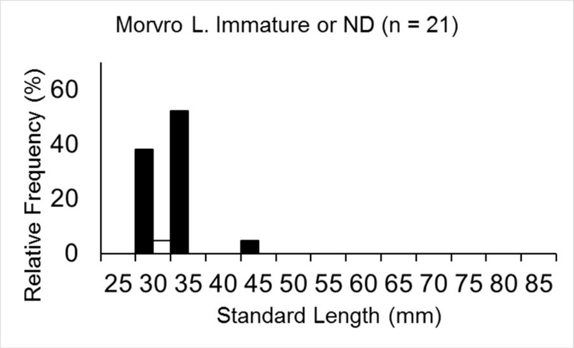
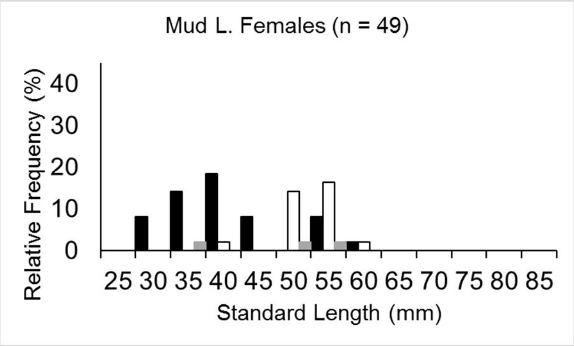
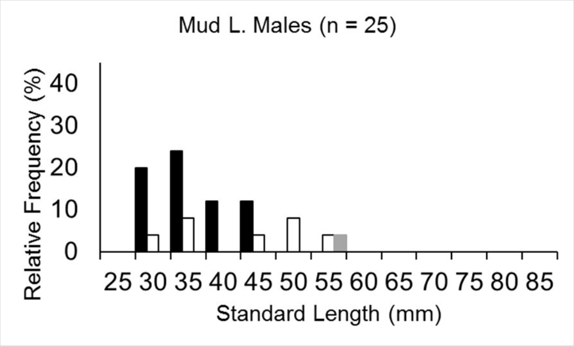
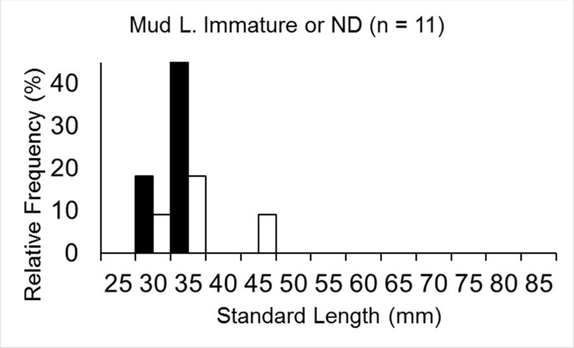
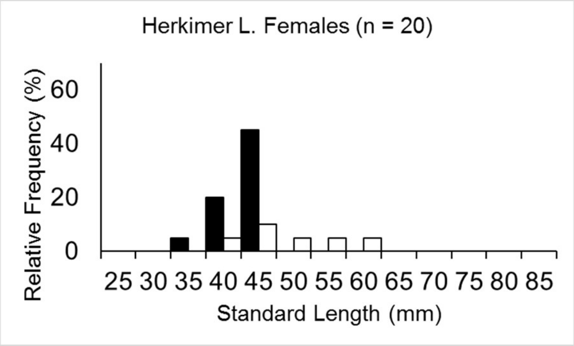
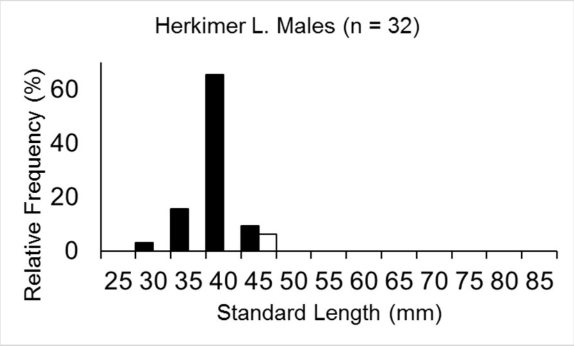

Morvro L. Immature or ND (n = 21)

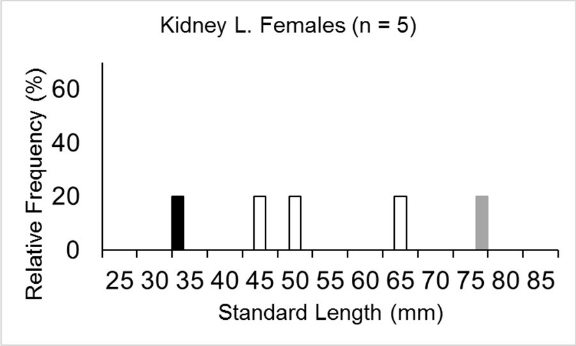

Mud L. Females (n = 49)

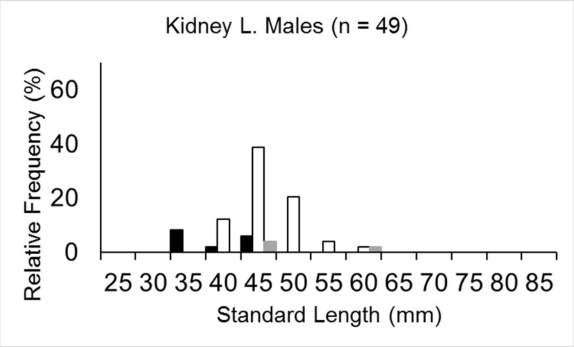

Mud L. Males (n = 25)

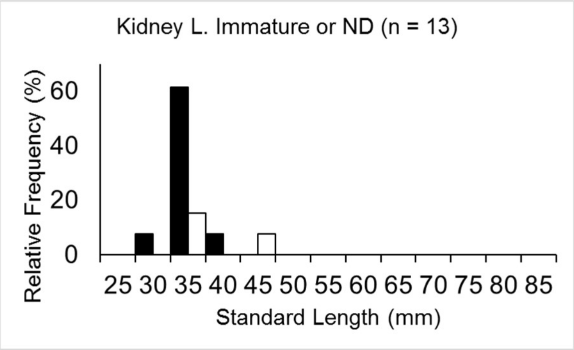

Mud L. Immature or ND (n = 11)

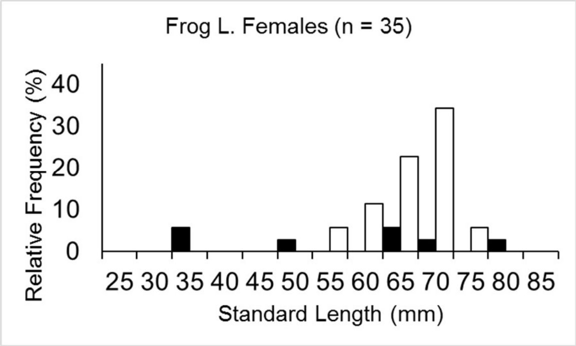

Herkimer L. Females (n = 20)

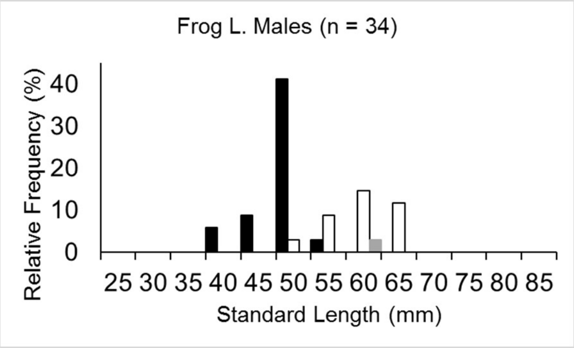

Herkimer L. Males (n = 32)

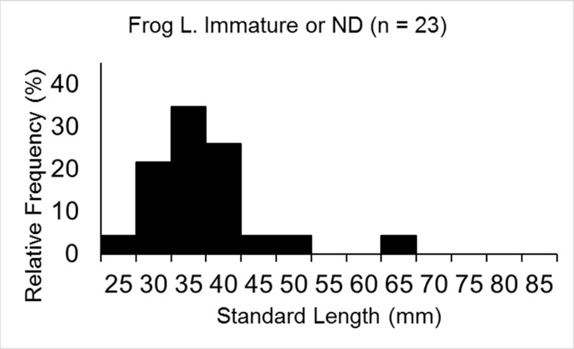

Kidney L. Females (n = 5)
Relative Frequency (%)
0
20
40
60
25 30 35 40 45 50 55 60 65 70 75 80 85
Standard Length (mm)

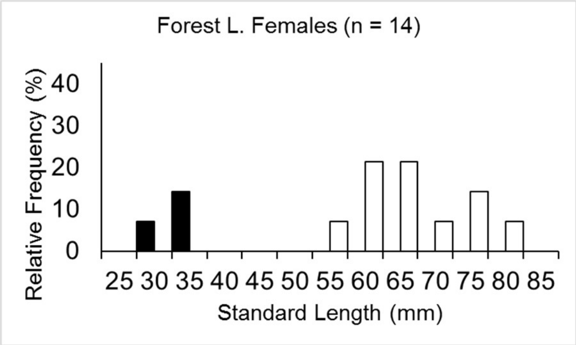

Kidney L. Males (n = 49)
Relative Frequency (%)
0
20
40
60
25 30 35 40 45 50 55 60 65 70 75 80 85
Standard Length (mm)

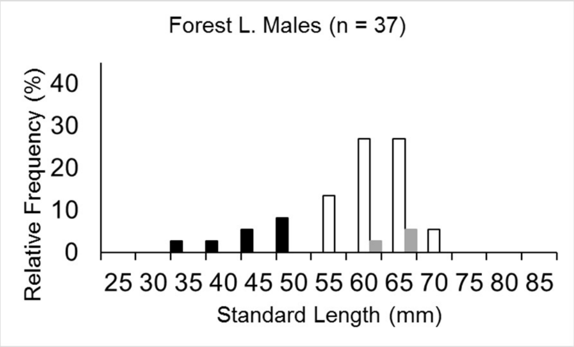

Kidney L. Immature or ND (n = 13)
Relative Frequency (%)
0
20
40
60
25 30 35 40 45 50 55 60 65 70 75 80 85
Standard Length (mm)

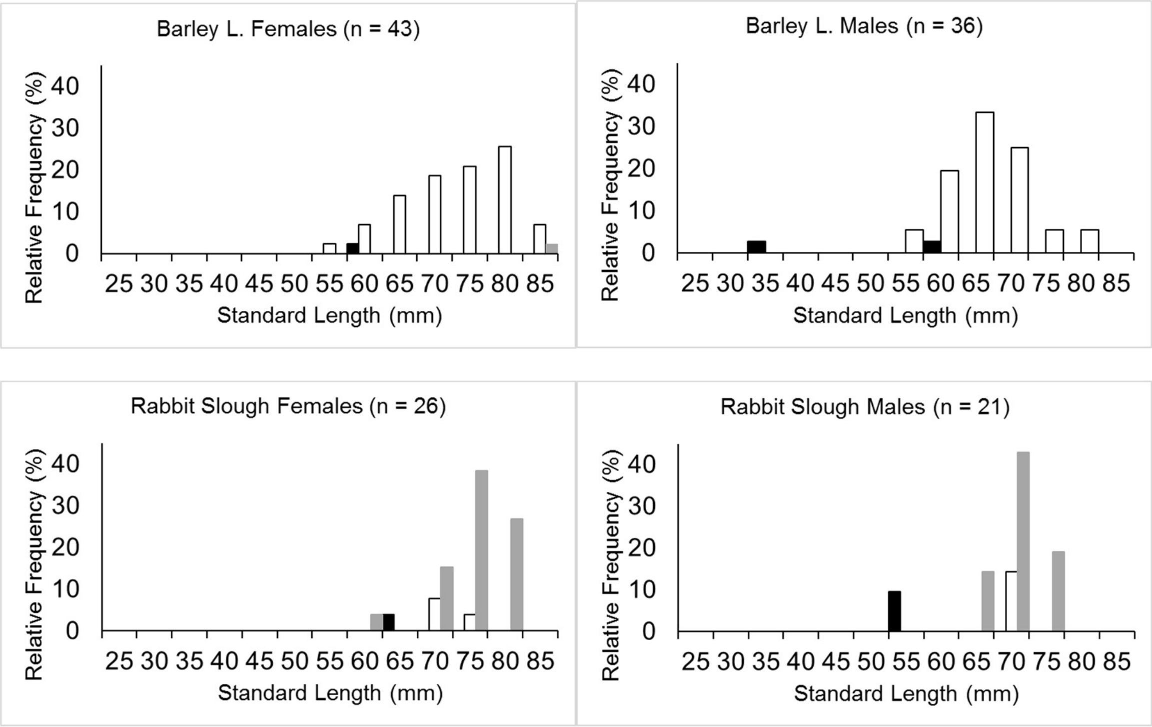

Frog L. Females (n = 35)
Relative Frequency (%)
0
10
20
30
40
25 30 35 40 45 50 55 60 65 70 75 80 85
Standard Length (mm)


Frog L. Males (n = 34)
Relative Frequency (%)
0
10
20
30
40
25 30 35 40 45 50 55 60 65 70 75 80 85
Standard Length (mm)


Frog L. Immature or ND (n = 23)
Relative Frequency (%)
0
10
20
30
40
25 30 35 40 45 50 55 60 65 70 75 80 85
Standard Length (mm)


Forest L. Females (n = 14)
Relative Frequency (%)
0
10
20
30
40
25 30 35 40 45 50 55 60 65 70 75 80 85
Standard Length (mm)


Forest L. Males (n = 37)
Relative Frequency (%)
0
10
20
30
40
25 30 35 40 45 50 55 60 65 70 75 80 85
Standard Length (mm)

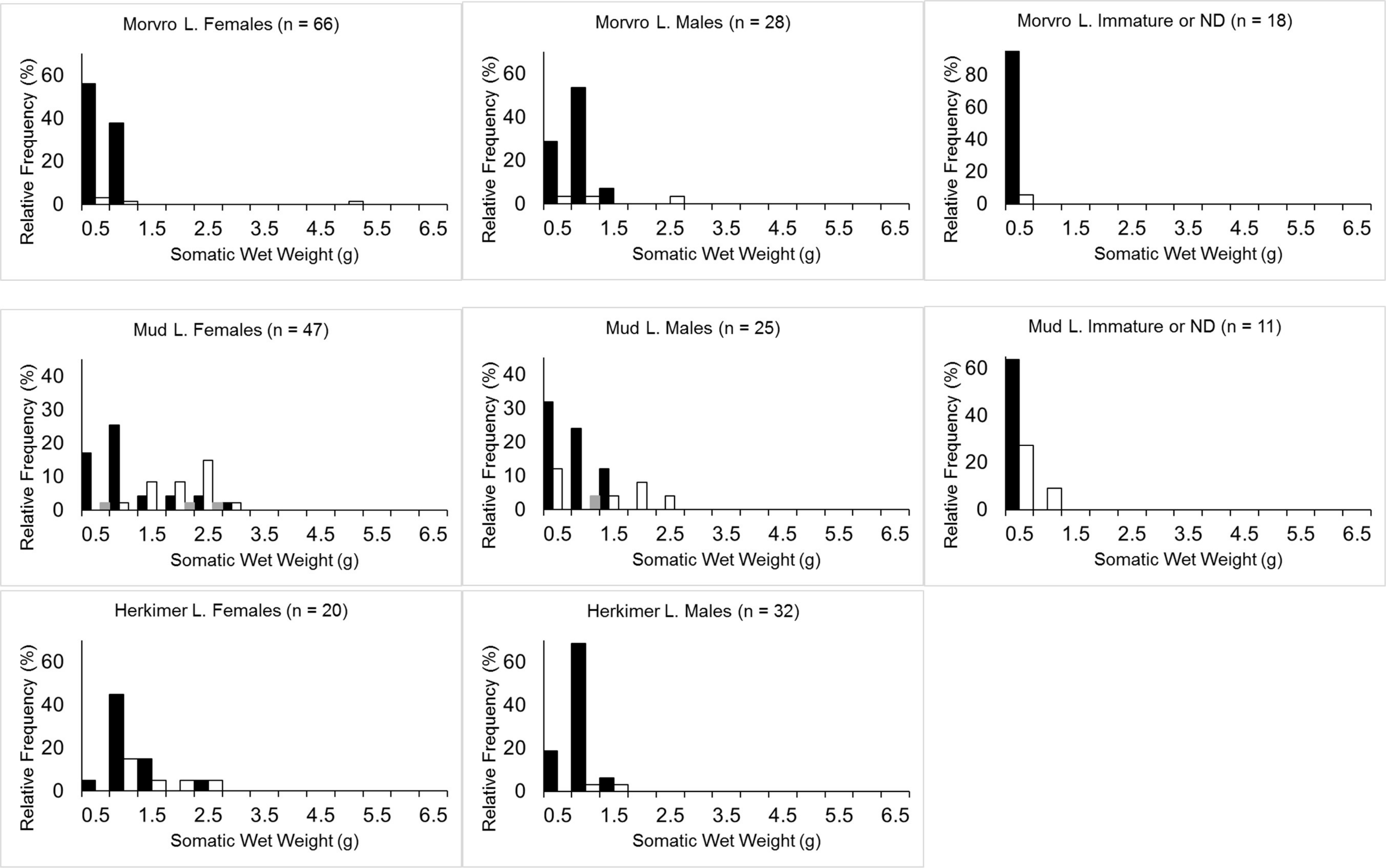

Barley L. Females (n = 43)

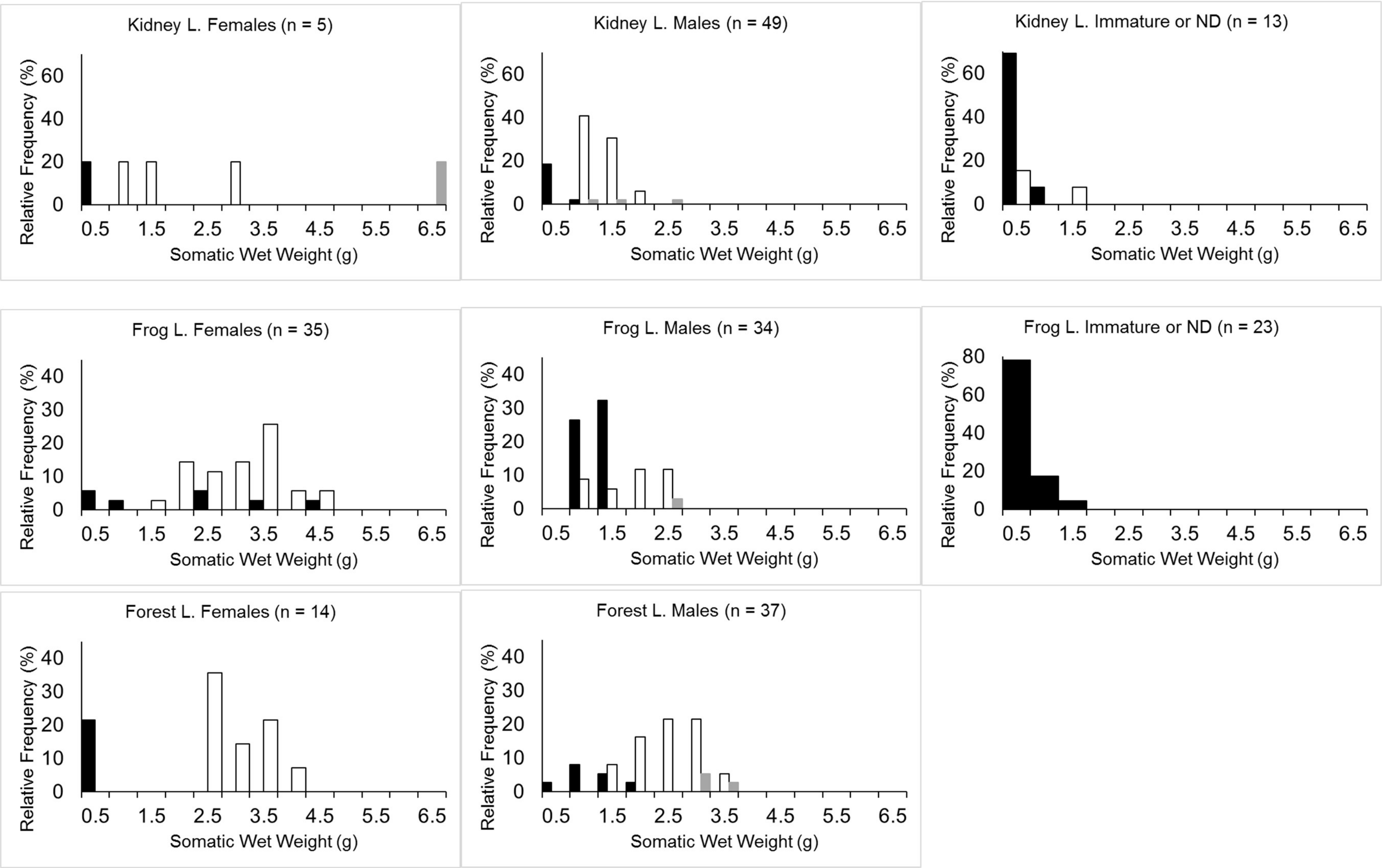
Barley L. Males (n = 36)

Rabbit Slough Females (n = 26)

Rabbit Slough Males (n = 21)

Figure S2.1. Length-frequency histograms of populations of Threespine Stickleback for age 1 (black), 2 (white), 3 and 4 (gray) fish.


Morvro L. Females (n = 66)
Morvro L. Males (n = 28)
Morvro L. Immature or ND (n = 18)
Mud L. Females (n = 47)
Mud L. Males (n = 25)
Mud L. Immature or ND (n = 11)
Herkimer L. Females (n = 20)
Herkimer L. Males (n = 32)
Relative Frequency (%)
Somatic Wet Weight (g)
0.5
1.5
2.5
3.5
4.5
5.5
6.5
0
10
20
30
40
60
80


Kidney L. Females (n = 5)
Kidney L. Males (n = 49)
Kidney L. Immature or ND (n = 13)
Frog L. Females (n = 35)
Frog L. Males (n = 34)
Frog L. Immature or ND (n = 23)
Forest L. Females (n = 14)
Forest L. Males (n = 37)
Relative Frequency (%)
0
10
20
30
40
60
80
Somatic Wet Weight (g)
0.5
1.5
2.5
3.5
4.5
5.5
6.5

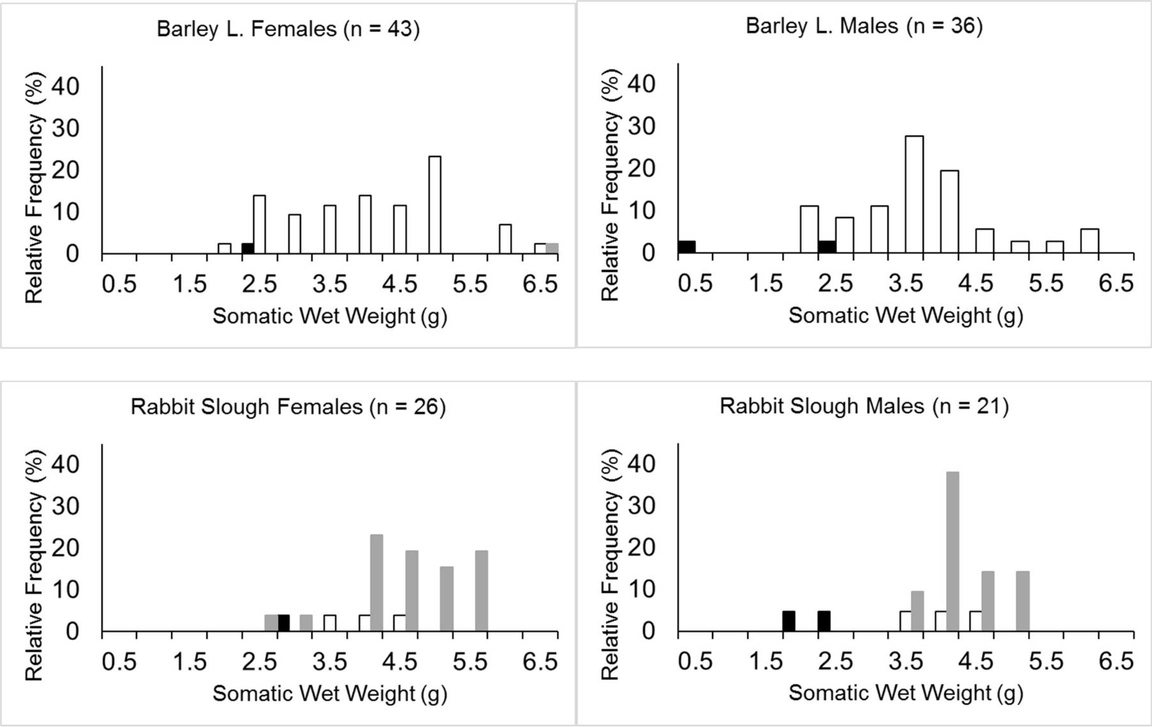


Figure S2.2. Frequency histograms for somatic wet weight of populations of Threespine Stickleback for age 1 (black), 2 (white), 3 and 4 (gray) fish.

Chapter 3

Table S3.1. SNP pools and primers used for Sequenom MassARRAY genotyping.

Pool	SNP_ID	2nd-PCRP	1st-PCRP
W1	chrXIII_5683842	AGCGGATAACGTTCCATTGTCCGATTCCTC	AGCGGATAACAGGAAGAGAGAGCCAGAAAG
W1	chrII_4557054	AGCGGATAACGCTGGGACAATAGTTTGACG	AGCGGATAACGTATAATTTCCGAGTAAGCAC
W1	chrXIX_16906593	AGCGGATAACAAACGCGGGCTACTTTAAGG	AGCGGATAACTTCCGTGACATCATTACAAG
W1	chrXVI_5891048	AGCGGATAACTTGAACTCCATCTACTAACC	AGCGGATAACAAAGACACAAACGGCCCAAC
W1	chrIV_32092920	AGCGGATAACAAGTCCCTGTGGGAACACTC	AGCGGATAACAAAACGAGGATCTCTACTGG
W1	chrXI_5775144	AGCGGATAACCAAAACAGGCAGCAGATTCG	AGCGGATAACTTGAGCAGCTCTTCAATGGG
W1	chrI_21600738	AGCGGATAACCTCAATGTCTGCTTGAATAC	AGCGGATAACATATTAGAGCTGCATGGCCC
W1	chrXVI_5893989	AGCGGATAACCCTCAGCACAGGTTCTAATC	AGCGGATAACTACCCCAGACGTTACTTTGC
W1	chrXIV_10392093	AGCGGATAACGAAAGCTTTGTGGAGGAAGG	AGCGGATAACTGCAGAGAGTAACGTGTGTG
W1	chrXVI_5909125	AGCGGATAACTTTGTATGAGGGGCAAGAGG	AGCGGATAACTTGCACTCCACGAGACTCAC
W1	chrXI_16070261	AGCGGATAACCATCTGATTAGTTCTCGGTG	AGCGGATAACCGACCAATCGATAGGAGATG
W1	chrXIX_9374274	AGCGGATAACTGGTCTGTTTGACTACATGG	AGCGGATAACGCCGTAAAGATTGTGGTCTC
W1	chrXVIII_3162328	AGCGGATAACATGTCAACCCACCTCTCCG	AGCGGATAACAGGAAGATAAACTGTGAGGG
W1	chrII_4541659	AGCGGATAACAATACCGGCTCATTCACCTC	AGCGGATAACTGATCCTAGAAGCCAAGAGC
W1	chrXVII_8129604	AGCGGATAACCCTCTCATTACAGGATCCAG	AGCGGATAACCACCAACCCGTCATATACAG
W1	chrXVIII_3156177	AGCGGATAACCTCGCAGCCCTTTTGGTTT	AGCGGATAACATCCTTATCTGAGGGAGAGC
W1	chrXI_5773228	AGCGGATAACTGAGGTCACTTTTCACTTGC	AGCGGATAACTCGCGGGTGGAACTAACAAA
W1	chrIV_3330514	AGCGGATAACAAAGGGAAGAAACCTCAGGG	AGCGGATAACTGTACACATGGCATCCATTG
W1	chrXIX_16948763	AGCGGATAACGGAACATGTGTTGTGAACGC	AGCGGATAACAAAGTCGAGGATGACAAACC
W1	chrXIII_5675328	AGCGGATAACTCCACCTCGATGAACTCCAC	AGCGGATAACCACCGACTTACGCAACAGAT
W1	chrII_4547932	AGCGGATAACAGAACATCACCGAGTTCGAG	AGCGGATAACTCTGAGTCCTTGTCCGGCCT
W1	chrXVI_5906684	AGCGGATAACATGTCTTTGAGGGTGAGGTC	AGCGGATAACGGGCACTCTTTCACTCAAAC
W1	chrXVI_5888503	AGCGGATAACAGCGCTTTTGGAAAAGAAT	AGCGGATAACCCCCCTATATTCAATCTATGG
W1	chrXIX_16924542	AGCGGATAACTACTATGTTGTTCTCCCAGC	AGCGGATAACCTGTAGCTACAAGCTTGAAC
W1	chrIV_3333740	AGCGGATAACAGACTCGTACTACTTTGGG	AGCGGATAACTTATTTGTTGTGCTGACTGG

W1	chrXVI_5896227	AGCGGATAACGTCCGTCGAGGTCACATATC	AGCGGATAACCCAAAAAACAATGAAGCCTG
W1	chrXIV_10394249	AGCGGATAACAGCTTTCACCTACAGCACAG	AGCGGATAACGCTTGTTTGTAGTGGATTC
W1	chrXIX_9371313	AGCGGATAACGGCTGAATTACATGTTGAAG	AGCGGATAACGGCAATACAAAAAGTAAATAC
W1	chrXIX_16959478	AGCGGATAACTTCTCCAAAATCCTCCGCTC	AGCGGATAACAGACCCCATAAGTGGCCAAG
W2	chrII_4553994	AGCGGATAACCTCTTCTTTGTGTTGGAGGC	AGCGGATAACAACGGTTGTAGAAAGAGCTG
W2	chrIV_3334738	AGCGGATAACTGCTCTCCTTCAGCTTCTAC	AGCGGATAACTGTTTCTTTCTTTCACTGGG
W2	chrXIX_16942698	AGCGGATAACTGGCGAGACGTGTTGTTTTG	AGCGGATAACTAGAGGGATTAGAGAGGGAC
W2	chrXVII_10618107	AGCGGATAACTCACCTGGGTGTTTGCACTG	AGCGGATAACCCAGTGCAGATGTAAGTGAG
W2	chrII_21927301	AGCGGATAACAATAACCGAGACTCGAAGGC	AGCGGATAACATGACGACGGACTGAACGAG
W2	chrI_21589802	AGCGGATAACGTTGACGCACCAGCACTCG	AGCGGATAACACACGCAGAAATGAACCCAG
W2	chrXIV_10387725	AGCGGATAACGATTTCCTTGCAGCTAACCG	AGCGGATAACTAATGCAGAGGCCCTTATGG
W2	chrXVIII_3139053	AGCGGATAACTCGTCGCTCTCCTCCTGTA	AGCGGATAACTCCAGGCGTCGTTGTTTTTC
W2	chrXVI_13150461	AGCGGATAACGACATAGTTGCACGGTTCAG	AGCGGATAACCTGTCAGACATTTGACTTCAC
W2	chrXIII_5678606	AGCGGATAACGCTCAATATAAGTCCCTCGG	AGCGGATAACTATTCAATGCGCATCCAGCC
W2	chrX_9534783	AGCGGATAACTTCCACCTGCCTCCTCTTTA	AGCGGATAACTGACCTCGAGGGAAAGGAAG
W2	chrXVI_5899818	AGCGGATAACTACATCTTGTTGTCTTCCTC	AGCGGATAACGAGCCTCTGTAAAACAAACC
W2	chrIV_32105368	AGCGGATAACTCGTCATGGCGACGGAAAC	AGCGGATAACAAACAACCTTTCCTGCCAGC
W2	chrXI_5763059	AGCGGATAACGGATCCAAGGATCATAAAAAC	AGCGGATAACAATTGGCCAAGAAGCGACCG
W2	chrXIII_5673292	AGCGGATAACGCAACAGGTGTGTCTGTTTG	AGCGGATAACGTACCCCGAACAAGTGCCTT
W2	chrXVIII_3149894	AGCGGATAACTGCTGAGCTATGTGGGAGAC	AGCGGATAACGGAGAAGTAGATCTCTGTGG
W2	chrXI_16076821	AGCGGATAACTAAAAGGGATGGATATTGC	AGCGGATAACATCGGTTCATAGGACACCTG
W2	chrXIII_5679721	AGCGGATAACGCTACTTCTACATGCTGTGG	AGCGGATAACGGGTAATTCTACTTCAATTCC
W2	chrI_21593750	AGCGGATAACTATTTCAGTCTGTGTGCCCC	AGCGGATAACGAAAACTCCTGTGCCATCTG
W2	chrII_4573177	AGCGGATAACATTCACCTTCATGTCAGCGG	AGCGGATAACACCGAGCGCGTTGAATAACC
W2	chrXIX_9375733	AGCGGATAACTTCTTTCCTTCTGGACCAGC	AGCGGATAACTTCAGAGTCCTCTCCTGTTG
W2	chrX_9527397	AGCGGATAACTAGTACTTGGCCAGTTCCAC	AGCGGATAACTCTGCCTGGGTGCCCTGAC
W2	chrXIX_13286373	AGCGGATAACCATGACAAGTGGCTGCATAG	AGCGGATAACATAAGGGAACCCTCCAACTG
W2	chrII_21928786	AGCGGATAACGCAGATACTACAAGCGTAGA	AGCGGATAACAGACACGAGCAGCCAGCCAA
W2	chrXVI_5912332	AGCGGATAACACACAGCTCAAGAGGAATGC	AGCGGATAACAATAGTTTGGGCCACTTGGG
W2	chrXI_5781688	AGCGGATAACGTTTTGGGAAGCAGGGTTTG	AGCGGATAACAACAGTCCTGACCCTTGACC
W2	chrXIX_13288787	AGCGGATAACCAAGTAAAAGTACCCCGAGG	AGCGGATAACCACCACTGCATTCCTTAACC

W3	chrII_21932462	AGCGGATAACCTTTTTCCAGATTCCAGCGG	AGCGGATAACCAGCTGGATCTGTGCTGATG
W3	chrII_4566452	AGCGGATAACAGTCGATGGAGTCCAGGTAG	AGCGGATAACCAACCTGAAGAACATCACCC
W3	chrXIX_16915746	AGCGGATAACACGCCGAGTTTAACAACGTC	AGCGGATAACGTTATCCTCCATGATGCCTG
W3	chrXI_5766794	AGCGGATAACGGTGTTTTGTGTCGTTTGGC	AGCGGATAACCGCTGTGACTTATTGGAGAG
W3	chrIV_3332621	AGCGGATAACTGCACTGTGATGTGTGGTTC	AGCGGATAACAAAAGGAAGAAGACACCGGC
W3	chrXVII_7561091	AGCGGATAACACTTTCTGTGTTCTGTTGGG	AGCGGATAACGATGGGTATGAAATTATTC
W3	chrXIX_9369810	AGCGGATAACAATGTCAAACTCCATCGACG	AGCGGATAACATGGAAGCTCATTCCTGACG
W3	chrXIX_13291868	AGCGGATAACTGTCGGTGACTGAAGGGGAG	AGCGGATAACAGGCACACAAACATGCCTTC
W3	chrXVI_13153214	AGCGGATAACCAAGCTTTCCGTTGAACGAC	AGCGGATAACCATTAAATGCAACTCTGCTG
W3	chrI_21598355	AGCGGATAACTCACACCTGGGTCTAAATCC	AGCGGATAACGAACCCAGTAGAGCCAAAAT
W3	chrXIV_10389873	AGCGGATAACAAGTAGCCTCCACATTCAGC	AGCGGATAACAGCCAGACTGATCTGTTCTC
W3	chrXVI_5905544	AGCGGATAACACATTGGCAGGAAACACACG	AGCGGATAACTACTATGGCGACAGTGAAGG
W3	chrXIII_5681784	AGCGGATAACATTCTCACCTTTCTCCCAGC	AGCGGATAACGGTTTAAGCATTGTCATATC
W3	chrX_9526306	AGCGGATAACCCACAATGACGGGTCATATC	AGCGGATAACGTGCAAACCTCAGTCACAAG
W3	chrXVIII_3146807	AGCGGATAACTTGACACGCTGGTCCTTTTC	AGCGGATAACTCCCGTGACTTATTTACTTC
W3	chrXIX_16928893	AGCGGATAACAAGCTCGGCCAGGGTTACTT	AGCGGATAACGCAGAGGAATCTTCGGTATG
W3	chrIV_3329457	AGCGGATAACCGGAGGGAGATATGATGCTG	AGCGGATAACAAATCCATTGTCGCCGTCTG
W3	chrXIX_16911266	AGCGGATAACAACACCGTGGCTCAAGTTTC	AGCGGATAACAGGGAGGTTCTTTGAGTGAG
W3	chrIV_3331520	AGCGGATAACGGCCCTTGTTCTGGAAAGTC	AGCGGATAACCACATTCCATCAGAGTGTGC
W3	chrXIV_10396321	AGCGGATAACAGTGACCATCCTGATGTTAG	AGCGGATAACTCCTCCACAGAAACACAGAG
W3	chrXIV_10398703	AGCGGATAACCTGCTATATTTACAATTACCG	AGCGGATAACCTACGAGTTCCACTTTTGAC
W3	chrIV_32100268	AGCGGATAACGCAAACCTAAATGAAATATGG	AGCGGATAACTTCCACTTAAAGCCCTTTGC
W3	chrX_9530904	AGCGGATAACCAACGCTATTGTTGTATGGG	AGCGGATAACAATCCAATCACCTGCCACTG
W4	chrXVIII_3168806	AGCGGATAACATCACGTGACTCTCACGATG	AGCGGATAACAACAAGTTACTTTGCGCGCC
W4	chrXVIII_3159265	AGCGGATAACACTTACAGCTCAAGGTGAGG	AGCGGATAACAGAGCCACAGCTGCAAAATG
W4	chrIV_32107470	AGCGGATAACTCGTTGCCCCAGAGATTAAG	AGCGGATAACAGTGTGCTGGCCGCCTTGT
W4	chrXI_5780656	AGCGGATAACCACCGCTTTGGCTTTAAGAC	AGCGGATAACATTGACTGCTGCATGACCAC
W4	chrXI_16071679	AGCGGATAACACAGGTCATTTAGCAGACGC	AGCGGATAACTAACCCCTAATTGCTCCCTG
W4	chrX_9528514	AGCGGATAACCCTCTGAGGAAGACGTGATA	AGCGGATAACATTTTGTGACCTCAGGTGGG
W4	chrIV_32103306	AGCGGATAACGGTTATATCTAAGCTCTCTC	AGCGGATAACAGTCCCAAGTCACTTCATAC
W4	chrXI_5779439	AGCGGATAACTTACCCGTCATTCCAGTGTG	AGCGGATAACACTTCACTTTCTGGCAGTGG

W4	chrIV_32097027	AGCGGATAACGAGGGTTCACAAAGAAGAGG	AGCGGATAACACATATGCACTCACTCGCAC
W4	chrII_4551986	AGCGGATAACCACGCACATTCTATCCCATA	AGCGGATAACGGTCACAACTTTTTCAACA
W4	chrXVIII_3144386	AGCGGATAACCTTACCTTCACCTCGCTTTC	AGCGGATAACTACAATGGCTGTGACGTGTG
W4	chrXIX_9383460	AGCGGATAACAAATCCTTAAATTAGGCAC	AGCGGATAACCTGATTTATGGAGTTATAG
W4	chrXVIII_3143344	AGCGGATAACCAGGTTGCAATTTGCAACCG	AGCGGATAACGAGAGTCAACTTCACTCCAG
W4	chrII_4564317	AGCGGATAACCGGCCGTGATGTCATATTAG	AGCGGATAACTCAAAGCACAACGCTCGCAC
W4	chrXVIII_3165611	AGCGGATAACCAGTTCCTTAATTCCGGTTG	AGCGGATAACTGTGGGTCTGTGAAGTAACG

Table S3.2. Genotype-phenotype association analyses and results for Barley Lake. Chr with Roman numerals indicate linkage group, and the number following indicates SNP position in the stickleback genome on that LG. If SNP location is followed by letters (F, M, or I), these indicate analysis within sex categories where F = female, M = male, and I = immature. If SNP location is followed by numbers (1 or 2), these indicate analysis within age categories where 1 = age 1 fish and 2 = age 2 fish. *F*-statistic, numerator and denominator degrees of freedom (df num and df den, respectively), unadjusted *P*-value, FDR significance threshold, and FDR-adjusted *P*-value (q) are given for each analysis. ns = Not significant at corrected Bonferonni threshold of $P < 0.0013$.

SNP	F	df num	df den	p	FDR sign thresholds	q	Corrected Bonferonni	η_p^2
chrIV_3334738	4.27	1	73	**0.0423**	0.000847458	0.7041	ns	0.056
chrXVI_5893989	3.76	1	71	0.0564	0.001694915	0.7041	ns	0.050
chrXIX_13286373_F	3.86	1	35	0.0574	0.002542373	0.7041	ns	0.100
chrII_4541659	3.4	1	70	0.0695	0.003389831	0.7041	ns	0.046
chrXVIII_3168806	2.72	2	67	0.073	0.004237288	0.7041	ns	0.075
chrXIV_10392093	2.98	1	70	0.0885	0.005084746	0.7041	ns	0.041
chrII_4551986_F	2.65	1	28	0.1148	0.005932203	0.7041	ns	0.086
chrXVIII_3159265	2.14	2	67	0.1259	0.006779661	0.7041	ns	0.059
chrII_4551986_M	2.52	1	23	0.126	0.007627119	0.7041	ns	0.099
chrII_4553994	2.02	2	70	0.1397	0.008474576	0.7041	ns	0.055
chrII_4557054	2.3	1	28	0.1408	0.009322034	0.7041	ns	0.078
chrII_4547932_F	2.23	1	40	0.1432	0.010169492	0.7041	ns	0.054
chrXVIII_3162328_F	2	1	16	0.1768	0.011016949	0.7379	ns	0.112
chrXVI_5896227	1.91	1	25	0.1792	0.011864407	0.7379	ns	0.073
chrII_4547932_M	1.82	1	30	0.1876	0.012711864	0.7379	ns	0.056
chrXIX_13286373_M	1.57	1	28	0.2201	0.013559322	0.8116	ns	0.054
chrIV_32105368	1.26	1	19	0.2752	0.01440678	0.9283	ns	0.062
chrXI_16076821	1.17	1	73	0.2832	0.015254237	0.9283	ns	0.016
chrXVI_13150461	0.99	1	73	0.3227	0.016101695	0.9349	ns	0.013
chrXIX_16942698	0.94	1	23	0.3418	0.016949153	0.9349	ns	0.039
chrXVI_13153214	0.79	1	72	0.3774	0.01779661	0.9349	ns	0.010
chrI_21598355	0.65	1	71	0.4231	0.018644068	0.9349	ns	0.009

chrXVIII_3139053	0.59	1	41	0.4473	0.019491525	0.9349	ns	0.013
chrXVII_8129604	0.8	2	67	0.453	0.020338983	0.9349	ns	0.023
chrXVI_5912332	0.51	1	70	0.4794	0.021186441	0.9349	ns	0.008
chrIV_32103306	0.5	1	68	0.4818	0.022033898	0.9349	ns	0.007
chrX_9534783	0.47	1	72	0.4947	0.022881356	0.9349	ns	0.006
chrX_9530904	0.66	2	65	0.5183	0.023728814	0.9349	ns	0.021
chrXVI_5891048	0.4	1	71	0.5271	0.024576271	0.9349	ns	0.006
chrII_21932462	0.6	2	70	0.5538	0.025423729	0.9349	ns	0.016
chrIV_32092920	0.34	1	70	0.5608	0.026271186	0.9349	ns	0.005
chrXVI_5888503	0.55	2	65	0.5787	0.027118644	0.9349	ns	0.016
chrXVI_5905544	0.3	1	58	0.5858	0.027966102	0.9349	ns	0.005
chrII_4566452	0.26	1	71	0.6121	0.028813559	0.9349	ns	0.003
chrXVIII_3162328_M	0.25	1	13	0.6287	0.029661017	0.9349	ns	0.020
chrXIII_5681784	0.23	1	71	0.6314	0.030508475	0.9349	ns	0.003
chrII_31927301	0.23	1	73	0.6358	0.031355932	0.9349	ns	0.003
chrIV_32100268	0.21	1	21	0.6481	0.03220339	0.9349	ns	0.010
chrXIX_16911266	0.18	1	66	0.6709	0.033050847	0.9349	ns	0.003
chrXIII_5673292	0.16	1	73	0.6935	0.033898305	0.9349	ns	0.001
chrXIV_10396321	0.13	1	71	0.715	0.034745763	0.9349	ns	0.001
chrX_9526306	0.11	1	71	0.7382	0.03559322	0.9349	ns	0.001
chrXVII_7561091	0.11	1	26	0.7407	0.036440678	0.9349	ns	0.003
chrXIV_10398703	0.09	1	48	0.7641	0.037288136	0.9349	ns	0.002
chrXVI_5909125	0.27	2	69	0.7668	0.038135593	0.9349	ns	0.008
chrXIII_5675328	0.07	1	72	0.7887	0.038983051	0.9349	ns	0.002
chrXIX_16906593	0.06	1	73	0.8008	0.039830508	0.9349	ns	0.001
chrIV_32107470	0.05	1	69	0.8167	0.040677966	0.9349	ns	0.001
chrI_21600738	0.05	1	32	0.8203	0.041525424	0.9349	ns	0.002
chrXVIII_3149894	0.05	1	73	0.8245	0.042372881	0.9349	ns	0.001
chrX_9527397	0.05	1	73	0.8318	0.043220339	0.9349	ns	0.001
chrXVIII_3146807	0.04	1	70	0.8383	0.044067797	0.9349	ns	0.001
chrXVIII_3143344	0.04	1	40	0.8398	0.044915254	0.9349	ns	0.001

chrX_9528514	0.03	1	69	0.8632	0.045762712	0.9424	ns	0.000
chrXVIII_3156177	0.13	2	67	0.8785	0.046610169	0.9424	ns	0.003
chrXIV_10394249	0.01	1	15	0.9238	0.047457627	0.9656	ns	0.001
chrXVIII_3165611	0.01	1	69	0.9329	0.048305085	0.9656	ns	0.000
chrXVIII_3144386	0	1	69	0.9694	0.049152542	0.9795	ns	0.000
chrII_4573177	0	1	72	0.9933	0.05	0.9795	ns	0.000
chrIV_3332621	0	1	71	0.9961	0.050847458	0.9795	ns	0.000

Table S3.3. Genotype-phenotype association analyses and results for Frog Lake. Chr with Roman numerals indicate linkage group, and the number following indicates SNP position in the stickleback genome on that LG. If SNP location is followed by letters (F, M, or I), these indicate analysis within sex categories where F = female, M = male, and I = immature. If SNP location is followed by numbers (1 or 2), these indicate analysis within age categories where 1 = age 1 fish and 2 = age 2 fish. *F*-statistic, numerator and denominator degrees of freedom (df num and df den, respectively), unadjusted *P*-value, FDR significance threshold, and FDR-adjusted *P*-value (q) are given for each analysis. ns = Not significant at corrected Bonferonni threshold of $P < 0.0011$.

SNP	F	df num	df den	p	FDR sign thresholds	q	Corrected Bonferroni	η_p^2
chrXI_5779439	8.99	1	42	**0.0046**	0.000413223	0.32065	ns	0.176
chrXI_16076821_2	8.82	1	36	**0.0053**	0.000826446	0.32065	ns	0.197
chrXIX_9369810_1	6.3	1	19	**0.0213**	0.001239669	0.54329	ns	0.248
chrXIII_5681784	5.11	1	56	**0.0276**	0.001652893	0.54329	ns	0.083
chrII_21932462_1	4.83	1	37	**0.0343**	0.002066116	0.54329	ns	0.116
chrII_4541659_1	4.68	1	37	**0.0371**	0.002479339	0.54329	ns	0.112
chrXVIII_3143344_F/I	3.54	2	34	**0.0401**	0.002892562	0.54329	ns	0.173
chrXIX_16959478	4.4	1	58	**0.0403**	0.003305785	0.54329	ns	0.071
chrXIV_10396321_F,1	7.27	1	5	**0.0429**	0.003719008	0.54329	ns	0.592
chrXVIII_3162328	4.52	1	22	**0.0449**	0.004132231	0.54329	ns	0.170
chrXVIII_3165611_F,1	6.17	1	5	0.0555	0.004545455	0.6105	ns	0.111
chrIV_32107470_I	3.76	1	14	0.0729	0.004958678	0.614075	ns	0.211
chrI_21593750	3.47	1	25	0.0744	0.005371901	0.614075	ns	0.122
chrXVII_8129604_F	3.39	1	31	0.0753	0.005785124	0.614075	ns	0.099
chrXIV_10387725_I	3.59	1	14	0.0789	0.006198347	0.614075	ns	0.204
chrXVI_5909125_2	3.21	1	37	0.0812	0.00661157	0.614075	ns	0.081
chrII_31927301_M,2	3.36	1	11	0.0938	0.007024793	0.667635	ns	0.230
chrII_4573177_M	2.5	1	28	0.1252	0.007438017	0.717057	ns	0.082
chrXVI_5909125_1	2.59	1	18	0.1253	0.00785124	0.717057	ns	0.128
chrIV_3332621_M	2.48	1	28	0.1268	0.008264463	0.717057	ns	0.082
chrXIV_10396321_F,2	2.49	1	24	0.1279	0.008677686	0.717057	ns	0.092
chrXI_16076821_I	2.54	1	14	0.133	0.009090909	0.717057	ns	0.153

chrIV_32103306_1	2.32	1	35	0.1363	0.009504132	0.717057	ns	0.062
chrXI_5763059	2.23	1	28	0.1465	0.009917355	0.730356	ns	0.076
chrXIX_9375733_M	2.18	1	28	0.1509	0.010330579	0.730356	ns	0.071
chrX_9526306_M	1.96	1	28	0.172	0.010743802	0.788517	ns	0.066
chrX_9527397_M	1.8	1	28	0.1902	0.011157025	0.788517	ns	0.059
chrXIII_5675328	1.64	1	57	0.2053	0.011570248	0.788517	ns	0.028
chrXIX_9374274_M	1.68	1	28	0.2061	0.011983471	0.788517	ns	0.056
chrII_31927301_F,2	1.65	1	25	0.2109	0.012396694	0.788517	ns	0.063
chrXIV_10387725_F	1.64	1	25	0.2125	0.012809917	0.788517	ns	0.063
chrXIII_5679721_F/I	1.58	1	39	0.2168	0.01322314	0.788517	ns	0.038
chrII_21932462_2	1.57	1	34	0.2192	0.013636364	0.788517	ns	0.044
chrXVIII_3144386_1	1.56	1	30	0.2219	0.014049587	0.788517	ns	0.049
chrXVIII_3139053_F/M	1.45	1	58	0.2338	0.01446281	0.788517	ns	0.024
chrII_21928786_I	1.55	1	13	0.2346	0.014876033	0.788517	ns	0.108
chrXI_5766794_F/I	1.46	2	36	0.246	0.015289256	0.792444	ns	0.076
chrII_4566452_I	1.54	2	13	0.2516	0.015702479	0.792444	ns	0.191
chrXIX_9369810_2	1.31	1	22	0.2642	0.016115702	0.792444	ns	0.055
chrXI_5766794_M	1.26	1	28	0.2715	0.016528926	0.792444	ns	0.042
chrII_4566452_F/M	1.22	1	57	0.2739	0.016942149	0.792444	ns	0.021
chrXIX_16924542	1.21	1	14	0.2902	0.017355372	0.792444	ns	0.080
chrXIX_16948763	1.21	1	14	0.2902	0.017768595	0.792444	ns	0.080
chrII_21928786_F	1.08	1	29	0.307	0.018181818	0.792444	ns	0.036
chrXI_16076821_M,1	1.09	1	17	0.3121	0.018595041	0.792444	ns	0.062
chrXIX_16942698	0.94	1	42	0.3367	0.019008264	0.792444	ns	0.021
chrIV_32097027_F/M	0.9	1	56	0.348	0.019421488	0.792444	ns	0.015
chrX_9527397_F/I	0.89	1	39	0.3525	0.019834711	0.792444	ns	0.022
chrXIX_13288787	0.92	1	13	0.3539	0.020247934	0.792444	ns	0.067
chrXIII_5673292_F/M	0.87	1	57	0.3561	0.020661157	0.792444	ns	0.015
chrXIV_10394249	0.92	1	12	0.3573	0.02107438	0.792444	ns	0.075
chrXVI_5888503_1	0.86	1	37	0.3597	0.021487603	0.792444	ns	0.022
chrXVI_5891048_1	0.86	1	37	0.3597	0.021900826	0.792444	ns	0.022

chrXVIII_3139053_I	0.89	1	14	0.3622	0.02231405	0.792444	ns	0.060
chrXI_16076821_F,1	0.98	1	5	0.367	0.022727273	0.792444	ns	0.165
chrII_4573177_F/I	0.81	1	39	0.373	0.023140496	0.792444	ns	0.020
chrXVII_7561091_F	0.86	1	11	0.3733	0.023553719	0.792444	ns	0.074
chrXVIII_3149894_M	0.78	1	27	0.3863	0.023966942	0.805902	ns	0.026
chrX_9528514_F/M	0.73	1	56	0.3955	0.024380165	0.81111	ns	0.013
chrXIX_13286373	0.72	1	27	0.4034	0.024793388	0.813523	ns	0.026
chrII_4557054	0.67	1	50	0.4167	0.025206612	0.8228	ns	0.014
chrXIV_10392093_M	0.66	1	29	0.4216	0.025619835	0.8228	ns	0.020
chrIV_32097027_I	0.89	2	13	0.4337	0.026033058	0.832979	ns	0.121
chrIV_3334738_F/I	0.61	1	39	0.4412	0.026446281	0.834144	ns	0.015
chrXIII_5673292_I	0.52	1	13	0.4816	0.026859504	0.84302	ns	0.039
chrXVII_7561091_M/I	0.51	1	15	0.4856	0.027272727	0.84302	ns	0.034
chrXVII_8129604_M	0.5	1	29	0.4868	0.02768595	0.84302	ns	0.015
chrXVIII_3165611_M,2	0.51	1	10	0.4903	0.028099174	0.84302	ns	0.055
chrXIX_9375733_F/I	0.46	1	39	0.5003	0.028512397	0.84302	ns	0.011
chrXVIII_3143344_M	0.71	2	24	0.5019	0.02892562	0.84302	ns	0.057
chrXIX_16911266_M	0.42	1	23	0.5223	0.029338843	0.84302	ns	0.018
chrXVIII_3168806_F	0.42	1	30	0.5223	0.029752066	0.84302	ns	0.014
chrXVIII_3156177_F/I	0.66	2	38	0.5226	0.030165289	0.84302	ns	0.034
chrXIV_10396321_I	0.42	1	13	0.5259	0.030578512	0.84302	ns	0.032
chrXIV_10396321_M,1	0.42	1	17	0.528	0.030991736	0.84302	ns	0.024
chrXVIII_3149894_F/I	0.4	1	35	0.5295	0.031404959	0.84302	ns	0.011
chrXVIII_3159265_M	0.39	1	26	0.5402	0.031818182	0.846069	ns	0.012
chrXVI_5888503_2	0.37	1	37	0.5454	0.032231405	0.846069	ns	0.011
chrII_31927301_1	0.29	1	31	0.5913	0.032644628	0.886694	ns	0.009
chrXVI_5906684_F	0.53	2	23	0.5941	0.033057851	0.886694	ns	0.044
chrXVIII_3146807	0.29	1	14	0.5996	0.033471074	0.886694	ns	0.019
chrXIV_10398703_I	0.55	2	7	0.6009	0.033884298	0.886694	ns	0.136
chrXVI_13150461	0.26	1	56	0.6132	0.034297521	0.893942	ns	0.004
chrXVIII_3144386_2	0.25	1	35	0.6224	0.034710744	0.894556	ns	0.008

chrXVIII_3159265_F/I	0.47	2	37	0.63	0.035123967	0.894556	ns	0.025
chrXIV_10387725_M	0.23	1	17	0.6358	0.03553719	0.894556	ns	0.010
chrII_21928786_M	0.2	1	27	0.6598	0.035950413	0.917653	ns	0.006
chrIV_3332621_F/I	0.16	1	38	0.69	0.036363636	0.945956	ns	0.005
chrXIII_5679721_M	0.15	1	28	0.699	0.03677686	0.945956	ns	0.006
chrXVI_5891048_2	0.14	1	36	0.7093	0.037190083	0.945956	ns	0.004
chrXVIII_3156177_M	0.13	1	29	0.7196	0.037603306	0.945956	ns	0.005
chrXVIII_3165611_F,2	0.13	1	25	0.7228	0.038016529	0.945956	ns	0.005
chrX_9526306_F/I	0.12	1	38	0.7327	0.038429752	0.945956	ns	0.003
chrXVI_13153214_M	0.1	1	28	0.7547	0.038842975	0.945956	ns	0.005
chrX_9534783_I	0.28	2	13	0.7638	0.039256198	0.945956	ns	0.041
chrII_4551986_M	0.09	1	19	0.7722	0.039669421	0.945956	ns	0.007
chrXIV_10398703_F/M	0.08	1	29	0.7776	0.040082645	0.945956	ns	0.003
chrII_4551986_F/I	0.08	1	27	0.7776	0.040495868	0.945956	ns	0.004
chrXIX_9374274_F/I	0.08	1	37	0.7808	0.040909091	0.945956	ns	0.002
chrIV_3334738_M	0.08	1	28	0.782	0.041322314	0.945956	ns	0.003
chrXIX_16911266_F/I	0.24	2	35	0.7896	0.041735537	0.945956	ns	0.014
chrXIV_10396321_M,2	0.05	1	10	0.8342	0.04214876	0.987726	ns	0.004
chrII_4553994_M	0.03	1	26	0.854	0.042561983	0.987726	ns	0.001
chrX_9528514_I	0.03	1	14	0.8615	0.042975207	0.987726	ns	0.002
chrII_4541659_2	0.03	1	26	0.8616	0.04338843	0.987726	ns	0.001
chrIV_3331520	0.03	1	24	0.8726	0.043801653	0.987726	ns	0.001
chrXVI_5906684_M	0.13	2	27	0.8813	0.044214876	0.987726	ns	0.010
chrIV_32092920_F/I	0.11	2	38	0.8925	0.044628099	0.987726	ns	0.006
chrIV_32103306_2	0.01	1	35	0.9079	0.045041322	0.987726	ns	0.000
chrXIX_16906593	0.01	1	58	0.9105	0.045454545	0.987726	ns	0.000
chrXVII_8129604_I	0.01	1	14	0.9121	0.045867769	0.987726	ns	0.001
chrXVIII_3165611_I	0.01	1	14	0.9182	0.046280992	0.987726	ns	0.001
chrXIV_10392093_F/I	0.01	1	39	0.9274	0.046694215	0.987726	ns	0.000
chrXVI_13153214_F/I	0.01	1	38	0.9339	0.047107438	0.987726	ns	0.000
chrX_9534783_F/M	0	1	58	0.9568	0.047520661	0.987726	ns	0.000

chrIV_32092920_M	0	1	29	0.9616	0.047933884	0.987726	ns	0.000
chrIV_32107470_F/M	0	1	56	0.9668	0.048347107	0.987726	ns	0.000
chrXVIII_3168806_M/I	0	1	28	0.9679	0.048760331	0.987726	ns	0.000
chrXVIII_3165611_M,1	0	1	16	0.9714	0.049173554	0.987726	ns	0.000
chrXIX_9383460	0	1	27	0.9837	0.049586777	0.991898	ns	0.000
chrII_4553994_F/I	0	1	37	0.9954	0.05	0.9954	ns	0.000

Table S3.4. Genotype-phenotype association analyses and results for Forest Lake. Chr with Roman numerals indicate linkage group, and the number following indicates SNP position in the stickleback genome on that LG. If SNP location is followed by letters (F, M, or I), these indicate analysis within sex categories where F = female, M = male, and I = immature. If SNP location is followed by numbers (1 or 2), these indicate analysis within age categories where 1 = age 1 fish and 2 = age 2 fish. *F*-statistic, numerator and denominator degrees of freedom (df num and df den, respectively), unadjusted *P*-value, FDR significance threshold, and FDR-adjusted *P*-value (q) are given for each analysis. ns = Not significant at corrected Bonferonni threshold of $P < 0.0036$.

SNP	F	df num	df den	p	FDR sign thresholds	q	Corrected Bonferroni	η_p^2
chrXI_5780656	5.73	1	30	**0.0231**	0.000925926	0.3265	ns	0.161
chrXIII_5683842	5.63	1	31	**0.024**	0.001851852	0.3265	ns	0.152
chrXI_5775144	4.85	1	32	**0.035**	0.002777778	0.3265	ns	0.132
chrXIX_16942698	4.14	1	31	0.0505	0.003703704	0.3265	ns	0.118
chrII_4566452	4.28	1	20	0.0516	0.00462963	0.3265	ns	0.180
chrI_21598355	4.04	1	29	0.0537	0.005555556	0.3265	ns	0.123
chrII_21932462	4.04	1	29	0.0537	0.006481481	0.3265	ns	0.123
chrXVI_5906684_F	4.86	1	9	0.0549	0.007407407	0.3265	ns	0.351
chrII_21928786_F	4.7	1	9	0.0584	0.008333333	0.3265	ns	0.340
chrXI_5763059	3.6	1	31	0.0671	0.009259259	0.3265	ns	0.104
chrIV_32092920	4.19	1	9	0.071	0.010185185	0.3265	ns	0.320
chrII_4564317_M	3.36	1	27	0.0779	0.011111111	0.3265	ns	0.109
chrIV_32107470	3.32	1	30	0.0786	0.012037037	0.3265	ns	0.100
chrXI_5781688	2.98	1	27	0.0959	0.012962963	0.3699	ns	0.100
chrIV_3334738_1	3.87	1	4	0.1206	0.013888889	0.4342	ns	0.489
chrII_31927301_2	2.31	1	31	0.1386	0.014814815	0.4678	ns	0.069
chrXI_5779439_F	2.28	1	9	0.1655	0.015740741	0.5257	ns	0.206
chrXIV_10387725	1.74	1	22	0.2005	0.016666667	0.6015	ns	0.070
chrXIX_16924542	1.75	1	8	0.2223	0.017592593	0.6287	ns	0.176
chrI_21600738	1.47	1	22	0.2384	0.018518519	0.6287	ns	0.066
chrXI_5779439_M	1.38	1	28	0.2496	0.019444444	0.6287	ns	0.046
chrIV_3331520	1.24	1	20	0.2791	0.02037037	0.6287	ns	0.058

chrI_21593750	1.15	1	31	0.2925	0.021296296	0.6287	ns	0.038
chrII_31927301_1	1.28	1	4	0.3204	0.022222222	0.6287	ns	0.241
chrXVI_13150461	0.26	1	56	0.3213	0.023148148	0.6287	ns	0.105
chrII_4541659	0.96	1	31	0.3356	0.024074074	0.6287	ns	0.031
chrXIV_10394249	0.93	1	28	0.3422	0.025	0.6287	ns	0.032
chrXVI_13153214	0.9	1	28	0.3505	0.025925926	0.6287	ns	0.033
chrXVI_5896227_2	0.9	1	24	0.3522	0.026851852	0.6287	ns	0.035
chrXVIII_3144386	0.88	1	32	0.3559	0.027777778	0.6287	ns	0.028
chrXVI_5896227_1	1.06	1	4	0.3609	0.028703704	0.6287	ns	0.214
chrII_4547932	0.8	1	31	0.3782	0.02962963	0.6309	ns	0.026
chrXIX_16911266	0.73	1	25	0.3995	0.030555556	0.6309	ns	0.030
chrXIX_9374274	0.72	1	31	0.4023	0.031481481	0.6309	ns	0.022
chrII_4564317_F	0.79	1	6	0.4089	0.032407407	0.6309	ns	0.114
chrIV_32103306	0.52	1	22	0.4773	0.033333333	0.7103	ns	0.026
chrX_9534783_M	0.5	1	22	0.4867	0.034259259	0.7103	ns	0.026
chrII_4553994	0.46	1	13	0.5092	0.035185185	0.7236	ns	0.032
chrXVIII_3162328	0.43	1	11	0.5272	0.036111111	0.7289	ns	0.037
chrXVII_8129604_M	0.38	1	26	0.5448	0.037037037	0.7289	ns	0.014
chrXVIII_3165611	0.36	1	22	0.5534	0.037962963	0.7289	ns	0.015
chrXVII_8129604_F	0.29	1	9	0.603	0.038888889	0.7753	ns	0.003
chrXI_16076821	0.22	1	25	0.6465	0.039814815	0.7913	ns	0.007
chrXVIII_3143344	0.21	1	14	0.6537	0.040740741	0.7913	ns	0.014
chrII_21928786_M	0.17	1	25	0.681	0.041666667	0.7913	ns	0.005
chrXIII_5681784	0.17	1	28	0.6864	0.042592593	0.7913	ns	0.005
chrII_4557054	0.15	1	26	0.7003	0.043518519	0.7913	ns	0.005
chrXVI_5909125_F	0.15	1	9	0.7034	0.044444444	0.7913	ns	0.021
chrXIX_9375733	0.13	1	31	0.718	0.04537037	0.7913	ns	0.005
chrIV_3332621	0.09	1	29	0.7624	0.046296296	0.8039	ns	0.005
chrXVIII_3168806	0.13	1	22	0.7814	0.047222222	0.8039	ns	0.008
chrXVI_5888503	0.08	1	31	0.7852	0.048148148	0.8039	ns	0.004
chrXVIII_3146807	0.07	1	27	0.789	0.049074074	0.8039	ns	0.005

chrXIII_5673292	0.04	1	21	0.847	0.05	0.8408	ns	0.002
chrXVI_5909125_M	0.03	1	26	0.8683	0.050925926	0.8408	ns	0.001
chrXIV_10392093	0.03	1	22	0.8719	0.051851852	0.8408	ns	0.001
chrXVI_5906684_M	0.01	1	26	0.9057	0.052777778	0.8469	ns	0.000
chrXIII_5679721	0.01	1	31	0.9096	0.053703704	0.8469	ns	0.000
chrX_9534783_F	0	1	9	0.9653	0.05462963	0.8637	ns	0.000
chrIV_3334738_2	0	1	31	0.9693	0.055555556	0.8637	ns	0.000
chrXVI_5891048	0	1	31	0.9757	0.056481481	0.8637	ns	0.000

Table S3.5. Genotype-phenotype association analyses and results for Herkimer Lake. Chr with Roman numerals indicate linkage group, and the number following indicates SNP position in the stickleback genome on that LG. If SNP location is followed by letters (F, M, or I), these indicate analysis within sex categories where F = female, M = male, and I = immature. If SNP location is followed by numbers (1 or 2), these indicate analysis within age categories where 1 = age 1 fish and 2 = age 2 fish. *F*-statistic, numerator and denominator degrees of freedom (df num and df den, respectively), unadjusted *P*-value, FDR significance threshold, and FDR-adjusted *P*-value (q) are given for each analysis. ns = Not significant at corrected Bonferonni threshold of $P < 0.0015$.

SNP	F	df num	df den	p	FDR sign thresholds	q	Corrected Bonferroni	η_p^2
chrXIII_5681784_F,2	16.18	1	5	**0.0101**	0.0005	0.3854	ns	0.765
chrXIII_5673292_M	5.39	2	27	**0.0108**	0.0011	0.3854	ns	0.286
chrXI_5781688_F	6.57	2	10	**0.015**	0.0016	0.3854	ns	0.566
chrIV_3334738_M	4.81	2	27	**0.0164**	0.0021	0.3854	ns	0.263
chrIV_32097027_M	3.7	2	25	**0.0392**	0.0027	0.65941	ns	0.228
chrXI_5780656_F	2.13	2	35	0.0502	0.0032	0.65941	ns	0.448
chrXIII_5681784_F,1	4.62	1	12	0.0528	0.0037	0.65941	ns	0.280
chrI_21600738	3.72	1	39	0.0611	0.0043	0.65941	ns	0.088
chrXIX_9375733_1	3.33	1	39	0.0757	0.0048	0.65941	ns	0.080
chrIV_32107470_1	3.2	1	39	0.0814	0.0053	0.65941	ns	0.075
chrXVI_5909125	3.23	1	27	0.0836	0.0059	0.65941	ns	0.109
chrXI_5780656_M	2.5	2	25	0.1027	0.0064	0.65941	ns	0.165
chrXIX_9375733_2	3.91	1	5	0.1048	0.0069	0.65941	ns	0.439
chrXVIII_3143344_F	2.82	1	17	0.1112	0.0074	0.65941	ns	0.142
chrII_31927301_F	2.67	2	11	0.1138	0.0080	0.65941	ns	0.323
chrIV_32097027_F	2.58	1	17	0.1267	0.0085	0.65941	ns	0.133
chrI_21598355	2.21	2	27	0.1296	0.0090	0.65941	ns	0.142
chrXI_5779439_M	2.19	2	25	0.1329	0.0096	0.65941	ns	0.146
chrXVII_8129604	2.31	1	39	0.1365	0.0101	0.65941	ns	0.055
chrIV_32107470_2	3.07	1	5	0.1403	0.0106	0.65941	ns	0.379
chrXIV_10387725_M	2.14	1	28	0.1546	0.0112	0.668255	ns	0.069
chrXIII_5679721	2.13	1	27	0.1564	0.0117	0.668255	ns	0.074

chrXVIII_3144386	1.9	2	26	0.1692	0.0122	0.674168	ns	0.129
chrXIX_16906593	1.9	1	39	0.1758	0.0128	0.674168	ns	0.046
chrIV_3332621	1.87	1	40	0.1793	0.0133	0.674168	ns	0.044
chrXI_5773228	1.81	1	39	0.1867	0.0138	0.674992	ns	0.044
chrXIII_5678606	1.73	1	11	0.2154	0.0144	0.730931	ns	0.136
chrXVI_5906684	1.53	1	38	0.2233	0.0149	0.730931	ns	0.037
chrXVIII_3139053	1.52	1	40	0.2255	0.0154	0.730931	ns	0.038
chrXIII_5681784_M	1.42	1	25	0.2441	0.0160	0.731676	ns	0.054
chrXVIII_3149894_M	1.4	2	26	0.2638	0.0165	0.731676	ns	0.097
chrII_21928786_F	1.3	1	17	0.2695	0.0170	0.731676	ns	0.072
chrXI_5766794	1.33	2	38	0.2762	0.0176	0.731676	ns	0.067
chrII_31927301_M	1.33	2	27	0.2803	0.0181	0.731676	ns	0.091
chrXIX_9369810	1.27	1	11	0.2832	0.0186	0.731676	ns	0.108
chrII_4547932_F	1.21	1	17	0.2866	0.0191	0.731676	ns	0.067
chrXVII_10618107	1.17	1	28	0.288	0.0197	0.731676	ns	0.040
chrI_21593750	1.03	1	28	0.3185	0.0202	0.748795	ns	0.034
chrX_9530904	1.03	1	29	0.3196	0.0207	0.748795	ns	0.032
chrIV_32092920	1	1	39	0.3243	0.0213	0.748795	ns	0.026
chrX_9528514	1.12	2	37	0.3357	0.0218	0.748795	ns	0.056
chrXIV_10394249	0.93	1	27	0.3425	0.0223	0.748795	ns	0.034
chrXIX_16911266	0.91	1	40	0.3464	0.0229	0.748795	ns	0.022
chrXI_5781688_M	1.09	2	26	0.3505	0.0234	0.748795	ns	0.080
chrXIII_5673292_F	0.85	1	17	0.3682	0.0239	0.769129	ns	0.049
chrXVIII_3165611	0.98	2	35	0.3862	0.0245	0.789191	ns	0.052
chrIV_3331520	0.73	1	37	0.3989	0.0250	0.7978	ns	0.019
chrII_4547932_M	0.64	1	27	0.4305	0.0255	0.843063	ns	0.023
chrX_9526306_M	0.82	2	26	0.452	0.0261	0.8554	ns	0.060
chrX_9534783	0.57	1	39	0.455	0.0266	0.8554	ns	0.015
chrII_4564317	0.54	1	20	0.4705	0.0271	0.867196	ns	0.025
chrXIX_13286373	0.5	1	22	0.4866	0.0277	0.879623	ns	0.022
chrII_4566452_M	0.7	2	27	0.5073	0.0282	0.888228	ns	0.051

chrXVIII_3156177	0.43	1	32	0.5189	0.0287	0.888228	ns	0.013
chrXVI_13150461_F	0.42	1	16	0.5266	0.0293	0.888228	ns	0.027
chrII_21928786_M	0.64	2	26	0.5373	0.0298	0.888228	ns	0.047
chrXIX_9374274	0.33	1	39	0.5694	0.0303	0.888228	ns	0.007
chrXI_16076821	0.32	1	28	0.5765	0.0309	0.888228	ns	0.011
chrII_4573177	0.31	1	38	0.5784	0.0314	0.888228	ns	0.008
chrIV_3334738_F	0.31	1	17	0.5846	0.0319	0.888228	ns	0.018
chrII_21932462	0.3	1	28	0.5891	0.0324	0.888228	ns	0.011
chrII_4557054	0.3	1	33	0.5906	0.0330	0.888228	ns	0.008
chrXIX_13288787	0.27	1	28	0.6107	0.0335	0.888228	ns	0.011
chrXIX_16915746	0.27	1	28	0.6107	0.0340	0.888228	ns	0.011
chrXVIII_3168806_F	0.26	1	17	0.6142	0.0346	0.888228	ns	0.017
chrXIX_16924542	0.24	1	27	0.625	0.0351	0.890152	ns	0.011
chrXVIII_3149894_F	0.23	1	17	0.6401	0.0356	0.898051	ns	0.014
chrXVI_5896227	0.2	1	35	0.655	0.0362	0.905441	ns	0.004
chrXVIII_3146807	0.17	1	34	0.6802	0.0367	0.926649	ns	0.004
chrX_9526306_F	0.15	1	17	0.706	0.0372	0.931867	ns	0.010
chrII_4541659_M	0.34	2	26	0.7144	0.0378	0.931867	ns	0.023
chrXI_5779439_F	0.14	1	17	0.7176	0.0383	0.931867	ns	0.009
chrXIII_5675328_M	0.11	1	13	0.7457	0.0388	0.931867	ns	0.007
chrXIII_5675328_F	0.09	1	12	0.7661	0.0394	0.931867	ns	0.010
chrXI_5763059	0.08	1	40	0.7797	0.0399	0.931867	ns	0.004
chrXVI_13153214	0.24	2	38	0.7874	0.0404	0.931867	ns	0.012
chrIV_32103306	0.07	1	39	0.7995	0.0410	0.931867	ns	0.002
chrII_4553994	0.21	2	36	0.8099	0.0415	0.931867	ns	0.011
chrXVIII_3143344_M	0.21	2	26	0.8105	0.0420	0.931867	ns	0.018
chrXI_16070261	0.05	1	39	0.8244	0.0426	0.931867	ns	0.001
chrXVIII_3159265	0.05	1	38	0.8257	0.0431	0.931867	ns	0.001
chrXIV_10392093	0.19	2	37	0.83	0.0436	0.931867	ns	0.011
chrXIV_10389873	0.04	1	28	0.8436	0.0441	0.931867	ns	0.001
chrXVI_13150461_M	0.16	2	27	0.8519	0.0447	0.931867	ns	0.011

chrXVI_5888503	0.04	1	39	0.852	0.0452	0.931867	ns	0.001
chrXIX_16948763	0.15	2	26	0.8616	0.0457	0.931867	ns	0.011
chrXVI_5891048	0.03	1	39	0.8688	0.0463	0.931867	ns	0.001
chrII_4541659_F	0.03	1	17	0.8745	0.0468	0.931867	ns	0.002
chrII_4566452_F	0.02	1	17	0.8823	0.0473	0.931867	ns	0.001
chrXVIII_3168806_M	0.02	1	26	0.8993	0.0479	0.935096	ns	0.001
chrXIV_10387725_F	0.01	1	17	0.91	0.0484	0.935096	ns	0.001
chrXIX_16942698	0.01	1	21	0.9152	0.0489	0.935096	ns	0.001
chrXIX_16959478	0.01	1	39	0.9397	0.0495	0.949804	ns	0.000
chrXIV_10396321	0	1	36	0.9733	0.0500	0.9733	ns	0.000

Table S3.6. Genotype-phenotype association analyses and results for Kidney Lake. Chr with Roman numerals indicate linkage group, and the number following indicates SNP position in the stickleback genome on that LG. If SNP location is followed by letters (F, M, or I), these indicate analysis within sex categories where F = female, M = male, and I = immature. If SNP location is followed by numbers (1 or 2), these indicate analysis within age categories where 1 = age 1 fish and 2 = age 2 fish. *F*-statistic, numerator and denominator degrees of freedom (df num and df den, respectively), unadjusted *P*-value, FDR significance threshold, and FDR-adjusted *P*-value (q) are given for each analysis. ns = Not significant at corrected Bonferonni threshold of $P < 0.0018$.

SNP	F	df num	df den	p	FDR sign thresholds	q	Corrected Bonferroni	η_p^2
chrXVIII_3149894_I	7.61	1	7	**0.0281**	0.00090909	0.7126	ns	0.533
chrXVI_5905544	3.12	1	20	0.0925	0.00181818	0.7126	ns	0.138
chrXVIII_3165611_I	3.58	1	7	0.1002	0.00272727	0.7126	ns	0.333
chrXIX_16942698	2.8	1	35	0.1034	0.00363636	0.7126	ns	0.074
chrXIV_10396321	2.56	1	34	0.1186	0.00454545	0.7126	ns	0.070
chrXVII_10618107	2.36	1	36	0.1329	0.00545455	0.7126	ns	0.061
chrXVIII_3149894_M	2.33	1	36	0.136	0.00636364	0.7126	ns	0.061
chrXIX_16906593	2.21	1	36	0.1455	0.00727273	0.7126	ns	0.058
chrXIX_16911266	2.08	1	33	0.159	0.00818182	0.7126	ns	0.061
chrX_9528514_2	2.07	1	34	0.1593	0.00909091	0.7126	ns	0.057
chrXVIII_3159265	1.76	1	40	0.1918	0.01000000	0.7126	ns	0.042
chrXIV_10392093	1.59	1	36	0.2159	0.01090909	0.7126	ns	0.043
chrXIV_10394249	1.45	1	34	0.2367	0.01181818	0.7126	ns	0.042
chrI_21598355_2	1.36	1	34	0.252	0.01272727	0.7126	ns	0.038
chrII_21932462_2	1.36	1	34	0.252	0.01363636	0.7126	ns	0.038
chrXI_5775144	1.27	1	42	0.2656	0.01454545	0.7126	ns	0.029
chrXIII_5683842	1.27	1	42	0.2656	0.01545455	0.7126	ns	0.029
chrXI_5763059	1.27	1	42	0.2656	0.01636364	0.7126	ns	0.029
chrX_9530904	1.13	1	38	0.2939	0.01727273	0.7126	ns	0.029
chrI_21598355_1	1.31	1	6	0.2958	0.01818182	0.7126	ns	0.176
chrII_21932462_1	1.31	1	6	0.2958	0.01909091	0.7126	ns	0.176
chrIV_3331520_M	1.08	1	33	0.3058	0.02000000	0.7126	ns	0.033

chrXVIII_3168806_M	1.04	1	40	0.314	0.02090909	0.7126	ns	0.026
chrXVI_5888503	0.97	1	34	0.3321	0.02181818	0.7126	ns	0.087
chrIV_32103306_M	0.95	1	39	0.3369	0.02272727	0.7126	ns	0.024
chrIV_32097027_M	0.89	1	40	0.3501	0.02363636	0.7126	ns	0.021
chrII_31927301	0.85	1	36	0.3609	0.02454545	0.7126	ns	0.024
chrXVIII_3168806_I	0.95	1	7	0.3628	0.02545455	0.7126	ns	0.133
chrXVIII_3156177_M	0.7	1	42	0.4086	0.02636364	0.7749	ns	0.015
chrXVI_5891048_M	0.57	1	41	0.4538	0.02727273	0.8320	ns	0.014
chrII_4547932	0.49	1	35	0.4866	0.02818182	0.8393	ns	0.015
chrXVI_5909125	0.49	1	43	0.4883	0.02909091	0.8393	ns	0.012
chrXVIII_3143344_M	0.45	1	40	0.507	0.03000000	0.8450	ns	0.011
chrXVI_5896227	0.29	1	29	0.5916	0.03090909	0.8766	ns	0.010
chrX_9528514_1	0.29	1	13	0.6013	0.03181818	0.8766	ns	0.022
chrIV_32092920	0.28	1	42	0.6025	0.03272727	0.8766	ns	0.006
chrXVI_5891048_I	0.3	1	7	0.6037	0.03363636	0.8766	ns	0.063
chrIV_32097027_I	0.27	1	7	0.6226	0.03454545	0.8766	ns	0.063
chrXVIII_3156177_I	0.25	1	7	0.6347	0.03545455	0.8766	ns	0.032
chrIV_3331520_I	0.24	1	7	0.6377	0.03636364	0.8766	ns	0.063
chrIV_32107470_M	0.19	1	40	0.6676	0.03727273	0.8766	ns	0.005
chrII_4541659	0.18	1	36	0.6728	0.03818182	0.8766	ns	0.006
chrXVIII_3139053	0.13	1	6	0.7269	0.03909091	0.8766	ns	0.020
chrXIX_9375733	0.11	1	36	0.7402	0.04000000	0.8766	ns	0.003
chrXIX_9369810	0.12	1	8	0.7414	0.04090909	0.8766	ns	0.012
chrIV_32107470_I	0.12	1	7	0.7414	0.04181818	0.8766	ns	0.020
chrIV_3332621	0.1	1	41	0.7492	0.04272727	0.8766	ns	0.005
chrXVIII_3143344_I	0.09	1	7	0.777	0.04363636	0.8766	ns	0.013
chrIV_32103306_I	0.08	1	6	0.7884	0.04454545	0.8766	ns	0.013
chrXVIII_3144386	0.07	1	41	0.7969	0.04545455	0.8766	ns	0.002
chrX_9534783	0.2	2	34	0.8159	0.04636364	0.8789	ns	0.013
chrIV_3334738	0.05	1	22	0.831	0.04727273	0.8789	ns	0.002
chrXVIII_3165611_M	0.03	1	40	0.8611	0.04818182	0.8936	ns	0.001

chrIV_3329457	0.02	1	32	0.8792	0.04909091	0.8955	ns	0.001
chrXIII_5675328	0	1	43	0.9933	0.05000000	0.9933	ns	0.000

Table S3.7. Genotype-phenotype association analyses and results for Mud Lake. Chr with Roman numerals indicate linkage group, and the number following indicates SNP position in the stickleback genome on that LG. If SNP location is followed by letters (F, M, or I), these indicate analysis within sex categories where F = female, M = male, and I = immature. If SNP location is followed by numbers (1 or 2), these indicate analysis within age categories where 1 = age 1 fish and 2 = age 2 fish. *F*-statistic, numerator and denominator degrees of freedom (df num and df den, respectively), unadjusted *P*-value, FDR significance threshold, and FDR-adjusted *P*-value (q) are given for each analysis. * = Significant at corrected Bonferonni threshold of $P < 0.0013$. ns = Not significant at corrected Bonferonni threshold of $P < 0.0013$.

SNP	F	df num	df den	p	FDR sign thresholds	q	Corrected Bonferroni	η_p^2
chrII_21932462_F,1	47.93	1	25	**0.00000029**	0.00044643	**0.00003248**	*	0.657
chrXI_5775144_F,1	28.52	1	24	**0.0000176**	0.00089286	**0.00098560**	*	0.543
chrXI_5763059_1	22.11	1	24	**0.00008856**	0.00133929	**0.00330624**	*	0.479
chrXIX_16942698_F,1	19.65	1	24	**0.0002**	0.00178571	**0.00560000**	*	0.451
chrXVIII_3156177_1	9.42	1	25	**0.0051**	0.00223214	0.11386667	*	0.273
chrXI_5773228_1	8.4	1	40	**0.0061**	0.00267857	0.11386667	*	0.174
chrI_21598355_1	7.5	1	26	**0.011**	0.00312500	0.17600000	ns	0.223
chrXIII_5683842_1	6.43	1	45	**0.0148**	0.00357143	0.20720000	ns	0.125
chrXI_5779439_M	6.59	1	15	**0.0214**	0.00401786	0.25830000	ns	0.304
chrX_9528514_I	10.12	1	5	**0.0245**	0.00446429	0.25830000	ns	0.667
chrIV_32103306_M	0.58	1	5	**0.0261**	0.00491071	0.25830000	ns	0.307
chrXI_5781688_M	5.55	1	15	**0.0325**	0.00535714	0.25830000	ns	0.270
chrXI_5780656_M	5.55	1	15	**0.0325**	0.00580357	0.25830000	ns	0.270
chrXI_5773228_2	5.41	1	15	**0.0344**	0.00625000	0.25830000	ns	0.265
chrIV_3329457	5.52	1	13	**0.0353**	0.00669643	0.25830000	ns	0.297
chrXVII_10618107	4.86	1	25	**0.0369**	0.00714286	0.25830000	ns	0.163
chrXIX_16924542_M	5.01	1	15	**0.0407**	0.00758929	0.26814118	ns	0.250
chrII_21932462_M	4.67	1	15	**0.0473**	0.00803571	0.28353684	ns	0.238
chrXI_5781688_F	4.18	1	37	**0.0481**	0.00848214	0.28353684	ns	0.102
chrXI_5780656_F	4.01	1	39	0.0524	0.00892857	0.28426667	ns	0.093
chrIV_32103306_F	3.98	1	38	0.0533	0.00937500	0.28426667	ns	0.095

chrX_9526306_F	3.09	2	36	0.0579	0.00982143	0.29476364	ns	0.147
chrXI_5779439_F	3.23	1	40	0.0797	0.01026786	0.38810435	ns	0.074
chrXIII_5675328_F	3.05	1	40	0.0883	0.01071429	0.39158519	ns	0.071
chrIV_32092920_1	3.02	1	45	0.0891	0.01116071	0.39158519	ns	0.063
chrX_9526306_M	0.18	1	14	0.0944	0.01160714	0.39158519	ns	0.011
chrXVIII_3159265_I	4.24	1	5	0.0944	0.01205357	0.39158519	ns	0.455
chrXVIII_3165611_M	2.9	1	20	0.1039	0.01250000	0.41560000	ns	0.126
chrXIX_16948763	2.63	1	40	0.113	0.01294643	0.43641379	ns	0.061
chrXVIII_3149894_F	2.4	1	39	0.1297	0.01339286	0.47148387	ns	0.058
chrXVI_5888503_M/I	2.49	1	20	0.1305	0.01383929	0.47148387	ns	0.111
chrXIX_9371313_M	2.26	1	12	0.1584	0.01428571	0.55440000	ns	0.158
chrXIX_16915746_F	1.85	2	37	0.1714	0.01473214	0.58172121	ns	0.091
chrXVI_13153214_M	1.7	1	15	0.2122	0.01517857	0.69901176	ns	0.102
chrII_4573177_M	1.65	2	14	0.2282	0.01562500	0.70178182	ns	0.190
chrI_21598355_2	1.58	1	14	0.23	0.01607143	0.70178182	ns	0.102
chrII_4553994_F	1.49	2	38	0.2386	0.01651786	0.70178182	ns	0.072
chrXVIII_3144386	1.42	1	59	0.2389	0.01696429	0.70178182	ns	0.023
chrII_4566452_M	1.49	2	14	0.259	0.01741071	0.70178182	ns	0.175
chrXIX_16842698_F,2	1.38	1	15	0.2591	0.01785714	0.70178182	ns	0.086
chrII_4566452_F	1.38	2	37	0.2634	0.01830357	0.70178182	ns	0.069
chrXVIII_3165611_F	1.23	1	39	0.274	0.01875000	0.70178182	ns	0.031
chrXIV_10387725_M	1.26	1	20	0.2757	0.01919643	0.70178182	ns	0.060
chrXIV_10389873_M	1.26	1	20	0.2757	0.01964286	0.70178182	ns	0.060
chrII_4553994_M	1.25	1	14	0.2823	0.02008929	0.70243478	ns	0.082
chrIV_32097027_M/I	1.19	1	20	0.2885	0.02053571	0.70243478	ns	0.055
chrXVIII_3168806_F	1.2	2	38	0.3131	0.02098214	0.74611064	ns	0.059
chrXIII_5681784	1	1	39	0.3244	0.02142857	0.75693333	ns	0.025
chrI_21593750_M/I	0.98	1	20	0.3332	0.02187500	0.76160000	ns	0.047
chrIV_3332621_M	0.95	1	20	0.3409	0.02232143	0.76361600	ns	0.045
chrXVI_5896227	0.76	1	36	0.388	0.02276786	0.82984533	ns	0.021
chrIV_32097027_F	0.75	1	40	0.392	0.02321429	0.82984533	ns	0.018

chrIV_3334738_M	0.74	1	15	0.4032	0.02366071	0.82984533	ns	0.048
chrXIX_9371313_F	0.68	1	35	0.4141	0.02410714	0.82984533	ns	0.019
chrXVIII_3168806_M	0.7	1	13	0.4176	0.02455357	0.82984533	ns	0.052
chrXVIII_3159265_F/M	0.65	1	60	0.4223	0.02500000	0.82984533	ns	0.011
chrIV_3332621_F	0.85	2	37	0.4344	0.02544643	0.82984533	ns	0.044
chrXVI_5909125_F	0.82	2	38	0.4487	0.02589286	0.82984533	ns	0.041
chrI_21589802_F	0.59	1	40	0.4487	0.02633929	0.82984533	ns	0.015
chrXIII_5673292_F/M	0.54	1	40	0.4663	0.02678571	0.82984533	ns	0.013
chrX_9534783_M	0.53	1	15	0.4758	0.02723214	0.82984533	ns	0.033
chrIV_32103306_I	0.58	1	5	0.4817	0.02767857	0.82984533	ns	0.094
chrXVIII_3165611_I	0.58	1	5	0.4817	0.02812500	0.82984533	ns	0.094
chrXVII_8129604_F/M	0.49	1	40	0.488	0.02857143	0.82984533	ns	0.012
chrXVIII_3149894_M	0.73	2	13	0.4986	0.02901786	0.82984533	ns	0.102
chrI_21589802_M/I	0.44	1	20	0.5144	0.02946429	0.82984533	ns	0.022
chrII_4541659_F/M	0.67	2	38	0.5176	0.02991071	0.82984533	ns	0.034
chrXIV_10392093_F/M	0.41	1	59	0.5239	0.03035714	0.82984533	ns	0.007
chrXIII_5683842_2	0.43	1	15	0.5241	0.03080357	0.82984533	ns	0.026
chrIV_3334738_F	0.39	1	39	0.5338	0.03125000	0.82984533	ns	0.010
chrXI_5775144_F,2	0.41	1	14	0.5345	0.03169643	0.82984533	ns	0.030
chrXIX_16959478	0.39	1	39	0.5367	0.03214286	0.82984533	ns	0.010
chrXIV_10389873_F	0.37	1	39	0.5445	0.03258929	0.82984533	ns	0.009
chrXIV_10392093_I	0.41	1	5	0.5486	0.03303571	0.82984533	ns	0.063
chrXI_5763059_2	0.36	1	15	0.5557	0.03348214	0.82984533	ns	0.026
chrXVI_5891048_M/I	0.33	1	18	0.5703	0.03392857	0.84044211	ns	0.017
chrI_21593750_F	0.3	1	40	0.585	0.03437500	0.84810667	ns	0.008
chrXVI_5912332_F	0.29	1	37	0.5954	0.03482143	0.84810667	ns	0.008
chrX_9530904_F	0.27	1	36	0.6049	0.03526786	0.84810667	ns	0.007
chrX_9530904_M	0.25	1	15	0.6232	0.03571429	0.84810667	ns	0.015
chrXIII_5675328_M/I	0.24	1	19	0.6277	0.03616071	0.84810667	ns	0.012
chrI_21600738_M	0.23	1	15	0.6365	0.03660714	0.84810667	ns	0.015
chrII_21928786_M	0.46	2	12	0.6392	0.03705357	0.84810667	ns	0.071

chrIV_32092920_2	0.2	1	15	0.6596	0.03750000	0.84810667	ns	0.013
chrXVI_5888503_F	0.19	1	40	0.6638	0.03794643	0.84810667	ns	0.005
chrII_31927301_M	0.43	2	10	0.6648	0.03839286	0.84810667	ns	0.079
chrXIX_9375733	0.19	1	25	0.6705	0.03883929	0.84810667	ns	0.007
chrXVI_5909125_M	0.38	2	14	0.6882	0.03928571	0.84810667	ns	0.051
chrIV_3331520_F	0.16	1	39	0.6936	0.03973214	0.84810667	ns	0.004
chrII_4541659_I	0.17	1	5	0.696	0.04017857	0.84810667	ns	0.030
chrXVII_8129604_I	0.17	1	5	0.7007	0.04062500	0.84810667	ns	0.030
chrXIX_16906593_M	0.13	1	15	0.7217	0.04107143	0.84810667	ns	0.009
chrXIV_10396321_M	0.12	1	14	0.7301	0.04151786	0.84810667	ns	0.009
chrXVIII_3139053_M/I	0.12	1	20	0.7328	0.04196429	0.84810667	ns	0.005
chrXVI_5912332_M	0.12	1	14	0.7382	0.04241071	0.84810667	ns	0.007
chrXIX_16911266	0.11	1	38	0.7415	0.04285714	0.84810667	ns	0.003
chrII_4573177_F	0.1	1	36	0.7481	0.04330357	0.84810667	ns	0.003
chrI_21600738_F	0.1	1	40	0.753	0.04375000	0.84810667	ns	0.002
chrXIX_16928893	0.1	1	58	0.7567	0.04419643	0.84810667	ns	0.002
chrXIX_16915746_M	0.09	1	15	0.768	0.04464286	0.84810667	ns	0.006
chrXIV_10396321_F	0.26	2	35	0.774	0.04508929	0.84810667	ns	0.015
chrXVIII_3143344_M/I	0.08	1	19	0.7857	0.04553571	0.84810667	ns	0.003
chrXVIII_3156177_2	0.07	1	15	0.7896	0.04598214	0.84810667	ns	0.007
chrII_31927301_F	0.07	1	38	0.7917	0.04642857	0.84810667	ns	0.002
chrII_21932462_F,2	0.07	1	14	0.7951	0.04687500	0.84810667	ns	0.004
chrXIX_16924542_F	0.06	1	40	0.8082	0.04732143	0.84942056	ns	0.002
chrX_9534783_F	0.06	1	40	0.8115	0.04776786	0.84942056	ns	0.002
chrXVI_13153214_F	0.05	1	39	0.8248	0.04821429	0.85534815	ns	0.001
chrX_9527397	0.04	1	39	0.8476	0.04866071	0.85765766	ns	0.001
chrXIV_10394249	0.04	1	57	0.8496	0.04910714	0.85765766	ns	0.001
chrII_21928786_F	0.16	2	38	0.85	0.04955357	0.85765766	ns	0.008
chrXVIII_3139053_F	0.02	1	40	0.8917	0.05000000	0.87515826	ns	0.001
chrXIX_13286373	0.02	1	13	0.8964	0.05044643	0.87515826	ns	0.001
chrII_4551986	0.02	1	5	0.8966	0.05089286	0.87515826	ns	0.007

chrXIX_16842698_M,1	0.02	1	10	0.8986	0.05133929	0.87515826	ns	0.002
chrXIX_16906593_F	0.01	1	40	0.9066	0.05178571	0.87533793	ns	0.000
chrXIII_5673292_I	0.01	1	5	0.9256	0.05223214	0.88172903	ns	0.003
chrXVIII_3168806_I	0.01	1	5	0.9296	0.05267857	0.88172903	ns	0.003
chrIV_32107470	0.01	1	60	0.9381	0.05312500	0.88172903	ns	0.000
chrIV_3331520_M	0	1	15	0.9558	0.05357143	0.88172903	ns	0.000
chrXVIII_3143344_F	0	1	39	0.9644	0.05401786	0.88172903	ns	0.000
chrXIV_10387725_F	0	1	40	0.9658	0.05446429	0.88172903	ns	0.000
chrXI_5775144_M	0	1	14	0.9713	0.05491071	0.88172903	ns	0.001
chrXVI_5891048_F	0.02	2	37	0.9762	0.05535714	0.88172903	ns	0.002
chrX_9528514_F/M	0	1	60	0.9979	0.05580357	0.89411840	ns	0.000

Table S3.8. Genotype-phenotype association analyses and results for Morvro Lake. Chr with Roman numerals indicate linkage group, and the number following indicates SNP position in the stickleback genome on that LG. If SNP location is followed by letters (F, M, or I), these indicate analysis within sex categories where F = female, M = male, and I = immature. If SNP location is followed by numbers (1 or 2), these indicate analysis within age categories where 1 = age 1 fish and 2 = age 2 fish. *F*-statistic, numerator and denominator degrees of freedom (df num and df den, respectively), unadjusted *P*-value, FDR significance threshold, and FDR-adjusted *P*-value (q) are given for each analysis. ns = Not significant at corrected Bonferonni threshold of $P < 0.0012$.

SNP	F	df num	df den	p	FDR sign thresholds	q	Corrected Bonferroni	η_p^2
chrII_4566452	4.64	1	98	**0.0336**	0.000847458	0.628022	ns	0.045
chrXIX_13286373	4.72	1	12	0.0506	0.001694915	0.628022	ns	0.286
chrIV_3329457	3.6	1	76	0.0615	0.002542373	0.628022	ns	0.046
chrX_9528514	3.13	1	98	0.0799	0.003389831	0.628022	ns	0.031
chrXIX_16942698	3.49	1	16	0.08	0.004237288	0.628022	ns	0.176
chrXIX_16924542	3.16	1	15	0.0958	0.005084746	0.628022	ns	0.172
chrXIX_16948763	3.16	1	15	0.0958	0.005932203	0.628022	ns	0.172
chrXIX_13288787	3.16	1	15	0.0958	0.006779661	0.628022	ns	0.172
chrXIX_16915746	3.16	1	15	0.0958	0.007627119	0.628022	ns	0.172
chrXIII_5675328	2.02	1	60	0.1601	0.008474576	0.892539	ns	0.033
chrIV_3332621	0.95	1	20	0.1799	0.009322034	0.892539	ns	0.019
chrXVI_5888503	1.56	1	95	0.2147	0.010169492	0.892539	ns	0.017
chrXVI_5891048	1.56	1	95	0.2147	0.011016949	0.892539	ns	0.017
chrXVI_5896227	1.55	1	81	0.2173	0.011864407	0.892539	ns	0.019
chrXVIII_3144386	1.27	1	81	0.2631	0.012711864	0.892539	ns	0.015
chrXVI_5909125	1.24	1	95	0.2685	0.013559322	0.892539	ns	0.012
chrXI_16076821	1.23	1	97	0.2699	0.01440678	0.892539	ns	0.012
chrII_21928786	1.22	1	97	0.2723	0.015254237	0.892539	ns	0.013
chrXVI_13150461	1.02	1	98	0.315	0.016101695	0.900149	ns	0.010
chrXVIII_3146807	1.16	2	91	0.3192	0.016949153	0.900149	ns	0.025
chrXIV_10392093	0.98	1	95	0.3245	0.01779661	0.900149	ns	0.010
chrII_31927301	0.85	1	96	0.3589	0.018644068	0.900149	ns	0.009

chrXIV_10394249	0.74	1	95	0.3914	0.019491525	0.900149	ns	0.007
chrIV_3334738	0.71	1	99	0.403	0.020338983	0.900149	ns	0.007
chrXIX_16959478	1.65	1	15	0.4134	0.021186441	0.900149	ns	0.009
chrII_4573177	0.61	1	97	0.4372	0.022033898	0.900149	ns	0.006
chrXIII_5681784	0.61	1	59	0.4373	0.022881356	0.900149	ns	0.011
chrXI_5763059	0.59	1	99	0.4459	0.023728814	0.900149	ns	0.006
chrXIII_5673292	0.58	1	98	0.4492	0.024576271	0.900149	ns	0.006
chrIV_32107470	0.51	1	98	0.4777	0.025423729	0.900149	ns	0.005
chrXIX_16906593	0.45	1	59	0.5073	0.026271186	0.900149	ns	0.007
chrXIII_5678606	0.43	1	84	0.5159	0.027118644	0.900149	ns	0.005
chrXI_5766794	0.39	1	97	0.5339	0.027966102	0.900149	ns	0.004
chrXIII_5679721	0.38	1	96	0.5394	0.028813559	0.900149	ns	0.004
chrXIX_16911266	0.36	1	58	0.5508	0.029661017	0.900149	ns	0.006
chrXI_16070261	0.36	1	95	0.5511	0.030508475	0.900149	ns	0.003
chrX_9534783	0.33	1	98	0.5645	0.031355932	0.900149	ns	0.003
chrIV_32092920	0.26	1	95	0.6087	0.03220339	0.912716	ns	0.003
chrX_9526306	0.24	1	96	0.6277	0.033050847	0.912716	ns	0.002
chrXVIII_3139053	0.21	1	96	0.6444	0.033898305	0.912716	ns	0.002
chrXVIII_3143344	0.21	1	98	0.6453	0.034745763	0.912716	ns	0.002
chrIV_32097027	0.19	1	97	0.662	0.03559322	0.912716	ns	0.002
chrII_4553994	0.19	1	24	0.6652	0.036440678	0.912716	ns	0.009
chrII_4557054	0.15	1	75	0.7032	0.037288136	0.927218	ns	0.003
chrXVII_8129604	0.14	1	95	0.7072	0.038135593	0.927218	ns	0.001
chrXVIII_3149894	0.11	1	96	0.7386	0.038983051	0.92788	ns	0.001
chrII_4541659	0.29	2	93	0.7502	0.039830508	0.92788	ns	0.006
chrXVIII_3159265	0.09	1	97	0.7633	0.040677966	0.92788	ns	0.001
chrXIV_10398703	0.08	1	6	0.7916	0.041525424	0.92788	ns	0.013
chrX_9527397	0.06	1	96	0.8003	0.042372881	0.92788	ns	0.001
chrXVI_13153214	0.05	1	98	0.8297	0.043220339	0.92788	ns	0.000
chrXVIII_3165611	0.17	2	95	0.8448	0.044067797	0.92788	ns	0.003
chrX_9530904	0.04	1	94	0.8499	0.044915254	0.92788	ns	0.000

chrII_4547932	0.03	1	95	0.864	0.045762712	0.92788	ns	0.000
chrII_4564317	0.03	1	70	0.8729	0.046610169	0.92788	ns	0.000
chrIV_32103306	0.02	1	97	0.8807	0.047457627	0.92788	ns	0.000
chrII_21932462	0.01	1	98	0.9236	0.048305085	0.956007	ns	0.000
chrXVIII_3156177	0	1	95	0.9678	0.049152542	0.984486	ns	0.000
chrXIV_10396321	0	1	83	0.9892	0.05	0.9892	ns	0.000

Table S3.9. Genotype-phenotype association analyses and results for Rabbit Slough. Chr with Roman numerals indicate linkage group, and the number following indicates SNP position in the stickleback genome on that LG. If SNP location is followed by letters (F, M, or I), these indicate analysis within sex categories where F = female, M = male, and I = immature. If SNP location is followed by numbers (1 or 2), these indicate analysis within age categories where 1 = age 1 fish and 2 = age 2 fish. *F*-statistic, numerator and denominator degrees of freedom (df num and df den, respectively), unadjusted *P*-value, FDR significance threshold, and FDR-adjusted *P*-value (q) are given for each analysis. ns = Not significant at corrected Bonferonni threshold of $P < 0.0016$.

SNP	F	df num	df den	p	FDR sign thresholds	q	Corrected Bonferonni	η_p^2
chrII_4547932	5.2	1	33	**0.0292**	0.0008	0.7221	ns	0.136
chrXVIII_3139053	3.81	1	34	0.0592	0.0016	0.7221	ns	0.096
chrXIV_10392093_M	4.11	1	13	0.0636	0.0024	0.7221	ns	0.238
chrXVI_13150461	3.99	1	14	0.0655	0.0032	0.7221	ns	0.217
chrXVIII_3144386	2.74	2	29	0.0811	0.0040	0.7221	ns	0.160
chrXIV_10392093_F	3.21	1	20	0.0882	0.0048	0.7221	ns	0.134
chrX_9527397	3.02	1	33	0.0914	0.0056	0.7221	ns	0.087
chrXIII_5675328	3.02	1	33	0.0917	0.0063	0.7221	ns	0.087
chrXVI_5909125	2.85	1	19	0.1078	0.0071	0.7546	ns	0.134
chrXIX_16959478	2.14	1	18	0.1611	0.0079	0.8912	ns	0.101
chrXVIII_3156177	1.94	1	32	0.1733	0.0087	0.8912	ns	0.059
chrXIX_9374274	1.75	1	32	0.1956	0.0095	0.8912	ns	0.049
chrXIX_16906593	1.63	1	32	0.2114	0.0103	0.8912	ns	0.050
chrXIX_16911266	1.53	1	17	0.2327	0.0111	0.8912	ns	0.081
chrXIII_5678606	1.45	1	34	0.2373	0.0119	0.8912	ns	0.038
chrXIX_16948763	1.45	1	19	0.244	0.0127	0.8912	ns	0.073
chrIV_3334738	1.44	2	31	0.2535	0.0135	0.8912	ns	0.089
chrXIII_5681784	1.25	1	27	0.2733	0.0143	0.8912	ns	0.046
chrXVIII_3149894	1.16	1	34	0.289	0.0151	0.8912	ns	0.029
chrXVI_5912332	1.31	2	19	0.2926	0.0159	0.8912	ns	0.122
chrXIX_16924542	1.12	1	20	0.3024	0.0167	0.8912	ns	0.049
chrIV_32103306	0.99	1	29	0.3291	0.0175	0.8912	ns	0.033

chrXVIII_3168806	1.14	2	29	0.3349	0.0183	0.8912	ns	0.071
chrII_4541659	0.89	1	33	0.3522	0.0190	0.8912	ns	0.030
chrXIV_10387725	0.84	1	34	0.3652	0.0198	0.8912	ns	0.020
chrIV_32107470	0.84	1	31	0.3678	0.0206	0.8912	ns	0.031
chrIV_32097027	0.66	1	20	0.4256	0.0214	0.8986	ns	0.037
chrXIX_13288787	0.65	1	20	0.4286	0.0222	0.8986	ns	0.037
chrXI_16076821	0.64	1	34	0.4296	0.0230	0.8986	ns	0.019
chrII_4557054	0.55	1	3	0.4654	0.0238	0.8986	ns	0.021
chrXVIII_3143344	0.54	1	31	0.4659	0.0246	0.8986	ns	0.020
chrXVI_5893989	0.54	1	33	0.4675	0.0254	0.8986	ns	0.019
chrII_4573177	0.51	1	34	0.4813	0.0262	0.8986	ns	0.019
chrII_4566452	0.48	1	29	0.4935	0.0270	0.8986	ns	0.020
chrXIV_10394249	0.47	1	3	0.4992	0.0278	0.8986	ns	0.020
chrII_4553994	0.33	1	22	0.5729	0.0286	0.9756	ns	0.026
chrIV_32092920	0.25	1	33	0.6196	0.0294	0.9756	ns	0.010
chrII_4564317	0.24	1	29	0.6307	0.0302	0.9756	ns	0.011
chrIV_3331520	0.23	1	28	0.6328	0.0310	0.9756	ns	0.010
chrI_21598355	0.22	1	16	0.6487	0.0317	0.9756	ns	0.014
chrI_21600738	0.42	2	31	0.6638	0.0325	0.9756	ns	0.022
chrXIII_5673292	0.17	1	34	0.6805	0.0333	0.9756	ns	0.010
chrII_21932462	0.17	1	29	0.6806	0.0341	0.9756	ns	0.010
chrII_21928786	0.17	1	19	0.6814	0.0349	0.9756	ns	0.012
chrXVI_5888503	0.15	1	13	0.7078	0.0357	0.9819	ns	0.009
chrII_31927301	0.05	1	33	0.8199	0.0365	0.9819	ns	0.002
chrX_9530904	0.05	1	25	0.8258	0.0373	0.9819	ns	0.002
chrXIV_10396321	0.04	1	26	0.8519	0.0381	0.9819	ns	0.001
chrXVII_8129604	0.03	1	33	0.8615	0.0389	0.9819	ns	0.001
chrIV_3332621	0.13	2	27	0.882	0.0397	0.9819	ns	0.011
chrXVIII_3159265	0.02	1	30	0.8849	0.0405	0.9819	ns	0.001
chrXIX_16915746	0.02	1	10	0.886	0.0413	0.9819	ns	0.002
chrXIII_5679721	0.02	1	34	0.9021	0.0421	0.9819	ns	0.000

chrXVI_5896227	0.01	1	11	0.9198	0.0429	0.9819	ns	0.001
chrX_9526306	0.01	1	19	0.9346	0.0437	0.9819	ns	0.000
chrXVIII_3146807	0.01	1	23	0.9369	0.0444	0.9819	ns	0.000
chrX_9534783	0	1	33	0.9664	0.0452	0.9819	ns	0.000
chrXVI_13153214	0	1	29	0.9694	0.0460	0.9819	ns	0.001
chrXVI_5891048	0	1	33	0.9704	0.0468	0.9819	ns	0.000
chrXVIII_3165611	0	1	30	0.9741	0.0476	0.9819	ns	0.000
chrXIX_9375733	0	1	34	0.9778	0.0484	0.9819	ns	0.000
chrXIX_16942698	0	1	20	0.9873	0.0492	0.9819	ns	0.000
chrXIX_16928893	0	1	17	0.9883	0.0500	0.9819	ns	0.000
chrX_9528514	0	1	31	0.9975	0.0508	0.9819	ns	0.000

Table S3.10. Pairwise distances among all populations (m; Table 3.1) from within the same drainage or in nearby drainages, with pairwise distances between populations not from the same drainage assigned an arbitrarily large distance of 1000000 m.

	FG	HK	MO	BL	MD	KN	RS	FT
FG	0							
HK	4710.09	0						
MO	3738.48	5622.35	0					
BL	1000000	1000000	1000000	0				
MD	1000000	1000000	1000000	64470.2	0			
KN	1000000	1000000	1000000	1000000	1000000	0		
RS	26248.01	24011.28	29213.71	48456.09	0	145813.2	0	
FT	1000000	1000000	1000000	1000000	1000000	23621.02	129089.9	0

Table S3.11. Results of sensitivity analyses to determine detectable effect sizes given the range of observed FDR-adjusted P-values (q) and degrees of freedom (df num = numerator degrees of freedom; df den = denominator degrees of freedom) used in genotype-phenotype association analyses for each population (see Tables S3.2-S3.9) and assuming power = 0.8. Effect size in original units (mm) was computed by converting Cohen's d to an effect size in standard deviation units using the standard deviation of ln(SL) for each population, adding and subtracting this value from the mean ln(SL) for each population, converting each of these values to their original units (mm), taking the difference between these two values, and dividing by two.

Pop	P	df num	df den	Cohen's d	effect size (mm)
BL	LOW	1	15	1.246	11.40
BL	LOW	1	73	0.502	4.57
BL	LOW	2	15	1.366	12.51
BL	LOW	2	73	0.540	4.92
BL	HIGH	1	15	0.727	6.63
BL	HIGH	1	73	0.328	2.99
BL	HIGH	2	15	0.811	7.40
BL	HIGH	2	73	0.363	3.31
FG	LOW	1	5	3.851	62.18
FG	LOW	1	58	0.597	8.15
FG	LOW	2	5	4.465	76.30
FG	LOW	2	58	0.641	8.76
FG	HIGH	1	5	1.328	18.44
FG	HIGH	1	58	0.368	5.01
FG	HIGH	2	5	1.528	21.37
FG	HIGH	2	58	0.408	5.56
FT	LOW	1	4	4.444	63.22
FT	LOW	1	31	0.794	9.67
FT	HIGH	1	4	1.535	18.97
FT	HIGH	1	31	0.503	6.11
HK	LOW	1	5	3.655	17.63
HK	LOW	1	40	0.718	3.36
HK	LOW	2	5	4.237	20.66
HK	LOW	2	40	0.774	3.62
HK	HIGH	1	5	1.328	6.23
HK	HIGH	1	40	0.443	2.07
HK	HIGH	2	5	1.528	7.18
HK	HIGH	2	40	0.492	2.30
KN	LOW	1	6	2.643	19.80
KN	LOW	1	43	0.663	4.80
KN	LOW	2	6	3.022	22.89
KN	LOW	2	43	0.715	5.18

KN	HIGH	1	6	1.190	8.67
KN	HIGH	1	43	0.427	3.09
KN	HIGH	2	6	1.357	9.90
KN	HIGH	2	43	0.474	3.43
MD	LOW	1	5	3.790	36.74
MD	LOW	1	60	0.583	5.04
MD	LOW	2	5	4.395	44.31
MD	LOW	2	60	0.626	5.41
MD	HIGH	1	5	1.328	11.61
MD	HIGH	1	60	0.362	3.12
MD	HIGH	2	5	1.528	13.43
MD	HIGH	2	60	0.401	3.46
MO	LOW	1	6	2.677	12.89
MO	LOW	1	98	0.430	2.03
MO	LOW	2	6	3.060	14.83
MO	LOW	2	98	0.462	2.18
MO	HIGH	1	6	1.190	5.63
MO	HIGH	1	98	0.283	1.33
MO	HIGH	2	6	1.357	6.43
MO	HIGH	2	98	0.314	1.48
RS	LOW	1	3	7.806	45.73
RS	LOW	1	34	0.763	4.20
RS	LOW	2	3	9.472	57.14
RS	LOW	2	34	0.825	4.54
RS	HIGH	1	3	1.903	10.51
RS	HIGH	1	34	0.481	2.65
RS	HIGH	2	3	2.274	12.57
RS	HIGH	2	34	0.534	2.94

Table S3.12. Results of correlation analyses between mean SWW and allele frequencies across eight populations at each SNP locus used in this study. r = Pearson product-moment correlation coefficient, df = degrees of freedom, p = unadjusted *P*-value, q = FDR-adjusted *P*-value to account for multiple comparisons.

SNP	r	t-stat	df	p	FDR adjusted thresholds	q	Corrected Bonferonni
chrXI_5763059	0.856	4.054	6	**0.007**	0.0005	0.6370	*
chrXIX_13286373	-0.748	-2.76	6	**0.033**	0.0011	0.9214	ns
chrXVI_5893989	-0.688	-2.321	6	0.059	0.0016	0.9214	ns
chrX_9534783	-0.681	-2.277	6	0.063	0.0022	0.9214	ns
chrXVIII_3144386	-0.625	-1.96	6	0.098	0.0027	0.9214	ns
chrXIX_9369810	0.62	1.936	6	0.101	0.0033	0.9214	ns
chrXIX_13291868	-0.611	-1.891	6	0.108	0.0038	0.9214	ns
chrXIX_16942698	-0.598	-1.828	6	0.117	0.0044	0.9214	ns
chrXI_16070261	0.586	1.774	6	0.127	0.0049	0.9214	ns
chrXIX_13288787	-0.572	-1.709	6	0.138	0.0055	0.9214	ns
chrII_4551986	-0.57	-1.698	6	0.140	0.0060	0.9214	ns
chrII_21928786	0.569	1.695	6	0.141	0.0066	0.9214	ns
chrI_21600738	-0.562	-1.664	6	0.147	0.0071	0.9214	ns
chrXIX_16948763	-0.551	-1.619	6	0.157	0.0077	0.9214	ns
chrXVI_5909125	0.55	1.611	6	0.158	0.0082	0.9214	ns
chrII_21927301	-0.546	-1.596	6	0.162	0.0088	0.9214	ns
chrIV_32097027	0.52	1.489	6	0.187	0.0093	0.9387	ns
chrXIII_5681784	-0.511	-1.455	6	0.196	0.0099	0.9387	ns
chrXVII_7561091	0.511	1.456	6	0.196	0.0104	0.9387	ns
chrXVIII_3162328	-0.528	-1.39	5	0.223	0.0110	0.9970	ns
chrXIX_16924542	-0.468	-1.297	6	0.242	0.0115	0.9970	ns
chrXIX_9383460	0.511	1.328	5	0.242	0.0121	0.9970	ns
chrI_21589802	-0.459	-1.267	6	0.252	0.0126	0.9970	ns
chrXVIII_3165611	0.443	1.212	6	0.271	0.0132	0.9970	ns
chrXVI_5912332	-0.434	-1.18	6	0.283	0.0137	0.9970	ns
chrXVIII_3139053	0.421	1.136	6	0.299	0.0143	0.9970	ns
chrIV_32105368	-0.508	-1.179	4	0.304	0.0148	0.9970	ns
chrIV_3329457	0.404	1.08	6	0.321	0.0154	0.9970	ns
chrXIX_16928893	-0.387	-1.028	6	0.344	0.0159	0.9970	ns
chrXIX_9375733	-0.383	-1.016	6	0.349	0.0165	0.9970	ns
chrXVIII_3143344	0.379	1.003	6	0.354	0.0170	0.9970	ns
chrXVIII_3168806	-0.357	-0.936	6	0.385	0.0176	0.9970	ns
chrII_4541659	0.315	-0.812	6	0.448	0.0181	0.9970	ns
chrXIV_10394249	-0.295	-0.756	6	0.479	0.0187	0.9970	ns
chrXI_16076821	-0.288	-0.737	6	0.489	0.0192	0.9970	ns
chrXI_5773228	-0.283	-0.722	6	0.497	0.0198	0.9970	ns

chrXIX_16911266	-0.281	-0.719	6	0.499	0.0203	0.9970	ns
chrXI_5781688	0.274	0.697	6	0.512	0.0209	0.9970	ns
chrII_4553994	-0.27	-0.686	6	0.518	0.0214	0.9970	ns
chrII_4557054	0.264	0.67	6	0.528	0.0220	0.9970	ns
chrII_4566452	-0.264	-0.67	6	0.528	0.0225	0.9970	ns
chrXIX_16959478	-0.263	-0.666	6	0.530	0.0231	0.9970	ns
chrI_21593750	0.249	0.629	6	0.553	0.0236	0.9970	ns
chrXI_5779439	-0.247	-0.623	6	0.556	0.0242	0.9970	ns
chrXIX_9374274	0.24	0.606	6	0.567	0.0247	0.9970	ns
chrXVI_5896227	0.234	0.602	6	0.569	0.0253	0.9970	ns
chrXVIII_3149894	0.234	0.59	6	0.577	0.0258	0.9970	ns
chrXI_5780656	-0.232	-0.585	6	0.580	0.0264	0.9970	ns
chrXVI_5905544	-0.226	-0.569	6	0.590	0.0269	0.9970	ns
chrXIX_9371313	-0.22	-0.554	6	0.600	0.0275	0.9970	ns
chrXIII_5679721	0.218	0.547	6	0.604	0.0280	0.9970	ns
chrXIV_10396321	-0.209	-0.524	6	0.619	0.0286	0.9970	ns
chrX_9530904	-0.206	-0.515	6	0.625	0.0291	0.9970	ns
chrXVIII_3146807	-0.206	-0.515	6	0.625	0.0297	0.9970	ns
chrIV_3331520	-0.203	-0.507	6	0.631	0.0302	0.9970	ns
chrXI_5775144	0.199	0.498	6	0.637	0.0308	0.9970	ns
chrXIV_10392093	0.178	0.444	6	0.672	0.0313	0.9970	ns
chrII_21932462	0.159	0.394	6	0.707	0.0319	0.9970	ns
chrXVI_13153214	-0.143	-0.353	6	0.736	0.0324	0.9970	ns
chrII_4547932	0.14	0.345	6	0.742	0.0330	0.9970	ns
chrI_21598355	0.138	0.342	6	0.744	0.0335	0.9970	ns
chrIV_32103306	0.133	0.329	6	0.754	0.0341	0.9970	ns
chrXVI_5906684	-0.132	-0.327	6	0.755	0.0346	0.9970	ns
chrIV_3334738	0.126	0.312	6	0.766	0.0352	0.9970	ns
chrIV_32092920	-0.119	-0.293	6	0.779	0.0357	0.9970	ns
chrII_4564317	-0.117	-0.29	6	0.782	0.0363	0.9970	ns
chrXVIII_3159265	0.117	0.29	6	0.782	0.0368	0.9970	ns
chrXIII_5678606	0.109	0.27	6	0.796	0.0374	0.9970	ns
chrXVII_10618107	-0.1	-0.245	6	0.815	0.0379	0.9970	ns
chrX_9528514	0.096	0.237	6	0.820	0.0385	0.9970	ns
chrXIX_16906593	-0.092	-0.227	6	0.823	0.0390	0.9970	ns
chrXIII_5673292	-0.095	-0.234	6	0.823	0.0396	0.9970	ns
chrXIV_10398703	0.086	0.21	6	0.840	0.0401	0.9970	ns
chrIV_32107470	-0.081	-0.198	6	0.850	0.0407	0.9970	ns
chrXVI_5891048	0.079	0.195	6	0.852	0.0412	0.9970	ns
chrXIV_10389873	0.074	0.183	6	0.861	0.0418	0.9970	ns
chrX_9527397	-0.068	-0.167	6	0.873	0.0423	0.9970	ns
chrXIII_5675328	0.063	0.154	6	0.882	0.0429	0.9970	ns
chrXVIII_3156177	-0.06	-0.148	6	0.887	0.0434	0.9970	ns

chrXI_5766794	-0.048	-0.118	6	0.910	0.0440	0.9970	ns
chrIV_3333740	-0.052	-0.117	5	0.911	0.0445	0.9970	ns
chrXIV_10387725	-0.033	-0.081	6	0.938	0.0451	0.9970	ns
chrXIII_5683842	0.03	0.073	6	0.944	0.0456	0.9970	ns
chrX_9526306	-0.022	-0.055	6	0.958	0.0462	0.9970	ns
chrIV_32100268	-0.023	-0.053	5	0.960	0.0467	0.9970	ns
chrXVI_5888503	-0.01	-0.024	6	0.982	0.0473	0.9970	ns
chrXVII_8129604	0.007	0.017	6	0.987	0.0478	0.9970	ns
chrII_4573177	0.007	0.017	6	0.987	0.0484	0.9970	ns
chrXVI_13150461	-0.006	-0.016	6	0.988	0.0489	0.9970	ns
chrXIX_16915746	-0.006	-0.016	6	0.988	0.0495	0.9970	ns
chrIV_3332621	0.002	0.004	6	0.997	0.0500	0.9970	ns

Table 3.13. Results of correlation analyses between mean SWW and allele frequencies across eight populations at each gene or linked set of genes within linkage groups (LG) used in this study. % var. PC1 = percent of variation in SNP allele frequency data accounted for by the first principal component used in correlation analyses (see text), r = Pearson product-moment correlation coefficient, df = degrees of freedom, p = unadjusted *P*-value. No correlations were significant after Bonferroni or FDR correction for multiple comparisons.

LG	Genes	% var. PC1	r	t-stat	df	p
I	*IGFBP2.1*	61	-0.513	-1.463	6	0.194
II	*IGF1r.2*	56	0.316	0.816	6	0.446
II	*MYOD1.2*	46	-0.727	-2.592	6	**0.041**
IV	*FGF2*	71	-0.228	-0.574	6	0.587
IV	*IGF1*	63	-0.262	-0.666	6	0.530
X	*NPYP*	70	0.010	0.025	6	0.981
XI	*STAT5*	63	0.322	0.833	6	0.437
XI	*GH2*	76	-0.362	-0.951	6	0.378
XIII	*GHr1*	43	0.105	0.260	6	0.804
XIV	*GHr2*	66	-0.200	-0.500	6	0.635
XVI	*IGF2, IGF1r.1*	46	0.316	0.816	6	0.446
XVI	*IGFBP2.2*	39	-0.037	-0.091	6	0.930
XVII	*PIT1*	87	-0.070	-0.173	6	0.868
XVIII	*LG17QTL*	53	-0.172	-0.427	6	0.684
XIX	*IGF2r*	49	0.581	1.749	6	0.131

Table S3.14. Results of pairwise association tests between SNPs. Not all associations are shown. Here, I report all associations between SNPs on the same LG (Roman numerals of locus names) and for chromosome and all those that were significant (without correction for multiple comparisons) between SNPs on different LGs. Distance indicates distance between SNPs when on the same LG. df = degrees of freedom for χ^2 (Chi2) tests of association. P-value = unadjusted *P*-value. q = FDR-adjusted *P*-value to account for multiple comparisons.

Locus 1	Locus 2	Chromosome	Distance	Chi2	df	P-Value	FDR sign threshold	q
I21600738	I21598355	I	**2383**	Infinity	10	**0.00000000**	0.00077640	**0.00000000**
I21589802	I21593750	I	3948	7.098313	4	0.13078300	0.00637940	0.96239103
I21593750	I21598355	I	4605	27.85633	6	**0.00010000**	0.00163043	0.00306667
I21600738	I21593750	I	6988	9.807225	8	0.27881800	0.01160714	0.96239103
I21589802	I21598355	I	8553	1.483154	6	0.96060600	0.04421584	0.96239103
I21600738	I21589802	I	10936	2.840795	6	0.82854700	0.03513199	0.96239103
II21927301	II21928786	II	**1485**	Infinity	12	**0.00000000**	0.00043996	**0.00000000**
II4553994	II4551986	II	**2008**	12.13918	8	0.14510100	0.00685818	0.96239103
II4566452	II4564317	II	**2135**	25.34896	12	**0.01325300**	0.00273033	0.24269949
II4557054	II4553994	II	**3060**	34.35374	14	**0.00183100**	0.00208333	0.04394400
II21928786	II21932462	II	**3676**	25.82698	12	**0.01135500**	0.00263975	0.21407685
II4547932	II4551986	II	**4054**	24.61126	10	**0.00613300**	0.00230331	0.13249850
II4557054	II4551986	II	**5068**	31.57758	10	**0.00047100**	0.00181159	0.01299960
II21927301	II21932462	II	**5161**	28.54903	16	**0.02716000**	0.00322205	0.42147084
II4547932	II4553994	II	**6062**	20.76497	14	0.10780900	0.00583592	0.92366735
II4541659	II4547932	II	**6273**	Infinity	16	**0.00000000**	0.00018116	**0.00000000**
II4573177	II4566452	II	**6725**	45.75513	12	**0.00000800**	0.00155280	**0.00025760**
II4557054	II4564317	II	**7263**	20.60939	10	**0.02398800**	0.00311853	0.38460428
II4573177	II4564317	II	**8860**	14.98723	10	0.13252800	0.00644410	0.96239103
II4547932	II4557054	II	**9122**	Infinity	14	**0.00000000**	0.00020704	**0.00000000**
II4557054	II4566452	II	**9398**	35.22162	14	**0.00136300**	0.00200569	0.03397827
II4553994	II4564317	II	**10323**	7.085483	12	0.85191300	0.03645186	0.96239103
II4541659	II4551986	II	**10327**	18.53257	10	**0.04661700**	0.00401139	0.58105835

II4551986	II4564317	II	**12331**	0	6	1.00000000	0.05172101	0.96239103
II4541659	II4553994	II	**12335**	Infinity	14	**0.00000000**	0.00045290	**0.00000000**
II4553994	II4566452	II	**12458**	Infinity	14	**0.00000000**	0.00090580	**0.00000000**
II4566452	II4551986	II	**14466**	13.91093	10	0.17709300	0.00813923	0.96239103
II4541659	II4557054	II	**15395**	Infinity	14	**0.00000000**	0.00019410	**0.00000000**
II4557054	II4573177	II	16123	19.08368	12	0.08652900	0.00537008	0.80306337
II4547932	II4564317	II	16385	20.76257	12	0.05396700	0.00427019	0.63087096
II4547932	II4566452	II	18520	14.23834	16	0.58096300	0.02386128	0.96239103
II4553994	II4573177	II	19183	21.6732	12	**0.04135000**	0.00380435	0.54216588
II4573177	II4551986	II	21191	4.175379	8	0.84096300	0.03581781	0.96239103
II4541659	II4564317	II	22658	6.775944	12	0.87205800	0.03768116	0.96239103
II4541659	II4566452	II	24793	28.47039	16	**0.02776200**	0.00323499	0.42908947
II4547932	II4573177	II	25245	26.40249	12	**0.00941000**	0.00254917	0.18456975
II4541659	II4573177	II	31518	32.93334	12	**0.00099100**	0.00191511	0.02587314
II21927301	II4573177	II	17354124	22.58684	12	**0.03144600**	0.00336439	0.46733594
II21928786	II4573177	II	17355609	12.91051	10	0.22871800	0.00991201	0.96239103
II4573177	II21932462	II	17359285	15.85729	12	0.19786000	0.00879917	0.96239103
II21927301	II4566452	II	17360849	17.61854	16	0.34670300	0.01401398	0.96239103
II21928786	II4566452	II	17362334	11.30951	12	0.50259600	0.02025104	0.96239103
II21927301	II4564317	II	17362984	14.71905	12	0.25716100	0.01079193	0.96239103
II21928786	II4564317	II	17364469	10.58329	12	0.56492700	0.02308489	0.96239103
II21932462	II4566452	II	17366010	21.1972	16	0.17105400	0.00789337	0.96239103
II21932462	II4564317	II	17368145	15.42273	12	0.21912800	0.00957557	0.96239103
II4557054	II21927301	II	17370247	12.2699	14	0.58463800	0.02401656	0.96239103
II4557054	II21928786	II	17371732	11.86772	12	0.45636100	0.01840062	0.96239103
II21927301	II4553994	II	17373307	17.69134	14	0.22120200	0.00965321	0.96239103
II21928786	II4553994	II	17374792	10.09809	12	0.60735600	0.02489648	0.96239103
II21927301	II4551986	II	17375315	7.514105	10	0.67618100	0.02782091	0.96239103
II4557054	II21932462	II	17375408	19.09356	14	0.16140500	0.00753106	0.96239103
II21928786	II4551986	II	17376800	7.092263	8	0.52671200	0.02144151	0.96239103
II4553994	II21932462	II	17378468	11.29928	14	0.66238100	0.02717391	0.96239103

II4547932	II21927301	II	17379369	14.1335	16	0.58876900	0.02418478	0.96239103
II21932462	II4551986	II	17380476	2.625001	10	0.98892600	0.04737319	0.96239103
II4547932	II21928786	II	17380854	4.915626	12	0.96073800	0.04422878	0.96239103
II4547932	II21932462	II	17384530	22.19572	16	0.13698400	0.00658644	0.96239103
II4541659	II21927301	II	17385642	7.553424	16	0.96106400	0.04426760	0.96239103
II4541659	II21928786	II	17387127	10.99095	12	0.52969400	0.02162267	0.96239103
II4541659	II21932462	II	17390803	11.42041	16	0.78282300	0.03293219	0.96239103
IV3333740	IV3334738	IV	**998**	1.441579	6	0.96328000	0.04455228	0.96239103
IV3331520	IV3332621	IV	**1101**	Infinity	14	**0.00000000**	0.00093168	**0.00000000**
IV3333740	IV3332621	IV	**1119**	6.485537	6	0.37105000	0.01493271	0.96239103
IV32105368	IV32103306	IV	**2062**	11.40717	2	**0.00333400**	0.00213509	0.07802487
IV3329457	IV3331520	IV	**2063**	3.090291	12	0.99487200	0.04842133	0.96239103
IV32105368	IV32107470	IV	**2102**	13.62853	2	**0.00109800**	0.00192805	0.02847431
IV3334738	IV3332621	IV	**2117**	Infinity	16	**0.00000000**	0.00091874	**0.00000000**
IV3333740	IV3331520	IV	**2220**	2.19184	4	0.70052300	0.02894669	0.96239103
IV32100268	IV32103306	IV	**3038**	3.052898	8	0.93099400	0.04179607	0.96239103
IV3329457	IV3332621	IV	**3164**	24.93803	14	**0.03518400**	0.00350673	0.50166412
IV3334738	IV3331520	IV	**3218**	11.18592	14	0.67137200	0.02754917	0.96239103
IV32100268	IV32097027	IV	**3241**	5.766852	8	0.67332900	0.02767857	0.96239103
IV32092920	IV32097027	IV	**4107**	7.693832	14	0.90465900	0.03997153	0.96239103
IV32103306	IV32107470	IV	**4164**	52.5005	16	**0.00000900**	0.00156573	**0.00028740**
IV3333740	IV3329457	IV	4283	1.451142	6	0.96267400	0.04446170	0.96239103
IV32105368	IV32100268	IV	5100	Not	possible			
IV3334738	IV3329457	IV	5281	2.868219	14	0.99928500	0.04957298	0.96239103
IV32097027	IV32103306	IV	6279	12.72721	14	0.54810700	0.02237319	0.96239103
IV32100268	IV32107470	IV	7202	3.404264	8	0.90649100	0.04012681	0.96239103
IV32092920	IV32100268	IV	7348	7.511211	8	0.48260900	0.01952640	0.96239103
IV32105368	IV32097027	IV	8341	Not	possible			
IV32092920	IV32103306	IV	10386	33.38315	16	**0.00657200**	0.00240683	0.13652800
IV32097027	IV32107470	IV	10443	17.47812	14	0.23159300	0.01006729	0.96239103
IV32092920	IV32105368	IV	12448	0.221448	2	0.89518600	0.03922101	0.96239103

IV32092920	IV32107470	IV	14550	13.92265	16	0.60447700	0.02476708	0.96239103
IV32092920	IV3334738	IV	28758182	9.591463	16	0.88707500	0.03862578	0.96239103
IV32092920	IV3333740	IV	28759180	1.124813	6	0.98043500	0.04632505	0.96239103
IV32092920	IV3332621	IV	28760299	13.63031	16	0.62623300	0.02549172	0.96239103
IV32092920	IV3331520	IV	28761400	13.81969	14	0.46322900	0.01864648	0.96239103
IV3334738	IV32097027	IV	28762289	11.68	14	0.63198400	0.02580228	0.96239103
IV3333740	IV32097027	IV	28763287	0	4	1.00000000	0.05173395	0.96239103
IV32092920	IV3329457	IV	28763463	10.90354	14	0.69359700	0.02858437	0.96239103
IV3332621	IV32097027	IV	28764406	9.482623	14	0.79894800	0.03370859	0.96239103
IV3331520	IV32097027	IV	28765507	6.619292	12	0.88171400	0.03822464	0.96239103
IV3334738	IV32100268	IV	28765530	6.6984	8	0.56949500	0.02331781	0.96239103
IV3333740	IV32100268	IV	28766528	0.786998	2	0.67469200	0.02773033	0.96239103
IV3329457	IV32097027	IV	28767570	7.647002	12	0.81207200	0.03432971	0.96239103
IV32100268	IV3332621	IV	28767647	6.432402	8	0.59891500	0.02456004	0.96239103
IV3334738	IV32103306	IV	28768568	16.86187	16	0.39458900	0.01576087	0.96239103
IV32100268	IV3331520	IV	28768748	3.361199	8	0.90969100	0.04032091	0.96239103
IV3333740	IV32103306	IV	28769566	3.032105	6	0.80480700	0.03395445	0.96239103
IV32105368	IV3334738	IV	28770630	0.472964	2	0.78940000	0.03322981	0.96239103
IV3332621	IV32103306	IV	28770685	16.61075	16	0.41120700	0.01642081	0.96239103
IV32100268	IV3329457	IV	28770811	0	4	1.00000000	0.05084110	0.96239103
IV3333740	IV32105368	IV	28771628	Not	possible			
IV3331520	IV32103306	IV	28771786	18.13848	14	0.20054600	0.00899327	0.96239103
IV3334738	IV32107470	IV	28772732	14.33613	16	0.57368800	0.02353778	0.96239103
IV32105368	IV3332621	IV	28772747	0.851977	2	0.65312400	0.02677277	0.96239103
IV3333740	IV32107470	IV	28773730	0.772562	4	0.94208800	0.04262422	0.96239103
IV32105368	IV3331520	IV	28773848	1.445712	2	0.48536400	0.01962992	0.96239103
IV3329457	IV32103306	IV	28773849	13.61778	14	0.47855700	0.01925466	0.96239103
IV3332621	IV32107470	IV	28774849	22.11634	16	0.13947500	0.00668996	0.96239103
IV32105368	IV3329457	IV	28775911	0.658381	2	0.71950600	0.02977484	0.96239103
IV3331520	IV32107470	IV	28775950	20.56529	14	0.11330300	0.00593944	0.95381872
IV3329457	IV32107470	IV	28778013	13.07113	14	0.52093100	0.02116977	0.96239103

X9527397	X9526306	X	**1091**	Infinity	12	**0.00000000**	0.00094462	**0.00000000**
X9527397	X9528514	X	**1117**	25.71447	12	**0.01177700**	0.00269151	0.21878042
X9526306	X9528514	X	**2208**	Infinity	14	**0.00000000**	0.00129400	**0.00000000**
X9530904	X9528514	X	**2390**	Infinity	16	**0.00000000**	0.00130694	**0.00000000**
X9527397	X9530904	X	**3507**	Infinity	12	**0.00000000**	0.00097050	**0.00000000**
X9534783	X9530904	X	**3879**	Infinity	16	**0.00000000**	0.00098344	**0.00000000**
X9526306	X9530904	X	**4598**	Infinity	14	**0.00000000**	0.00099638	**0.00000000**
X9534783	X9528514	X	**6269**	Infinity	16	**0.00000000**	0.00128106	**0.00000000**
X9527397	X9534783	X	**7386**	Infinity	12	**0.00000000**	0.00046584	**0.00000000**
X9534783	X9526306	X	**8477**	Infinity	14	**0.00000000**	0.00095756	**0.00000000**
XI5781688	XI5780656	XI	**1032**	Infinity	6	**0.00000000**	0.00134576	**0.00000000**
XI5779439	XI5780656	XI	**1217**	Infinity	6	**0.00000000**	0.00135870	**0.00000000**
XI5773228	XI5775144	XI	**1916**	4.845059	4	0.30356800	0.01253882	0.96239103
XI5781688	XI5779439	XI	**2249**	Infinity	6	**0.00000000**	0.00133282	**0.00000000**
XI5775144	XI5779439	XI	**4295**	2.310378	4	0.67888000	0.02798913	0.96239103
XI5775144	XI5780656	XI	**5512**	0.613509	4	0.96155100	0.04430642	0.96239103
XI5773228	XI5779439	XI	**6211**	Infinity	6	**0.00000000**	0.00131988	**0.00000000**
XI5773228	XI5766794	XI	**6434**	27.04289	8	**0.00069500**	0.00186335	0.01864917
XI5775144	XI5781688	XI	**6544**	3.993363	4	0.40690500	0.01623965	0.96239103
XI16070261	XI16076821	XI	**6560**	Infinity	10	**0.00000000**	0.00047878	**0.00000000**
XI5773228	XI5780656	XI	**7428**	19.28272	6	**0.00371200**	0.00218685	0.08487082
XI5775144	XI5766794	XI	**8350**	2.142057	4	0.70965000	0.02934783	0.96239103
XI5773228	XI5781688	XI	**8460**	14.73063	6	**0.02245900**	0.00306677	0.36616699
XI5773228	XI5763059	XI	**10169**	27.72969	8	**0.00052800**	0.00183747	0.01436755
XI5775144	XI5763059	XI	**12085**	Infinity	6	**0.00000000**	0.00049172	**0.00000000**
XI5766794	XI5779439	XI	12645	11.12612	8	0.19465500	0.00869565	0.96239103
XI5766794	XI5780656	XI	13862	6.455675	6	0.37412400	0.01508799	0.96239103
XI5781688	XI5766794	XI	14894	3.100689	6	0.79610700	0.03355331	0.96239103
XI5763059	XI5779439	XI	16380	6.517181	8	0.58950300	0.02419772	0.96239103
XI5763059	XI5780656	XI	17597	7.023464	6	0.31868300	0.01308230	0.96239103
XI5763059	XI5781688	XI	18629	7.286144	6	0.29519300	0.01215062	0.96239103

XI16070261	XI5781688	XI	10288573	3.54267	4	0.47142000	0.01906056	0.96239103
XI16070261	XI5780656	XI	10289605	1.809636	6	0.93634700	0.04223602	0.96239103
XI16070261	XI5779439	XI	10290822	1.406649	6	0.96545300	0.04477226	0.96239103
XI16070261	XI5775144	XI	10295117	1.368351	2	0.50450600	0.02040631	0.96239103
XI16076821	XI5781688	XI	10295133	5.495618	6	0.48198600	0.01948758	0.96239103
XI16076821	XI5780656	XI	10296165	1.620846	8	0.99051500	0.04756729	0.96239103
XI16070261	XI5773228	XI	10297033	8.14137	4	0.08653500	0.00538302	0.80306337
XI16076821	XI5779439	XI	10297382	5.179703	8	0.73820800	0.03064182	0.96239103
XI5775144	XI16076821	XI	10301677	3.321215	6	0.76758400	0.03216874	0.96239103
XI16070261	XI5766794	XI	10303467	4.391681	8	0.82017000	0.03473085	0.96239103
XI5773228	XI16076821	XI	10303593	3.193238	8	0.92165200	0.04113613	0.96239103
XI16070261	XI5763059	XI	10307202	5.920444	8	0.65614300	0.02697981	0.96239103
XI16076821	XI5766794	XI	10310027	8.252074	12	0.76513400	0.03193582	0.96239103
XI16076821	XI5763059	XI	10313762	6.347832	14	0.95694800	0.04387940	0.96239103
XIII5678606	XIII5679721	XIII	**1115**	15.06562	12	0.23786800	0.01024845	0.96239103
XIII5675328	XIII5673292	XIII	**2036**	Infinity	14	**0.00000000**	0.00051760	**0.00000000**
XIII5683842	XIII5681784	XIII	**2058**	1.92687	6	0.92630000	0.04149845	0.96239103
XIII5679721	XIII5681784	XIII	**2063**	54.08316	12	**0.00000000**	0.00010352	**0.00000000**
XIII5678606	XIII5681784	XIII	**3178**	10.53314	12	0.56929400	0.02330487	0.96239103
XIII5675328	XIII5678606	XIII	**3278**	9.85651	10	0.45317100	0.01819358	0.96239103
XIII5683842	XIII5679721	XIII	**4121**	14.31854	6	**0.02627300**	0.00317029	0.41354224
XIII5675328	XIII5679721	XIII	**4393**	18.3718	10	**0.04900700**	0.00410197	0.59662347
XIII5683842	XIII5678606	XIII	**5236**	5.052632	6	0.53708100	0.02188147	0.96239103
XIII5673292	XIII5678606	XIII	**5314**	31.84212	12	**0.00146400**	0.00203157	0.03603118
XIII5673292	XIII5679721	XIII	**6429**	13.87293	12	0.30889700	0.01269410	0.96239103
XIII5675328	XIII5681784	XIII	**6456**	21.64766	12	**0.04166400**	0.00383023	0.54216588
XIII5673292	XIII5681784	XIII	**8492**	21.03397	14	0.10076500	0.00562888	0.89507117
XIII5675328	XIII5683842	XIII	**8514**	15.87807	6	**0.01442400**	0.00278209	0.25881677
XIII5683842	XIII5673292	XIII	**10550**	Infinity	8	**0.00000000**	0.00053054	**0.00000000**
XIV10394249	XIV10396321	XIV	**2072**	Infinity	16	**0.00000000**	0.00103520	**0.00000000**
XIV10387725	XIV10389873	XIV	**2148**	Infinity	8	**0.00000000**	0.00102226	**0.00000000**

XIV10392093	XIV10394249	XIV	**2156**	Infinity	16	**0.00000000**	0.00023292	**0.00000000**
XIV10392093	XIV10389873	XIV	**2220**	Infinity	8	**0.00000000**	0.00100932	**0.00000000**
XIV10396321	XIV10398703	XIV	**2382**	31.92041	14	**0.00411200**	0.00223861	0.09142579
XIV10392093	XIV10396321	XIV	**4228**	73.43889	16	**0.00000000**	0.00011646	**0.00000000**
XIV10392093	XIV10387725	XIV	**4368**	Infinity	10	**0.00000000**	0.00054348	**0.00000000**
XIV10394249	XIV10389873	XIV	**4376**	3.143494	8	0.92503200	0.04135611	0.96239103
XIV10394249	XIV10398703	XIV	**4454**	27.83717	14	**0.01495300**	0.00283385	0.26226465
XIV10389873	XIV10396321	XIV	**6448**	19.72853	8	**0.01141300**	0.00266563	0.21407685
XIV10394249	XIV10387725	XIV	**6524**	6.066275	10	0.80966500	0.03422619	0.96239103
XIV10392093	XIV10398703	XIV	**6610**	Infinity	16	**0.00000000**	0.00104814	**0.00000000**
XIV10387725	XIV10396321	XIV	8596	32.71202	10	**0.00030400**	0.00177277	0.00857413
XIV10389873	XIV10398703	XIV	8830	1.389008	2	0.49932200	0.02006988	0.96239103
XIV10387725	XIV10398703	XIV	10978	3.33072	10	0.97253600	0.04547101	0.96239103
XIX9374274	XIX9375733	XIX	**1459**	Infinity	8	**0.00000000**	0.00073758	**0.00000000**
XIX9371313	XIX9369810	XIX	**1503**	6.524193	2	**0.03830800**	0.00366201	0.51983680
XIX13286373	XIX13288787	XIX	**2414**	Infinity	12	**0.00000000**	0.00062112	**0.00000000**
XIX9371313	XIX9374274	XIX	**2961**	Not	possible			
XIX13288787	XIX13291868	XIX	**3081**	1.634774	2	0.44158400	0.01775362	0.96239103
XI5763059	XI5766794	XI	**3735**	57.08171	12	**0.00000000**	0.00009058	**0.00000000**
XIX16924542	XIX16928893	XIX	**4351**	Infinity	8	**0.00000000**	0.00121636	**0.00000000**
XIX9371313	XIX9375733	XIX	**4420**	14.27139	4	**0.00647700**	0.00238095	0.13601700
XIX9374274	XIX9369810	XIX	**4464**	Infinity	8	**0.00000000**	0.00124224	**0.00000000**
XIX16911266	XIX16915746	XIX	**4480**	Infinity	16	**0.00000000**	0.00120342	**0.00000000**
XIX16906593	XIX16911266	XIX	**4673**	Infinity	14	**0.00000000**	0.00106108	**0.00000000**
XIX13286373	XIX13291868	XIX	**5495**	1.757075	2	0.41539000	0.01657609	0.96239103
XIX9375733	XIX9369810	XIX	**5923**	Infinity	14	**0.00000000**	0.00125518	**0.00000000**
XIX16948763	XIX16942698	XIX	**6065**	Infinity	16	**0.00000000**	0.00065994	**0.00000000**
XIX9375733	XIX9383460	XIX	**7727**	Infinity	4	**0.00000000**	0.00138458	**0.00000000**
XIX16924542	XIX16915746	XIX	**8796**	Infinity	16	**0.00000000**	0.00111284	**0.00000000**
XIX16906593	XIX16915746	XIX	**9153**	74.78467	14	**0.00000000**	0.00014234	**0.00000000**
XIX9374274	XIX9383460	XIX	**9186**	Infinity	4	**0.00000000**	0.00137164	**0.00000000**

XIX16948763	XIX16959478	XIX	**10715**	Infinity	12	**0.00000000**	0.00028468	**0.00000000**
XIX9371313	XIX9383460	XIX	**12147**	Not	possible			
XIX16915746	XIX16928893	XIX	**13147**	Infinity	8	**0.00000000**	0.00122930	**0.00000000**
XIX16924542	XIX16911266	XIX	**13276**	Infinity	16	**0.00000000**	0.00107402	**0.00000000**
XIX9369810	XIX9383460	XIX	**13650**	Infinity	4	**0.00000000**	0.00139752	**0.00000000**
XIX16942698	XIX16928893	XIX	**13805**	5.90309	8	0.65808600	0.02700569	0.96239103
XIX16959478	XIX16942698	XIX	**16780**	16.91548	12	0.15280400	0.00720756	0.96239103
XIX16911266	XIX16928893	XIX	**17627**	9.087253	8	0.33498900	0.01370342	0.96239103
XIX16906593	XIX16924542	XIX	**17949**	75.73581	14	**0.00000000**	0.00001294	**0.00000000**
XIX16924542	XIX16942698	XIX	**18156**	Infinity	16	**0.00000000**	0.00064700	**0.00000000**
XIX16948763	XIX16928893	XIX	**19870**	25.36865	8	**0.00134600**	0.00199275	0.03377236
XIX16906593	XIX16928893	XIX	**22300**	6.236739	6	0.39720000	0.01586439	0.96239103
XIX16924542	XIX16948763	XIX	**24221**	Infinity	16	**0.00000000**	0.00025880	**0.00000000**
XIX16942698	XIX16915746	XIX	**26952**	Infinity	16	**0.00000000**	0.00117754	**0.00000000**
XIX16959478	XIX16928893	XIX	**30585**	5.547618	8	0.69775900	0.02877847	0.96239103
XIX16942698	XIX16911266	XIX	**31432**	27.46759	14	**0.01672700**	0.00292443	0.28598729
XIX16948763	XIX16915746	XIX	**33017**	Infinity	16	**0.00000000**	0.00112578	**0.00000000**
XIX16924542	XIX16959478	XIX	**34936**	Infinity	12	**0.00000000**	0.00027174	**0.00000000**
XIX16906593	XIX16942698	XIX	**36105**	25.16497	14	**0.03297300**	0.00342909	0.48078367
XIX16948763	XIX16911266	XIX	**37497**	Infinity	16	**0.00000000**	0.00108696	**0.00000000**
XIX16906593	XIX16948763	XIX	**42170**	Infinity	14	**0.00000000**	0.00024586	**0.00000000**
XIX16959478	XIX16915746	XIX	**43732**	Infinity	12	**0.00000000**	0.00113872	**0.00000000**
XIX16959478	XIX16911266	XIX	**48212**	22.02358	12	**0.03725600**	0.00358437	0.51736347
XIX16906593	XIX16959478	XIX	**52885**	18.64262	10	**0.04504400**	0.00393375	0.57253295
XIX16906593	XIX13291868	XIX	**3614725**	0	2	1.00000000	0.05113872	0.96239103
XIX16906593	XIX13288787	XIX	**3617806**	Infinity	14	**0.00000000**	0.00058230	**0.00000000**
XIX13291868	XIX16911266	XIX	**3619398**	2.411375	2	0.29948600	0.01235766	0.96239103
XIX16906593	XIX13286373	XIX	**3620220**	44.63319	10	**0.00000300**	0.00150104	**0.00009993**
XIX13288787	XIX16911266	XIX	**3622479**	Infinity	16	**0.00000000**	0.00109990	**0.00000000**
XIX13291868	XIX16915746	XIX	**3623878**	0	2	1.00000000	0.05143634	0.96239103
XIX13286373	XIX16911266	XIX	**3624893**	59.63414	12	**0.00000000**	0.00012940	**0.00000000**

XIX13288787	XIX16915746	XIX	**3626959**	Infinity	16	**0.00000000**	0.00116460	**0.00000000**
XIX13286373	XIX16915746	XIX	**3629373**	Infinity	12	**0.00000000**	0.00115166	**0.00000000**
XIX16924542	XIX13291868	XIX	**3632674**	0	2	1.00000000	0.05115166	0.96239103
XIX16924542	XIX13288787	XIX	**3635755**	Infinity	16	**0.00000000**	0.00059524	**0.00000000**
XIX13291868	XIX16928893	XIX	**3637025**	Not	possible			
XIX16924542	XIX13286373	XIX	**3638169**	Infinity	12	**0.00000000**	0.00055642	**0.00000000**
XIX13288787	XIX16928893	XIX	**3640106**	31.01073	8	**0.00014000**	0.00164337	0.00425953
XIX13286373	XIX16928893	XIX	**3642520**	15.08527	6	**0.01960400**	0.00300207	0.32650800
XIX16942698	XIX13291868	XIX	**3650830**	0	2	1.00000000	0.05134576	0.96239103
XIX13288787	XIX16942698	XIX	**3653911**	Infinity	16	**0.00000000**	0.00069876	**0.00000000**
XIX13286373	XIX16942698	XIX	**3656325**	Infinity	12	**0.00000000**	0.00068582	**0.00000000**
XIX16948763	XIX13291868	XIX	**3656895**	0	2	1.00000000	0.05116460	0.96239103
XIX16948763	XIX13288787	XIX	**3659976**	Infinity	16	**0.00000000**	0.00060818	**0.00000000**
XIX16948763	XIX13286373	XIX	**3662390**	Infinity	12	**0.00000000**	0.00056936	**0.00000000**
XIX16959478	XIX13291868	XIX	**3667610**	1.372335	2	0.50350200	0.02028986	0.96239103
XIX16959478	XIX13288787	XIX	**3670691**	72.72427	12	**0.00000000**	0.00003882	**0.00000000**
XIX16959478	XIX13286373	XIX	**3673105**	40.54037	10	**0.00001400**	0.00157867	**0.00044341**
XIX13286373	XIX9383460	XIX	**3902913**	11.85446	4	**0.01846700**	0.00296325	0.31160038
XIX13288787	XIX9383460	XIX	**3905327**	16.33522	4	**0.00260100**	0.00210921	0.06165806
XIX13291868	XIX9383460	XIX	**3908408**	Not	possible			
XIX13286373	XIX9375733	XIX	**3910640**	Infinity	10	**0.00000000**	0.00075052	**0.00000000**
XIX9374274	XIX13286373	XIX	**3912099**	47.82966	6	**0.00000000**	0.00002588	**0.00000000**
XIX13288787	XIX9375733	XIX	**3913054**	92.01948	14	**0.00000000**	0.00007764	**0.00000000**
XIX9374274	XIX13288787	XIX	**3914513**	51.83778	8	**0.00000000**	0.00005176	**0.00000000**
XIX9371313	XIX13286373	XIX	**3915060**	10.76309	6	0.09598000	0.00557712	0.85979367
XIX9375733	XIX13291868	XIX	**3916135**	3.01537	2	0.22142200	0.00966615	0.96239103
XIX13286373	XIX9369810	XIX	**3916563**	8.980167	10	0.53398700	0.02175207	0.96239103
XIX9371313	XIX13288787	XIX	**3917474**	26.80109	6	**0.00015800**	0.00165631	0.00476963
XIX9374274	XIX13291868	XIX	**3917594**	2.932667	2	0.23077000	0.01002847	0.96239103
XIX13288787	XIX9369810	XIX	**3918977**	14.13636	14	0.43960300	0.01765010	0.96239103
XIX9371313	XIX13291868	XIX	**3920555**	Not	possible			

XIX13291868	XIX9369810	XIX	**3922058**	Not	possible			
XIX16906593	XIX9383460	XIX	**7523133**	1.629509	2	0.44274800	0.01779244	0.96239103
XIX16906593	XIX9375733	XIX	**7530860**	27.50315	12	**0.00653600**	0.00239389	0.13651408
XIX16915746	XIX9383460	XIX	**7532286**	17.96231	4	**0.00125500**	0.00195393	0.03211470
XIX16906593	XIX9374274	XIX	**7532319**	26.63951	6	**0.00016900**	0.00166925	0.00506214
XIX16906593	XIX9371313	XIX	**7535280**	1.570525	6	0.95467300	0.04365942	0.96239103
XIX9375733	XIX16911266	XIX	**7535533**	54.41882	14	**0.00000100**	0.00148810	**0.00003360**
XIX16906593	XIX9369810	XIX	**7536783**	9.304314	12	0.67674200	0.02784679	0.96239103
XIX9374274	XIX16911266	XIX	**7536992**	19.73146	8	**0.01140100**	0.00265269	0.21407685
XIX9371313	XIX16911266	XIX	**7539953**	2.147515	6	0.90564400	0.04007505	0.96239103
XIX9375733	XIX16915746	XIX	**7540013**	Infinity	14	**0.00000000**	0.00119048	**0.00000000**
XIX16924542	XIX9383460	XIX	**7541082**	17.84051	4	**0.00132600**	0.00197981	0.03348800
XIX16911266	XIX9369810	XIX	**7541456**	9.074666	14	0.82623800	0.03504141	0.96239103
XIX9374274	XIX16915746	XIX	**7541472**	58.73709	8	**0.00000000**	0.00015528	**0.00000000**
XIX9371313	XIX16915746	XIX	**7544433**	7.689403	6	0.26175300	0.01093427	0.96239103
XIX16928893	XIX9383460	XIX	**7545433**	1.616147	2	0.44571600	0.01785714	0.96239103
XIX16915746	XIX9369810	XIX	**7545936**	14.50103	14	0.41308900	0.01649845	0.96239103
XIX16924542	XIX9375733	XIX	**7548809**	Infinity	14	**0.00000000**	0.00071170	**0.00000000**
XIX16924542	XIX9374274	XIX	**7550268**	Infinity	8	**0.00000000**	0.00031056	**0.00000000**
XIX9375733	XIX16928893	XIX	**7553160**	17.21616	8	**0.02793500**	0.00324793	0.43004319
XIX16924542	XIX9371313	XIX	**7553229**	4.534805	6	0.60470100	0.02478002	0.96239103
XIX9374274	XIX16928893	XIX	**7554619**	13.52601	6	**0.03540300**	0.00351967	0.50293085
XIX16924542	XIX9369810	XIX	**7554732**	9.599893	16	0.88667100	0.03858696	0.96239103
XIX9371313	XIX16928893	XIX	**7557580**	1.015408	2	0.60187600	0.02470238	0.96239103
XIX16928893	XIX9369810	XIX	**7559083**	10.02358	8	0.26337500	0.01096014	0.96239103
XIX16942698	XIX9383460	XIX	**7559238**	9.624806	4	**0.04724500**	0.00402433	0.58699254
XIX16948763	XIX9383460	XIX	**7565303**	20.01121	4	**0.00049700**	0.00182453	0.01361991
XIX16942698	XIX9375733	XIX	**7566965**	39.83605	14	**0.00027100**	0.00172101	0.00787326
XIX9374274	XIX16942698	XIX	**7568424**	30.25178	8	**0.00019100**	0.00168219	0.00567711
XIX9371313	XIX16942698	XIX	**7571385**	4.60441	6	0.59545400	0.02440476	0.96239103
XIX16948763	XIX9375733	XIX	**7573030**	Infinity	14	**0.00000000**	0.00072464	**0.00000000**

XIX16948763	XIX9374274	XIX	**7574489**	Infinity	8	**0.00000000**	0.00032350	**0.00000000**
XIX16959478	XIX9383460	XIX	**7576018**	1.466255	4	0.83260200	0.03533903	0.96239103
XIX16948763	XIX9371313	XIX	**7577450**	Infinity	6	**0.00000000**	0.00029762	**0.00000000**
XIX16948763	XIX9369810	XIX	7578953	10.73119	16	0.82576600	0.03500259	0.96239103
XIX16959478	XIX9375733	XIX	7583745	22.29214	10	**0.01368400**	0.00275621	0.24823932
XIX16959478	XIX9374274	XIX	7585204	18.96513	8	**0.01504700**	0.00284679	0.26226465
XIX16959478	XIX9371313	XIX	7588165	5.106662	4	0.27652700	0.01143892	0.96239103
XIX16959478	XIX9369810	XIX	7589668	5.084169	12	0.95510800	0.04372412	0.96239103
XVI5893989	XVI5912332	XV	18343	20.86922	8	**0.00750300**	0.00243271	0.15421060
XVI5906684	XVI5905544	XVI	**1140**	6.110623	4	0.19103800	0.00860507	0.96239103
XVI5893989	XVI5896227	XVI	**2238**	15.45619	6	**0.01699000**	0.00293737	0.28920423
XVI5906684	XVI5909125	XVI	**2441**	Infinity	8	**0.00000000**	0.00041408	**0.00000000**
XVI5888503	XVI5891048	XVI	**2545**	Infinity	16	**0.00000000**	0.00033644	**0.00000000**
XVI13150461	XVI13153214	XVI	**2753**	31.34313	14	**0.00496200**	0.00226449	0.10956096
XVI5891048	XVI5893989	XVI	**2941**	20.72517	8	**0.00791400**	0.00245859	0.16094577
XVI5909125	XVI5912332	XVI	**3207**	22.1582	10	**0.01431900**	0.00276915	0.25854493
XVI5909125	XVI5905544	XVI	**3581**	36.30186	12	**0.00029000**	0.00173395	0.00835306
XVI5891048	XVI5896227	XVI	**5179**	Infinity	16	**0.00000000**	0.00036232	**0.00000000**
XVI5888503	XVI5893989	XVI	**5486**	14.07719	8	0.07977600	0.00512422	0.77842036
XVI5906684	XVI5912332	XVI	**5648**	0	6	1.00000000	0.05069876	0.96239103
XVI5912332	XVI5905544	XVI	**6788**	21.27738	8	**0.00644600**	0.00235507	0.13601700
XVI5888503	XVI5896227	XVI	**7724**	Infinity	16	**0.00000000**	0.00034938	**0.00000000**
XVI5896227	XVI5905544	XVI	**9317**	16.94034	10	0.07569500	0.00498188	0.75676490
XVI5896227	XVI5906684	XVI	**10457**	2.654564	8	0.95413400	0.04360766	0.96239103
XVI5893989	XVI5905544	XVI	**11555**	11.49748	8	0.17507200	0.00800983	0.96239103
XVI5893989	XVI5906684	XVI	**12695**	0	4	1.00000000	0.05038820	0.96239103
XVI5896227	XVI5909125	XVI	**12898**	Infinity	16	**0.00000000**	0.00040114	**0.00000000**
XVI5891048	XVI5905544	XVI	**14496**	19.66535	12	0.07368700	0.00491718	0.74738482
XVI5893989	XVI5909125	XVI	**15136**	25.19829	8	**0.00143900**	0.00201863	0.03564292
XVI5891048	XVI5906684	XVI	**15636**	8.071856	8	0.42648300	0.01710663	0.96239103
XVI5896227	XVI5912332	XVI	**16105**	6.883662	8	0.54923500	0.02241201	0.96239103

XVI5888503	XVI5905544	XVI	**17041**	13.67571	12	0.32189400	0.01318582	0.96239103
XVI5891048	XVI5909125	XVI	**18077**	Infinity	16	**0.00000000**	0.00038820	**0.00000000**
XVI5888503	XVI5906684	XVI	**18181**	4.05234	8	0.85237100	0.03647774	0.96239103
XVI5888503	XVI5909125	XVI	**20622**	Infinity	16	**0.00000000**	0.00037526	**0.00000000**
XVI5891048	XVI5912332	XVI	**21284**	Infinity	10	**0.00000000**	0.00076346	**0.00000000**
XVI5888503	XVI5912332	XVI	23829	24.52265	10	**0.00632800**	0.00234213	0.13509056
XVI13150461	XVI5912332	XVI	7238129	3.967982	8	0.86000100	0.03686594	0.96239103
XVI5912332	XVI13153214	XVI	7240882	6.066912	10	0.80961100	0.03420031	0.96239103
XVI5909125	XVI13150461	XVI	7241336	8.328738	14	0.87150600	0.03757764	0.96239103
XVI5906684	XVI13150461	XVI	7243777	5.412495	10	0.86197700	0.03693064	0.96239103
XVI5909125	XVI13153214	XVI	7244089	23.37981	16	0.10395200	0.00574534	0.90466335
XVI13150461	XVI5905544	XVI	7244917	1.122919	10	0.99970800	0.04979296	0.96239103
XVI5906684	XVI13153214	XVI	7246530	2.06988	10	0.99577600	0.04860248	0.96239103
XVI13153214	XVI5905544	XVI	7247670	3.559287	8	0.89454000	0.03914337	0.96239103
XVI5896227	XVI13150461	XVI	7254234	4.826459	14	0.98808400	0.04729555	0.96239103
XVI5893989	XVI13150461	XVI	7256472	7.728197	8	0.46046100	0.01858178	0.96239103
XVI5896227	XVI13153214	XVI	7256987	11.05003	14	0.68210200	0.02814441	0.96239103
XVI5893989	XVI13153214	XVI	7259225	4.508966	8	0.80853500	0.03416149	0.96239103
XVI5891048	XVI13150461	XVI	7259413	12.63545	14	0.55540800	0.02264493	0.96239103
XVI5888503	XVI13150461	XVI	7261958	7.626231	14	0.90787600	0.04024327	0.96239103
XVI5891048	XVI13153214	XVI	7262166	16.3499	16	0.42881900	0.01722308	0.96239103
XVI5888503	XVI13153214	XVI	7264711	18.80829	16	0.27873000	0.01159420	0.96239103
XVII8129604	XVII7561091	XVII	568513	2.874021	10	0.98423500	0.04679089	0.96239103
XVII8129604	XVII10618107	XVII	2488503	0	6	1.00000000	0.05075052	0.96239103
XVII10618107	XVII7561091	XVII	3057016	1.635182	2	0.44149400	0.01772774	0.96239103
XVIII3143344	XVIII3144386	XVIII	**1042**	Infinity	16	**0.00000000**	0.00143634	**0.00000000**
XVIII3146807	XVIII3144386	XVIII	**2421**	33.62323	12	**0.00077300**	0.00187629	0.02059912
XVIII3162328	XVIII3159265	XVIII	**3063**	22.91064	6	**0.00082700**	0.00188923	0.02188718
XVIII3149894	XVIII3146807	XVIII	**3087**	22.45984	10	**0.01292600**	0.00270445	0.23840880
XVIII3156177	XVIII3159265	XVIII	**3088**	Infinity	14	**0.00000000**	0.00144928	**0.00000000**
XVIII3165611	XVIII3168806	XVIII	**3195**	22.76854	14	0.06414200	0.00463251	0.69111012

XVIII3162328	XVIII3165611	XVIII	**3283**	7.249413	6	0.29839600	0.01230590	0.96239103
XVIII3146807	XVIII3143344	XVIII	**3463**	37.23915	12	**0.00020400**	0.00170807	0.00597164
XVIII3139053	XVIII3143344	XVIII	**4291**	Infinity	14	**0.00000000**	0.00141046	**0.00000000**
XVIII3139053	XVIII3144386	XVIII	**5333**	20.33175	16	0.20564900	0.00918737	0.96239103
XVIII3149894	XVIII3144386	XVIII	**5508**	16.91046	14	0.26098100	0.01092133	0.96239103
XVIII3156177	XVIII3162328	XVIII	**6151**	8.415051	10	0.58836400	0.02412008	0.96239103
XVIII3156177	XVIII3149894	XVIII	**6283**	40.63598	14	**0.00020300**	0.00169513	0.00597164
XVIII3159265	XVIII3165611	XVIII	**6346**	33.3208	16	**0.00669900**	0.00241977	0.13842212
XVIII3162328	XVIII3168806	XVIII	**6478**	25.45277	8	**0.00130200**	0.00196687	0.03309821
XVIII3149894	XVIII3143344	XVIII	**6550**	Infinity	14	**0.00000000**	0.00142340	**0.00000000**
XVIII3139053	XVIII3146807	XVIII	**7754**	12.07865	12	0.43938400	0.01763716	0.96239103
XVIII3156177	XVIII3146807	XVIII	**9370**	13.35793	10	0.20434200	0.00910973	0.96239103
XVIII3149894	XVIII3159265	XVIII	**9371**	15.97785	14	0.31472900	0.01290114	0.96239103
XVIII3156177	XVIII3165611	XVIII	**9434**	31.92067	14	**0.00411100**	0.00222567	0.09142579
XVIII3159265	XVIII3168806	XVIII	**9541**	Infinity	12	**0.00000000**	0.00146222	**0.00000000**
XVIII3139053	XVIII3149894	XVIII	**10841**	39.23204	14	**0.00033600**	0.00178571	0.00940800
XVIII3156177	XVIII3144386	XVIII	**11791**	30.68289	14	**0.00613800**	0.00231625	0.13249850
XVIII3162328	XVIII3149894	XVIII	**12434**	5.792244	10	0.83240500	0.03530021	0.96239103
XVIII3146807	XVIII3159265	XVIII	**12458**	6.310092	10	0.78857300	0.03317805	0.96239103
XVIII3156177	XVIII3168806	XVIII	**12629**	58.09639	12	**0.00000000**	0.00016822	**0.00000000**
XVIII3156177	XVIII3143344	XVIII	**12833**	13.07581	14	0.52056400	0.02115683	0.96239103
XVIII3144386	XVIII3159265	XVIII	**14879**	46.13087	16	**0.00009300**	0.00161749	0.00287482
XVIII3162328	XVIII3146807	XVIII	**15521**	5.881812	6	0.43655900	0.01759834	0.96239103
XVIII3149894	XVIII3165611	XVIII	**15717**	50.92257	14	**0.00000400**	0.00152692	**0.00013098**
XVIII3143344	XVIII3159265	XVIII	15921	11.18858	16	0.79769800	0.03364389	0.96239103
XVIII3156177	XVIII3139053	XVIII	17124	9.935421	14	0.76688900	0.03209110	0.96239103
XVIII3162328	XVIII3144386	XVIII	17942	13.99939	8	0.08178100	0.00515010	0.79206233
XVIII3146807	XVIII3165611	XVIII	18804	17.16376	12	0.14354000	0.00681936	0.96239103
XVIII3149894	XVIII3168806	XVIII	18912	9.301822	12	0.67695700	0.02789855	0.96239103
XVIII3162328	XVIII3143344	XVIII	18984	15.71464	10	0.10809800	0.00584886	0.92409441
XVIII3139053	XVIII3159265	XVIII	20212	7.51076	16	0.96211600	0.04439700	0.96239103

XVIII3144386	XVIII3165611	XVIII	21225	24.6734	16	0.07579400	0.00500776	0.75676490
XVIII3146807	XVIII3168806	XVIII	21999	10.56389	10	0.39248800	0.01569617	0.96239103
XVIII3143344	XVIII3165611	XVIII	22267	21.80471	16	0.14961000	0.00703934	0.96239103
XVIII3162328	XVIII3139053	XVIII	23275	3.324113	10	0.97273600	0.04549689	0.96239103
XVIII3144386	XVIII3168806	XVIII	24420	25.98171	14	**0.02602600**	0.00315735	0.41214944
XVIII3143344	XVIII3168806	XVIII	25462	13.27254	14	0.50518500	0.02045807	0.96239103
XVIII3139053	XVIII3165611	XVIII	26558	31.67184	16	**0.01103000**	0.00261387	0.21042621
XVIII3139053	XVIII3168806	XVIII	29753	17.01335	14	0.25546700	0.01076605	0.96239103
XIII5683842	XIX16942698			53.77549	8	**0.00000000**	0.00006470	**0.00000000**
XI5775144	XIII5683842			Infinity	6	**0.00000000**	0.00021998	**0.00000000**
XIII5683842	XVIII3162328			Infinity	4	**0.00000000**	0.00042702	**0.00000000**
XIII5683842	XI5763059			Infinity	6	**0.00000000**	0.00050466	**0.00000000**
XI5775144	XIX16942698			Infinity	6	**0.00000000**	0.00063406	**0.00000000**
XI5763059	XIX16942698			Infinity	14	**0.00000000**	0.00067288	**0.00000000**
XI5775144	I21598355			Infinity	6	**0.00000000**	0.00078934	**0.00000000**
XIII5683842	I21598355			Infinity	8	**0.00000000**	0.00080228	**0.00000000**
XI5763059	I21598355			Infinity	10	**0.00000000**	0.00081522	**0.00000000**
XIX16942698	I21598355			Infinity	12	**0.00000000**	0.00082816	**0.00000000**
XI5775144	II21932462			Infinity	6	**0.00000000**	0.00084110	**0.00000000**
XIII5683842	II21932462			Infinity	8	**0.00000000**	0.00085404	**0.00000000**
XI5763059	II21932462			Infinity	14	**0.00000000**	0.00086698	**0.00000000**
XIX16942698	II21932462			Infinity	16	**0.00000000**	0.00087992	**0.00000000**
I21598355	II21932462			Infinity	12	**0.00000000**	0.00089286	**0.00000000**
XVII8129604	II4564317			Infinity	12	**0.00000000**	0.00126812	**0.00000000**
XI5775144	XVIII3162328			34.79473	4	**0.00000100**	0.00147516	**0.00003360**
XI5775144	XIX13286373			30.44454	4	**0.00000400**	0.00151398	**0.00013098**
XVIII3162328	I21598355			29.03895	4	**0.00000800**	0.00153986	**0.00025760**
XVIII3162328	II21932462			39.18113	10	**0.00002400**	0.00159161	**0.00075395**
XIII5683842	XIX13286373			24.07049	4	**0.00007700**	0.00160455	0.00239942
XVIII3162328	XI5763059			29.19426	8	**0.00029300**	0.00174689	0.00835306
XIX13286373	II21932462			36.26256	12	**0.00029400**	0.00175983	0.00835306

XI5763059	XIX13286373	35.84516	12	**0.00034300**	0.00179865	0.00953491
XI5775144	XIII5675328	19.40464	4	**0.00065400**	0.00185041	0.01767172
XVI13150461	XI5779439	26.34555	8	**0.00091600**	0.00190217	0.02407771
X9526306	XIII5681784	32.32268	12	**0.00123400**	0.00194099	0.03178784
XVI5906684	XIX9383460	17.58602	4	**0.00148600**	0.00204451	0.03634116
XI5775144	II4553994	17.45983	4	**0.00157300**	0.00205745	0.03822687
X9527397	IV3329457	28.25017	10	**0.00164600**	0.00207039	0.03975090
XVI5891048	XVIII3146807	30.89642	12	**0.00204300**	0.00209627	0.04872933
XI5775144	XVI5905544	16.05643	4	**0.00294400**	0.00212215	0.06936351
XVI5896227	X9528514	35.53836	16	**0.00335200**	0.00214803	0.07802487
XI5781688	XIX16942698	19.46435	6	**0.00344700**	0.00216097	0.07975574
XIX9375733	II21932462	32.29192	14	**0.00364000**	0.00217391	0.08372000
IV3331520	XVI13153214	32.15697	14	**0.00380500**	0.00219979	0.08648541
XIII5683842	XVI5905544	19.13769	6	**0.00393700**	0.00221273	0.08896239
II21932462	XVI5905544	25.73257	10	**0.00411700**	0.00225155	0.09142579
IV32097027	XVIII3159265	31.1862	14	**0.00522000**	0.00227743	0.11460273
XIX16959478	X9534783	27.90748	12	**0.00570700**	0.00229037	0.12458671
IV3334738	XVIII3165611	33.57165	16	**0.00620200**	0.00232919	0.13313627
XIII5675328	XI5763059	27.54015	12	**0.00645500**	0.00236801	0.13601700
XIII5679721	X9530904	27.03716	12	**0.00763300**	0.00244565	0.15605244
XIII5673292	IV3332621	32.71996	16	**0.00804800**	0.00247153	0.16281399
II4547932	XI5781688	17.34606	6	**0.00809200**	0.00248447	0.16285150
XVI13153214	XVIII3159265	32.47191	16	**0.00867600**	0.00249741	0.17219569
XIV10392093	XVIII3143344	32.47071	16	**0.00867900**	0.00251035	0.17219569
IV32092920	XIX16906593	29.58887	14	**0.00869000**	0.00252329	0.17219569
XIV10389873	XI5779439	17.05823	6	**0.00907200**	0.00253623	0.17884800
XIV10394249	X9534783	32.12822	16	**0.00962200**	0.00256211	0.18777479
II4573177	XIX13286373	23.24962	10	**0.00986200**	0.00257505	0.19149130
XI5763059	XVI13153214	29.09228	14	**0.01015400**	0.00258799	0.19617528
I21593750	X9534783	19.92075	8	**0.01064000**	0.00260093	0.20454209
XI5775144	XIX9375733	16.5574	6	**0.01105500**	0.00262681	0.21042621

XVII8129604	XVI5905544	22.77214	10	**0.01162000**	0.00267857	0.21690667
XIV10389873	XI5780656	19.37936	8	**0.01295700**	0.00271739	0.23840880
II21932462	XVIII3165611	30.95296	16	**0.01364400**	0.00274327	0.24823932
XVI5906684	II21928786	19.0673	8	**0.01450400**	0.00279503	0.25881677
XVI5912332	XIX16928893	15.85836	6	**0.01453500**	0.00280797	0.25881677
XIX16924542	II4553994	27.85598	14	**0.01486700**	0.00282091	0.26226465
I21593750	IV32107470	18.96153	8	**0.01506700**	0.00285973	0.26226465
X9534783	XIX16915746	30.61363	16	**0.01506800**	0.00287267	0.26226465
XIX16906593	XVIII3139053	27.67144	14	**0.01572500**	0.00288561	0.27247265
XIII5675328	II21932462	27.64691	14	**0.01584300**	0.00289855	0.27329175
XVIII3139053	X9530904	30.41333	16	**0.01597300**	0.00291149	0.27430965
II4541659	XIV10396321	29.92588	16	**0.01839000**	0.00295031	0.31160038
XIV10394249	XVIII3149894	27.10773	14	**0.01864200**	0.00297619	0.31318560
XIV10394249	X9530904	29.81722	16	**0.01897300**	0.00298913	0.31736655
II4541659	XVI5909125	29.60373	16	**0.02016900**	0.00301501	0.33447646
I21593750	XI5779439	18.02487	8	**0.02104100**	0.00302795	0.34609930
XI5775144	XIX16924542	14.90003	6	**0.02104900**	0.00304089	0.34609930
XVI13153214	XVIII3143344	29.41559	16	**0.02128000**	0.00305383	0.34841492
XIV10394249	XVI5891048	29.19462	16	**0.02265600**	0.00307971	0.36782682
XVI13153214	IV32103306	29.0862	16	**0.02336100**	0.00309265	0.37768579
XIII5673292	II21932462	29.03628	16	**0.02369300**	0.00310559	0.38145730
II4573177	IV32103306	23.3058	12	**0.02523900**	0.00313147	0.40298965
XVI5888503	XVIII3146807	23.28667	12	**0.02538800**	0.00314441	0.40370054
XVIII3156177	IV32107470	25.94225	14	**0.02632800**	0.00318323	0.41354224
II4547932	XVIII3146807	23.12083	12	**0.02671800**	0.00319617	0.41656413
XIII5675328	XVIII3168806	23.1186	12	**0.02673600**	0.00320911	0.41656413
XVI5891048	IV3334738	28.39727	16	**0.02833200**	0.00326087	0.43442400
IV32097027	XI5779439	17.14279	8	**0.02865600**	0.00327381	0.43765527
XI5781688	XIV10389873	14.0222	6	**0.02938900**	0.00328675	0.44708306
XI5775144	XIX9374274	7.021801	2	**0.02987000**	0.00329969	0.45175594
IV32105368	XVI13153214	7.017788	2	**0.02993000**	0.00331263	0.45175594

XIX16915746	XVIII3146807	22.66137	12	**0.03074200**	0.00332557	0.46220657
XIII5675328	XIX16928893	13.89132	6	**0.03087400**	0.00333851	0.46239200
XVI5893989	XIX16928893	13.85704	6	**0.03127500**	0.00335145	0.46658919
I21600738	XVI5906684	19.64821	10	**0.03276100**	0.00337733	0.48078367
XIX16924542	X9528514	27.86505	16	**0.03281300**	0.00339027	0.48078367
XIX9371313	X9526306	13.72658	6	**0.03284400**	0.00340321	0.48078367
XIX16906593	X9528514	25.17704	14	**0.03285900**	0.00341615	0.48078367
XIX9374274	II21932462	16.71943	8	**0.03316700**	0.00344203	0.48095266
XI5763059	XVI5905544	19.60161	10	**0.03325400**	0.00345497	0.48095266
XIV10392093	XVI5909125	27.80496	16	**0.03335800**	0.00346791	0.48095266
XIV10392093	XIII5673292	27.68079	16	**0.03450900**	0.00348085	0.49569805
XI5781688	XIX9369810	13.5586	6	**0.03497500**	0.00349379	0.50053111
II4553994	XIX16942698	24.88642	14	**0.03570500**	0.00353261	0.50536308
XIX13286373	I21598355	16.48682	8	**0.03591900**	0.00354555	0.50653655
XVIII3162328	IV32107470	19.26281	10	**0.03704900**	0.00355849	0.51736347
XIV10392093	XIII5681784	24.75299	14	**0.03708600**	0.00357143	0.51736347
II4547932	X9527397	22.00941	12	**0.03741400**	0.00359731	0.51736347
XVI5906684	IV32100268	10.18061	4	**0.03749300**	0.00361025	0.51736347
II4547932	XIII5679721	21.99719	12	**0.03755100**	0.00362319	0.51736347
XVI5906684	II4573177	16.35093	8	**0.03762400**	0.00363613	0.51736347
IV3331520	XI5780656	16.31343	8	**0.03810700**	0.00364907	0.51983680
I21600738	XIX9371313	6.523149	2	**0.03832800**	0.00367495	0.51983680
XIX16924542	XI5781688	13.31189	6	**0.03834200**	0.00368789	0.51983680
XIII5678606	XVIII3159265	21.91123	12	**0.03852700**	0.00370083	0.52051863
XIX16906593	XVIII3144386	24.52664	14	**0.03953800**	0.00371377	0.53231649
XI5775144	X9527397	6.421518	2	**0.04032600**	0.00372671	0.54104050
X9526306	XI5766794	18.98394	10	**0.04046700**	0.00373965	0.54105359
I21593750	XIX13288787	16.1075	8	**0.04086700**	0.00375259	0.54216588
II21928786	IV3331520	18.9317	10	**0.04113900**	0.00376553	0.54216588
XIII5679721	XIX16911266	21.68778	12	**0.04117200**	0.00377847	0.54216588
XIV10392093	XVI13153214	27.01995	16	**0.04126200**	0.00379141	0.54216588

XVII8129604	XVIII3149894	21.6596	12	**0.04151700**	0.00381729	0.54216588
II21927301	XIII5681784	24.33764	14	**0.04169800**	0.00384317	0.54216588
I21598355	XVI5905544	18.88004	10	**0.04181300**	0.00385611	0.54216588
XIII5683842	XVIII3156177	13.00464	6	**0.04296200**	0.00386905	0.55392558
X9526306	XVIII3168806	21.53699	12	**0.04304900**	0.00388199	0.55392558
XVIII3149894	XI5780656	12.99284	6	**0.04315000**	0.00389493	0.55392558
XIV10392093	X9530904	26.76114	16	**0.04421600**	0.00390787	0.56573054
XVII10618107	XVIII3139053	12.88262	6	**0.04493800**	0.00392081	0.57253295
XIX13288787	XVIII3146807	21.35686	12	**0.04539300**	0.00394669	0.57507722
XIX9371313	X9530904	12.81738	6	**0.04602900**	0.00395963	0.58105835
XVI5909125	XIX13286373	21.29391	12	**0.04623900**	0.00397257	0.58105835
XI5781688	IV32097027	12.78854	6	**0.04652000**	0.00398551	0.58105835
XIV10387725	XIX16911266	18.53624	10	**0.04656400**	0.00399845	0.58105835
XVII8129604	II21927301	23.84867	14	**0.04778700**	0.00403727	0.59182362
XIX16942698	XVI13153214	23.82392	14	**0.04811600**	0.00405021	0.59264408
XI5781688	XIX16928893	12.69406	6	**0.04816000**	0.00406315	0.59264408
II21927301	X9534783	26.41652	16	**0.04844200**	0.00407609	0.59329519
XIX9374274	XVIII3162328	12.67372	6	**0.04852000**	0.00408903	0.59329519
II4547932	XI5780656	15.56168	8	**0.04910100**	0.00411491	0.59662347
II4557054	XIII5681784	23.70641	14	**0.04970300**	0.00412785	0.60048975
I21600738	X9526306	18.3245	10	**0.04973000**	0.00414079	0.60048975

Table S3.15. F_{IS} within loci and populations. Bolded values indicate SNPs for which significant genotype-phenotype associations were found within Mud Lake.

SNP	BL	FG	FT	HK	KN	MD	MO	RS
chrI_21589802	NA	NA	1.000	0.000	NA	0.270	1.000	NA
chrI_21593750	NA	-0.031	-0.051	-0.061	NA	-0.008	NA	NA
chrI_21598355	-0.039	NA	-0.449	0.176	-0.542	0.042	NA	-0.059
chrI_21600738	0.556	0.000	-0.140	0.019	NA	-0.082	NA	0.189
chrII_4541659	-0.047	-0.120	-0.003	0.260	-0.109	-0.053	0.207	-0.089
chrII_4547932	-0.068	-0.018	0.176	-0.045	-0.053	-0.018	-0.025	0.424
chrII_4551986	1.000	1.000	1.000	NA	NA	1.000	NA	1.000
chrII_4553994	0.377	0.405	0.757	0.504	NA	0.538	0.092	0.596
chrII_4557054	0.927	0.737	0.381	0.135	NA	0.664	0.757	0.724
chrII_4564317	NA	0.000	0.010	0.067	NA	-0.007	-0.054	0.109
chrII_4566452	0.005	0.137	-0.064	0.247	0.000	0.110	-0.026	-0.043
chrII_4573177	0.045	0.012	NA	-0.144	NA	0.051	-0.048	-0.128
chrII_21927301	-0.093	-0.182	-0.085	-0.099	-0.053	0.142	-0.195	-0.043
chrII_21928786	NA	0.134	0.030	0.078	NA	0.161	-0.280	0.093
chrII_21932462	**-0.141**	**0.329**	**-0.449**	**-0.020**	**-0.542**	**-0.059**	**0.111**	**0.109**
chrIV_3329457	0.796	NA	1.000	0.793	0.310	0.862	0.537	0.661
chrIV_3331520	-0.013	-0.051	0.179	0.072	0.091	0.034	NA	-0.253
chrIV_3332621	0.078	-0.028	0.334	-0.130	0.083	0.052	0.046	0.165
chrIV_3333740	NA	NA	0.000	NA	NA	0.643	0.478	1.000
chrIV_3334738	0.192	0.112	0.060	-0.064	-0.051	0.050	-0.028	0.102
chrIV_32092920	0.087	-0.052	-0.050	-0.068	-0.057	0.110	0.101	-0.238
chrIV_32097027	NA	-0.057	-0.020	-0.250	-0.114	-0.069	-0.072	0.501
chrIV_32100268	NA	0.147	-0.692	NA	NA	0.438	NA	0.000
chrIV_32103306	0.008	-0.065	0.300	-0.269	0.087	-0.024	0.168	0.177
chrIV_32105368	0.598	NA	NA	NA	NA	NA	NA	NA
chrIV_32107470	-0.182	0.060	-0.241	-0.032	-0.102	0.002	0.052	0.019
chrX_9526306	0.113	-0.029	NA	-0.109	-0.008	-0.036	-0.067	-0.103
chrX_9527397	0.088	-0.077	NA	-0.040	NA	-0.053	-0.061	0.334
chrX_9528514	0.083	-0.160	-0.020	-0.069	0.114	-0.223	-0.061	-0.210
chrX_9530904	0.045	-0.006	-0.020	0.351	0.045	0.010	-0.137	-0.051
chrX_9534783	-0.043	0.149	0.177	0.059	0.203	0.051	-0.236	-0.049
chrXI_5763059	**-0.012**	**-0.077**	**-0.397**	**-0.079**	**-0.517**	**-0.122**	**-0.012**	**NA**
chrXI_5766794	-0.013	-0.131	0.000	-0.314	NA	-0.012	-0.109	NA
chrXI_5773228	**-0.013**	**NA**	**1.000**	**-0.041**	**NA**	**-0.093**	**NA**	**NA**
chrXI_5775144	**NA**	**NA**	**-0.410**	**NA**	**-0.517**	**-0.182**	**NA**	**NA**
chrXI_5779439	NA	0.177	-0.078	0.023	NA	-0.013	NA	NA
chrXI_5780656	NA	NA	-0.105	0.000	NA	-0.007	NA	0.000

chrXI_5781688	NA	NA	0.085	0.095	NA	0.001	NA	NA
chrXI_16070261	NA	-0.006	-0.009	0.083	NA	NA	0.084	0.000
chrXI_16076821	-0.077	0.240	-0.028	0.488	-0.008	0.664	-0.001	0.095
chrXIII_5673292	0.014	0.016	0.241	0.052	-0.016	-0.106	-0.073	0.270
chrXIII_5675328	0.010	0.040	NA	0.105	-0.041	-0.096	-0.068	-0.238
chrXIII_5678606	NA	-0.012	-0.010	-0.087	NA	-0.018	-0.046	-0.085
chrXIII_5679721	NA	0.020	0.074	0.182	NA	0.492	0.040	0.123
chrXIII_5681784	0.035	-0.090	0.036	0.106	NA	0.056	-0.028	0.024
chrXIII_5683842	NA	NA	-0.395	NA	-0.517	-0.189	NA	-0.043
chrXIV_10387725	NA	-0.098	0.487	0.257	NA	-0.007	NA	0.043
chrXIV_10389873	NA	NA	1.000	-0.030	NA	-0.017	NA	-0.036
chrXIV_10392093	-0.104	0.120	-0.082	0.218	-0.031	-0.017	0.056	0.023
chrXIV_10394249	-0.085	-0.062	0.142	-0.053	-0.041	-0.041	0.049	-0.253
chrXIV_10396321	0.106	-0.160	-0.020	0.172	-0.032	0.001	-0.061	0.024
chrXIV_10398703	-0.082	0.087	-0.077	1.000	0.000	-0.200	0.100	0.661
chrXVI_5888503	0.634	0.103	0.084	-0.097	-0.113	-0.131	-0.010	-0.065
chrXVI_5891048	-0.082	0.156	-0.018	0.003	-0.083	-0.031	-0.010	-0.093
chrXVI_5893989	-0.200	NA	NA	0.000	NA	-0.043	NA	-0.101
chrXVI_5896227	0.012	0.333	0.015	-0.084	-0.025	-0.177	-0.032	-0.111
chrXVI_5905544	0.122	1.000	NA	-0.011	-0.711	0.382	NA	-0.070
chrXVI_5906684	1.000	0.077	-0.112	-0.011	NA	NA	NA	-0.010
chrXVI_5909125	-0.148	0.216	-0.040	0.019	-0.065	0.030	-0.122	0.250
chrXVI_5912332	0.128	NA	NA	0.000	NA	-0.043	1.000	-0.048
chrXVI_13150461	-0.101	-0.063	-0.093	-0.100	NA	-0.006	-0.148	-0.063
chrXVI_13153214	-0.014	-0.075	-0.140	0.028	0.000	-0.071	0.036	-0.181
chrXVII_7561091	-0.048	0.092	-0.053	NA	NA	0.043	0.000	0.000
chrXVII_8129604	0.125	0.077	-0.101	0.040	NA	0.159	0.017	0.197
chrXVII_10618107	NA	NA	NA	-0.061	-0.150	-0.024	1.000	NA
chrXVIII_3139053	-0.031	0.018	0.000	-0.030	-0.040	-0.134	0.007	0.098
chrXVIII_3143344	-0.013	0.056	0.219	0.196	0.158	0.087	-0.020	-0.033
chrXVIII_3144386	0.241	0.000	-0.172	-0.189	-0.094	0.046	-0.099	0.135
chrXVIII_3146807	0.038	-0.052	-0.396	0.053	NA	NA	0.100	0.089
chrXVIII_3149894	0.063	0.087	NA	0.084	-0.047	-0.084	-0.127	-0.242
chrXVIII_3156177	**0.246**	**-0.076**	**NA**	**0.043**	**0.110**	**-0.063**	**0.072**	**0.141**
chrXVIII_3159265	0.075	0.052	0.000	-0.142	-0.092	-0.050	-0.045	-0.100
chrXVIII_3162328	0.131	0.508	-0.414	NA	-0.833	-1.000	1.000	-0.333
chrXVIII_3165611	-0.077	-0.155	0.243	0.117	0.164	0.064	0.122	-0.100
chrXVIII_3168806	-0.033	-0.008	-0.021	-0.091	-0.117	0.031	NA	0.421
chrXIX_9369810	0.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
chrXIX_9371313	0.000	NA	NA	NA	NA	0.348	0.000	NA
chrXIX_9374274	NA	0.498	0.641	0.555	NA	NA	NA	0.185
chrXIX_9375733	0.492	0.514	0.684	0.576	0.863	-0.037	NA	0.158
chrXIX_9383460	NA	0.536	0.593	NA	NA	NA	NA	NA

chrXIX_13286373	-0.379	-0.396	-0.725	-0.304	-1.000	-0.273	-0.156	NA
chrXIX_13288787	-0.237	-0.270	-0.538	-0.351	-0.857	-0.217	-0.163	-0.041
chrXIX_13291868	NA	0.000	NA	NA	NA	NA	NA	NA
chrXIX_16906593	0.581	0.432	NA	0.345	0.954	0.424	0.123	0.468
chrXIX_16911266	0.512	0.286	0.731	0.661	0.852	0.342	0.224	0.212
chrXIX_16915746	-0.270	-0.267	-0.471	-0.351	-0.829	0.084	-0.171	0.110
chrXIX_16924542	-0.254	-0.250	-0.577	-0.360	-0.808	-0.181	-0.158	0.155
chrXIX_16928893	NA	NA	0.155	-0.068	NA	0.045	NA	-0.298
chrXIX_16942698	**-0.169**	**-0.255**	**-0.672**	**0.834**	**-0.821**	**-0.177**	**-0.045**	**-0.099**
chrXIX_16948763	-0.279	-0.259	-0.493	-0.095	-0.808	-0.084	-0.158	-0.067
chrXIX_16959478	NA	0.345	0.488	0.541	NA	0.298	0.187	0.395

Table S3.16. Locus-specific F_{ST} (Nei's G_{ST}') and major allele frequencies (MAF). Values for locus-specific F_{ST} which are greater than the average G_{ST}' across all loci are indicated with an asterisk next to the G_{ST}' column and values for locus-specific MAF which are greater than the average MAF are indicated with an asterisk next to the MAF column. SNPs with significant genotype-phenotype associations within Mud Lake or among populations are indicated in bold. SNPs that were detected as Fst outliers are indicated in italics.

Gene	SNP	Gst'	> average	MAF	> average
IGFBP2.1	*chrI_21589802*	*0.830*	*	*0.879*	*
	chrI_21593750	0.483	*	0.870	*
	chrI_21598355	0.269		0.834	*
	chrI_21600738	0.303		0.892	*
IGF1r.2	chrII_4541659	0.300		0.540	
	chrII_4547932	*0.060*		*0.866*	*
	chrII_4551986	0.355		0.868	*
	chrII_4553994	0.238		0.618	
	chrII_4557054	*0.500*	*	*0.582*	
	chrII_4564317	*0.620*	*	*0.888*	*
	chrII_4566452	0.248		0.612	
	chrII_4573177	0.183		0.801	*
MYOD1.2	chrII_21927301	0.284		0.503	
	chrII_21928786	0.283		0.693	
	chrII_21932462	**0.227**		**0.626**	
FGF2	chrIV_3329457	0.630	*	0.836	*
	chrIV_3331520	0.187		0.850	*
	chrIV_3332621	*0.099*		*0.690*	
	chrIV_3333740	0.541	*	0.681	
	chrIV_3334738	0.275		0.509	
IGF1	chrIV_32092920	0.364	*	0.604	
	chrIV_32097027	0.402	*	0.559	
	chrIV_32100268	0.350		0.873	*
	chrIV_32103306	0.262		0.515	
	chrIV_32105368	0.595	*	0.830	*
	chrIV_32107470	*0.170*		*0.504*	
NPYP	chrX_9526306	0.438	*	0.517	
	chrX_9527397	*0.074*		*0.908*	*
	chrX_9528514	0.184		0.694	
	chrX_9530904	0.330		0.577	
	chrX_9534783	*0.060*		*0.687*	
STAT5	**chrXI_5763059**	**0.428**	*	**0.686**	
	chrXI_5766794	0.604	*	0.679	
	chrXI_5773228	***0.782***	*	***0.763***	*

	chrXI_5775144	**0.229**		**0.910**	*
	chrXI_5779439	*0.709*	*	*0.654*	
	chrXI_5780656	*0.739*	*	*0.673*	
	chrXI_5781688	*0.615*	*	*0.744*	*
GH2	*chrXI_16070261*	*0.573*	*	*0.835*	*
	chrXI_16076821	*0.663*	*	*0.548*	
GHr1	chrXIII_5673292	0.307		0.659	
	chrXIII_5675328	0.136		0.786	*
	chrXIII_5678606	*0.030*		*0.968*	*
	chrXIII_5679721	0.237		0.811	*
	chrXIII_5681784	0.222		0.805	*
	chrXIII_5683842	0.204		0.906	*
GHr2	chrXIV_10387725	0.109		0.923	*
	chrXIV_10389873	0.162		0.954	*
	chrXIV_10392093	0.291		0.598	
	chrXIV_10394249	0.497	*	0.648	
	chrXIV_10396321	*0.119*		*0.810*	*
	chrXIV_10398703	*0.140*		*0.694*	
IGFBP2.2	chrXVI_5888503	0.296		0.629	
	chrXVI_5891048	0.264		0.582	
	chrXVI_5893989	0.177		0.945	*
	chrXVI_5896227	0.383	*	0.543	
	chrXVI_5905544	0.334		0.907	*
	chrXVI_5906684	0.495	*	0.828	*
	chrXVI_5909125	0.309		0.571	
	chrXVI_5912332	0.289		0.909	*
PIT1	*chrXVI_13150461*	*0.615*	*	*0.527*	
	chrXVI_13153214	*0.152*		*0.778*	*
LG17QTL	chrXVII_7561091	0.442	*	0.588	
	chrXVII_8129604	0.409	*	0.532	
	chrXVII_10618107	*0.837*	*	*0.739*	*
IGF2r	chrXVIII_3139053	0.336		0.622	
	chrXVIII_3143344	0.162		0.708	
	chrXVIII_3144386	0.265		0.637	
	chrXVIII_3146807	0.284		0.822	*
	chrXVIII_3149894	0.234		0.673	
	chrXVIII_3156177	**0.214**		**0.733**	*
	chrXVIII_3159265	*0.147*		*0.659*	
	chrXVIII_3162328	0.013		0.541	
	chrXVIII_3165611	0.328		0.572	
	chrXVIII_3168806	0.176		0.719	*
MYOD1.1	*chrXIX_9369810*	*0.435*	*	*0.720*	*
	chrXIX_9371313	*0.859*	*	*0.812*	*

	chrXIX_9374274	*0.520*	*	*0.637*	
	chrXIX_9375733	0.316		0.763	*
	chrXIX_9383460	0.686	*	0.733	*
IGF2	*chrXIX_13286373*	*0.292*		*0.642*	
	chrXIX_13288787	*0.297*		*0.695*	
	chrXIX_13291868	*0.994*	*	*0.924*	*
IGF1r.1	chrXIX_16906593	0.471	*	0.629	
	chrXIX_16911266	0.335		0.571	
	chrXIX_16915746	*0.113*		*0.678*	
	chrXIX_16924542	*0.249*		*0.650*	
	chrXIX_16928893	0.510	*	0.881	*
	chrXIX_16942698	***0.317***		***0.715***	
	chrXIX_16948763	*0.257*		*0.684*	
	chrXIX_16959478	0.489	*	0.805	*

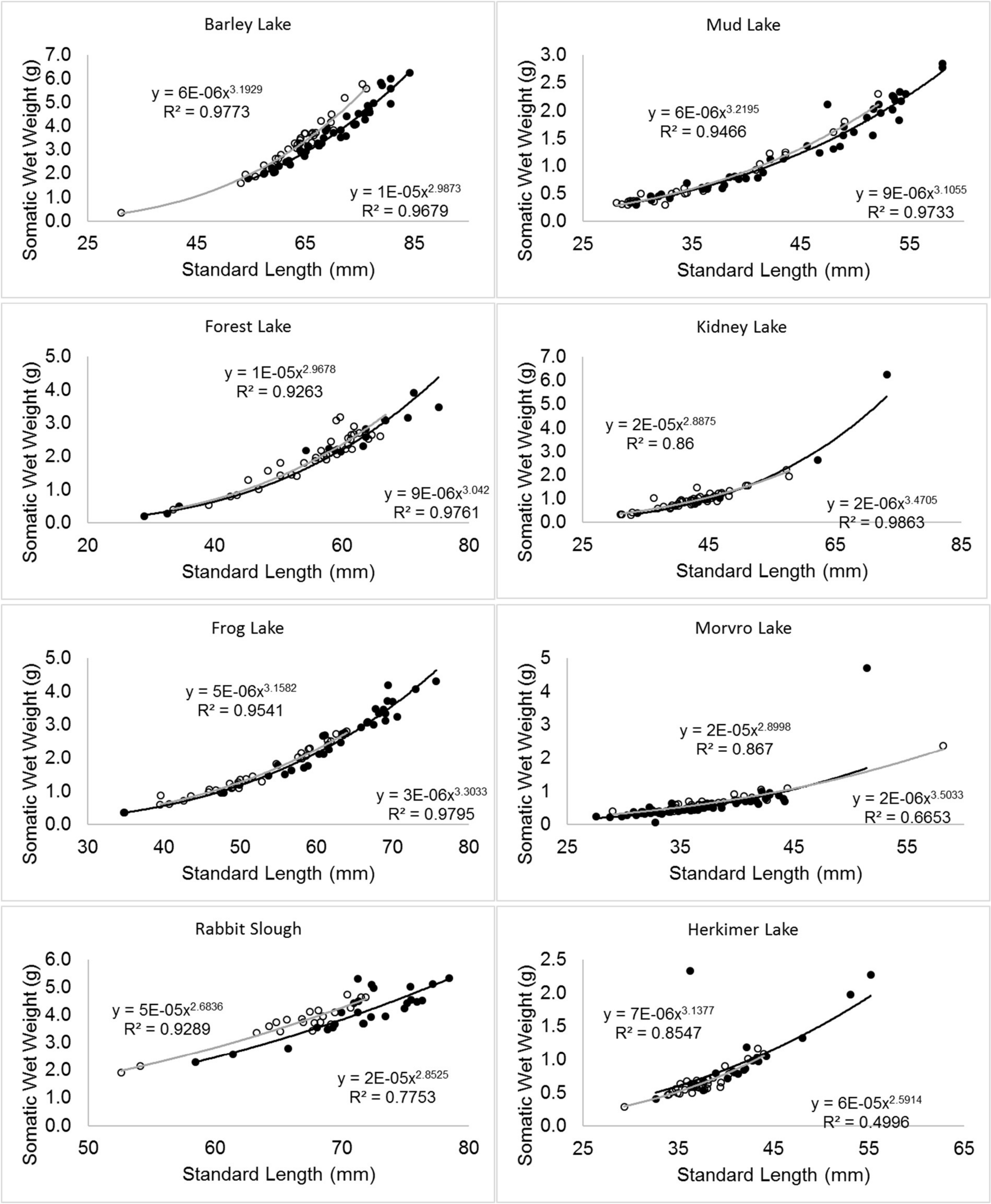


Figure S3.1. Power regression analysis results showing correlation between SWW and SL within populations. Note axis values differ among plots. Closed and open points, and black and gray regression lines, represent females and males, respectively. Regression equations are given in plots above regression lines for males and below regression lines for females.