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Deviation from Panmixia via Assortative Mating and Divergent Habitat Preferences

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Deviation from Panmixia via Assortative Mating and Divergent Habitat Preferences

by

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Dedication

This work is dedicated to my family, George, Meilan, Junjia, Yuzhen, Sue, Francis, Melba and Artie, in memory, to Xingshu.

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Deviation From Panmixia via Assortative Mating and Divergent Habitat Preferences

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Supervisors: Daniel Bolnick and Mark Kirkpatrick

The speciation process is often viewed to start from panmictic populations. Understanding the evolutionary mechanisms that cause populations to deviate from panmixia is essential to understanding the initial stage of population divergence that may lead to speciation. My dissertation focuses on the evolution of two mechanisms that cause deviation from panmixia: assortative mating and divergent habitat preferences. The first chapter is a meta-analysis on published measures of the strength of assortative mating within natural animal populations. Results showed that deviation from panmixia via weak positive assortative mating was typical within natural animal populations, while disassortative mating was rare or absent. Results also suggested that assortative mating did not typically evolve adaptively, but instead as an incidental consequence of other mechanisms, such as spatial segregation. Divergent habitat uses are important drivers of spatial segregation. The second chapter revealed a behavioral mechanism of divergent habitat uses between parapatric lake and stream threespine stickleback populations. The results showed strong divergent rheotaxis between lake and stream fish during their breeding season. The divergence is likely to contribute to the sorting of lake and stream fish into their natal habitats and promote habitat-based assortative mating. The third chapter focused on the neuroanatomical and morphological mechanisms of rheotaxis. Results showed significant correlations between the numbers of neuromasts (functional units of the lateral line) and rheotaxis in both lab-reared and wild-caught threespine stickleback. Results also showed heritable divergence in lateral line structure between parapatric lake and stream stickleback, suggesting that divergent rheotaxis and the resulting divergent habitat uses are likely to have a heritable component. In summary, my dissertation revealed ultimate evolutionary mechanisms of assortative mating and proximate evolutionary mechanisms of divergent habitat uses. These results shed light on the understanding of the beginning of population divergence and ultimately speciation.

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Chapter 1: Assortative Mating In Animals[1]

ABSTRACT

Assortative mating occurs when there is a correlation (positive or negative) between male and female phenotypes or genotypes across mated pairs. To determine the typical strength and direction of assortative mating in animals, we carried out a meta-analysis of published measures of assortative mating for a variety of phenotypic and genotypic traits in a diverse set of animal taxa. We focused on the strength of assortment within populations, excluding reproductively isolated populations and species. We collected 1116 published correlations between mated pairs from 254 species (360 unique species-trait combinations) in five phyla. The mean correlation between mates was 0.28, showing an overall tendency towards positive assortative mating within populations. Although 19% of the correlations were negative, simulations suggest that these could represent type I error and that negative assortative mating may be rare. We also find significant differences in the strength of assortment among major taxonomic groups and among trait categories. We discuss various possible reasons for the evolution of assortative mating and its implications for speciation.

INTRODUCTION

Assortative mating is used to describe a variety of patterns of non-random mating. In the speciation literature, assortative mating is treated as a mechanism of premating reproductive isolation between distinct species or divergent populations (Johannesson et al. 1995; Seehausen et al. 1997; Coyne and Orr 2004). In the behavioral literature, assortative mating has been used to describe a particular form of mate choice in which

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individuals select mates on the basis of phenotypic similarity to themselves (Crespi 1989; Harari et al. 1999; Shine et al. 2001). More generally, assortative mating can be defined as a pattern of non-random mating, without making specific assumptions regarding its behavioral mechanism or evolutionary role (Lewontin et al. 1968; Kondrashov and Shpak 1998). Adopting this general view, assortative mating can be measured as a correlation between the values of a homologous phenotypic or genotypic trait across members of mated pairs (Wright 1921; Lipsey and Wilson 2001; Redden and Allison 2006). Assortative mating may be either positive, implying a tendency to mate with phenotypically similar individuals, or negative (also called disassortative) implying the converse (Partridge 1983; Hooper and Miller 2008). There are many empirical examples of both positive and negative assortative mating (Johnston and Johnson 1989; Follett et al. 2007; Pryke and Griffth 2007; Lu et al. 2009), but it remains unclear what the distribution of the strength of assortative mating is in nature, especially whether there is a systematic tendency towards assortment, random mating, or disassortment.

Assortative mating has several important evolutionary consequences. Positive assortment increases homozygosity within loci, promotes linkage disequilibrium between loci, and consequently inflates the variance of quantitative traits (Lynch and Walsh 1998). The resulting deviations from Hardy-Weinberg equilibrium can cause statistical biases in association mapping studies (Redden and Allison 2006) and estimates of quantitative genetic parameters (Gimelfarb 1986). Assortative mating also plays a key role in speciation, contributing to premating isolation between phenotypically divergent populations (Felsenstein 1981; Kondrashov and Shpak 1998; Coyne and Orr 2004; Bolnick and Kirkpatrick 2012). In models of adaptive speciation, reproductive isolation via positive assortative mating evolves in response to disruptive selection (Kirkpatrick 2000; Dieckmann et al. 2004; Gavrilets 2004; Bank et al. 2011). Assortment is

hypothesized to reduce the production of less fit phenotypically intermediate offspring. Conversely, stabilizing selection favors the evolution of disassortative mating, which reduces the production of less fit phenotypic extremes (Kondrashov and Shpak 1998; Kirkpatrick and Ravigné 2002). Disassortative mating also increases heterozygosity, decreases inbreeding depression (Waser 1993; Pusey and Wolf 1996), and can facilitate the maintenance of sexually antagonistic variation (Arnqvist 2011). Given these multifarious evolutionary effects of assortative mating, it would be valuable to know the distribution of its strength in natural populations, as well as its evolutionary origins.

Two general hypotheses could explain the occurrence of assortative mating. The first asserts that the strength of assortative mating evolves adaptively in response to direct or indirect selection on mating preferences. Selection can act directly on mate choice if fitness depends on the similarity of mated pairs. For example, conjugation in the marine nudibranch Chromodoris zebra is facilitated when the partners are of similar size (Crozier 1917; Crozier 1918). Alternatively, assortative mating can affect the fitness of a pair's offspring, resulting in indirect selection on the parents' mating behavior. For example, Heliconius butterflies that mate assortatively based on mimetic color patterns avoid producing offspring with maladaptive patterns (Chamberlain et al. 2009). More generally, disruptive selection will indirectly favor the evolution of positive assortative mating to avoid producing less fit offspring; conversely, stabilizing selection will favor negative assortment (Dieckmann and Doebeli 1999; Kirkpatrick and Ravigné 2002; Gavrilets 2004; De Cara et al. 2008; Otto et al. 2008). For similar reasons, assortative mating can evolve in response to inbreeding or outbreeding depression (Epinat and Lenormand 2009). Regardless of the type of selection involved, assortment can result from mutual mate choice or by the behavior of only males or females (McNamara and Collins 1990).

Under the second general hypothesis, assortative mating is an incidental consequence of temporal, mechanical, and physiological constraints. In this case, assortment may be neutral, selectively favored, or even deleterious. Several kinds of mechanisms may contribute to these constraints, such as temporal segregation, spatial segregation, intrasexual competition and intersexual conflict (see Discussion for details; Crespi 1989; Arnqvist et al. 1996; Cézilly 2004). Quantifying the direction and strength of assortative mating may shed light on the prevalence of adaptive and incidental assortative mating, a point to which we return in the Discussion.

Knowledge about patterns of assortment in nature could also be useful in developing more realistic models of speciation. Models have shown how assortative mating can lead to sympatric speciation (Udovic 1980; Felsenstein 1981; Doebeli 1996) and influence the outcome of secondary contact (Kondrashov and Shpak 1998; Kirkpatrick 2000; Bolnick and Kirkpatrick 2012). Unfortunately, such models have generally not been parameterized with empirical data, with a few exceptions (Gavrilets and Vose 2007; Gavrilets et al. 2007; Duenez-Guzman et al. 2009; Sadedin et al. 2009). The typical assumption is that populations initially exhibit random mating, but this may not be empirically justified. Meta-analysis can determine the distribution of assortment within populations, which can be treated as a range of biologically realistic initial conditions in future models.

Despite these needs for an overview of the strength and direction of assortative mating, no comprehensive review currently exists. Many studies focus on a single species or clade, and are typically based on a single phenotypic trait (Olson et al. 1986; Arnqvist et al. 1996; Bernstein and Bernstein 2003; Cézilly 2004; Roulin 2004; Wogel et al. 2005). It is therefore unclear what general patterns might exist regarding the strength of assortative mating in animal populations. Outstanding questions include: What is the

distribution of the strength of assortative mating, how frequent is negative versus positive assortative mating, are there differences in the strengths of assortment among taxa and among different kinds of phenotypic traits, and are the data consistent with the hypothesis that assortment evolves adaptively in response to indirect effects of stabilizing and disruptive selection? Here we address these questions using a meta-analysis of the strength of assortative mating across diverse taxa of animals based on a variety of assortment traits.

METHODS

We conducted a mixed-model meta-analysis of assortative mating based on phenotypic traits within natural animal populations. As our measure for the strength of assortative mating, we used the correlation coefficient for the values of a homologous trait in mated pairs. This statistic is appropriate for a meta-analysis because it is a natural measure of effect size that quantifies the magnitude and direction of assortative mating, in a manner comparable across diverse published studies (Lipsey and Wilson 2001). For studies that report other effect size metrics (F statistics, $\chi 2$ statistics, t statistics, or appropriate descriptive data), we converted these into correlation coefficients using standard methods (Hedges et al. 1985; Cook 1994).

Literature search

We searched for publications reporting suitable measures of assortative mating, using keywords searches in multiple databases, including Google Scholar, JSTOR and Web of Science. We also examined the reference sections of relevant publications to find additional studies. To minimize the risk of bias in effect direction, for each keyword search term we also searched for its antonym when possible, for instance searching for both "assortative" and "disassortative". Appendix A gives details of our search methods,

including keywords and criteria for including studies in our database. Online Supplementary Material gives a full list of studies included in our final database.

We excluded studies of assortative mating between incipient species, populations undergoing secondary contact or other forms of hybridization, host races, as well as populations whose conspecific status is ambiguous. Our focus is on the strength of assortment within single populations, rather than reproductive isolation between divergent populations or incipient species. By focusing on within-population assortment, we are documenting the strength of a potentially important population genetic process, and the range of reasonable initial conditions preceding any steps towards speciation. There is an important pragmatic reason to exclude assortment between highly diverged populations: those situations arguably encompass cases of perfect assortment, which then includes all pairs of species on the planet that do not interbreed. In judging whether to exclude case studies from our dataset, we relied on the taxonomic status of taxa described by the publication providing relevant assortment data. In cases where no information was provided, we used a Google Scholar search to check the taxonomic status in recent published descriptions. Plants are not included in our study simply because too few studies reported appropriate effect size statistics. We did not include humans because strong cultural influences and substantial recent admixture make human assortative mating hard to compare with other species (Spuhler 1968; Merikangas 1982; Wolański 1994; Courtiol et al. 2010).

We reviewed more than 13,000 publications. While the search was thorough, it is certain that there are publications that our search did not find. However, an exhaustive search is not necessary for meta-analysis, which is fundamentally a sampling activity intended to retrieve studies that are representative of the question of interest. Thus, a meta-analysis can yield accurate results if it is an unbiased sample from a large and

representative literature. Conversely, exhaustive samples of the relevant publications are not guaranteed to be representative, due to publication or reporting bias (Cooper et al. 2009).

Data collection

For each study, we recorded the scientific name of the focal species, the trait that is subject to assortative mating, the correlation coefficient or other metric of effect size that could be converted into a correlation coefficient, the statistical significance of reported metric, and the sample size (number of mated pairs). We divided the species into eleven commonly represented taxonomic groups (amphibians, annelids, birds, chelicerates, crustaceans, fishes, gastropods, insects, mammals, protists, and reptiles) and into five phyla (annelids, arthropods, chordates, ciliophores, and mollusks). Assortment traits were divided into ten general trait categories (defined in Table A2). We also recorded whether the focal trait is reported as a categorical trait or a continuous trait. Note that a trait may be listed as categorical either because it takes discrete values, or because the researchers divided a continuous trait into discrete categories (e.g. size or age class). The database used for meta-analysis is available on Dryad.

For some combinations of species and trait categories, we found more than one estimate for the correlation of mated pairs, for instance if the correlation was measured in multiple years or within each of multiple populations. To avoid pseudoreplication, we calculated a weighted mean correlation coefficient for each combination of species and trait category, where the weight is the square root of the sample size. We refer to these values as "species-trait means", and denote them as $\bar{r}$. The sample size associated with each species-trait mean, which we denote as N, is the sum of sample sizes of the amalgamated studies for a given species-trait combination (Borenstein et al. 2009). (We found that using the average sample size of amalgamated studies produces very similar

results.) Note that some pseudoreplication remains because traits within a species are phenotypically correlated, and related species can have similar breeding systems because of shared phylogenetic history. Unfortunately, the data are not adequate to eliminate these associations, and we return to this issue in the Discussion. We did not calculate means using Fisher's z-transform because that can lead to positive bias (Hunter and Schmidt 2004; Cooper et al. 2009). The meta-analysis was performed based on the species-trait means, and so each combination of species and trait category therefore appears only once in our analyses. We did not average the assortment strengths for different trait categories within a given species, because the strength of assortment is likely to vary across trait categories.

Meta analysis

To summarize the strengths of assortment across taxa and trait categories, we used the weighted average of the species-trait means, where the weight assigned to each species-trait mean effect is the reciprocal of its sampling variance v (Borenstein et. al 2009; Viechtbauer 2010):

$$v = \frac{\left(1-\bar{r}^2\right)^2}{N-1}.$$

We used restricted maximum-likelihood with a mixed model (Viechtbauer 2010) to test if the average of the species-trait means differs from zero, and to test for significant variation among factors (taxon, trait category). Each species-trait mean is modeled as the sum of a fixed factor that represents the effect of a category (for example, taxon or trait category) and a random effect. We report the statistics QM and QE (sometimes called Qbet and QW respectively). QM indicates the amount of heterogeneity in $\bar{r}$ that is explained by the model (Cooper et al. 2009). A significant QM indicates that the strength of assortment differs significantly between the levels of the factor included in

the model (e.g., taxon or trait type). QE indicates the amount of residual error heterogeneity. A significant QE indicates heterogeneity among observations within groups (i.e. taxa and trait categories) not explained by the model (Cooper et al. 2009). All analyses were performed in R (R Development Core Team 2009) using the package "metafor" (Viechtbauer 2010).

Meta Estimating the underlying distribution of the strength of assortment

The estimates for the correlations between mated pairs in our dataset inevitably include sampling variance and measurement error. These errors will cause the observed distribution of $\bar{r}$ to have different distribution than the actual underlying distribution of correlations in nature. To illustrate this point, imagine a world without negative assortative mating. Due to sampling error, some studies of species with positive assortative mating will estimate negative values for the correlation between mated pairs, and some of these will even be statistically significant (type I error). The frequency of negative assortative mating thus is inflated. Similarly, if negative assortative mating were the general rule, error would instead inflate the observed frequency of positive assortative mating.

It does not seem possible to correct for this effect precisely. That is because there are unknowable sources of measurement error in meta-analyses of diverse studies, and because the data are not independent (as the result of phylogenetic relations between species and phenotypic correlations between different traits in the same species; see Discussion for details). Nevertheless, we used a heuristic approach to estimate the underlying distribution of the strength of assortment while accounting for sampling error.

We began by assuming that the true values of assortment for a given species and trait combination, which we denote by r, are drawn from a beta distribution that is modified to range from a lower bound of b to an upper bound of 1 (rather than from 0 to

1). We fixed the upper bound because as populations progress towards speciation they must inevitably approach very strong assortative mating. We let the lower bound vary because we have no prior notion about what the smallest value of r in nature might be. This distribution is very flexible: for example, it can take a form similar to a normal distribution, an exponential distribution, or even a bimodal U-shaped distribution. The modified beta distribution is characterized by three parameters: its lower bound b, its mean m, and its variance s2. We used simulations to determine what values of these three parameters would yield an observed distribution of species-trait means $\bar{r}$ that most closely matches our data, given the sample sizes and pseudoreplication in our dataset. The result is an estimate for the true underlying distribution of assortment in nature.

An outline of the algorithm we used follows (further details are given in Appendix C). Given values for the three parameters for the modified beta , we randomly sampled 360 values of r to represent the species-trait means. Each value of r was then paired at random with one of the species-trait combinations in our database. For each of these pairings, we determined the number of studies n for that species-trait combination, and the sample size N_i for the i^{th} study in that combination. We then simulated n estimates of the correlation; each estimate was obtained by drawing N_i mated pairs of values from a bivariate normal distribution with the given value of r . These simulated correlations (corresponding to the individual studies in the data base) were then averaged to give a simulated value for a species-trait mean $\bar{r}$ in the same way that we did for the real data. This process is repeated for each of the 360 values of r to give a simulated distribution of observed species trait means, with realistic sampling error. We calculated a measure of how well the simulated distribution matches the observed distribution of $\bar{r}$ (see Appendix C for details). This was repeated five times for each combination the three

parameters for the distribution of r, to identify the combination that gave the best fit to the data.

RESULTS

Our database contains 1116 measurements of the strength of assortative mating from 254 species in five phyla collected from 269 publications (Table 1.1, Online Supplementary Material). Our final data set consists of 360 species-trait means. Of those values, 89% are positive and 11% negative (80% and 19% respectively in the 1116 raw estimates). Birds, insects, crustaceans, and amphibians are better represented than other taxonomic groups (Table 1.1 and Online Supplementary Material). At the level of phylum, arthropods and chordates (46% and 52% of the raw estimates respectively) together represent almost all of the dataset. These studies measured assortment on a wide variety of traits (102 different traits; Table A2). A majority of these fall into three trait categories: size (47% of the raw estimates), structural characters that are not a direct measure of overall body size (30% of the raw estimates), and visual signals that are mostly measures of color, pattern, and sexually selected traits such as crest size (47%, 30%, and 14% of raw estimates respectively). The complete list of categories and specific traits is given in Table 1.1. Note that the measures of individual morphological traits frequently covary with body size. Nearly all (95%) of the traits in our databases are continuous.

Phylum	N	Taxon	N	Trait category	N
Annelida	1 (1)	Annelid	1 (1)	Age	35 (25)
Arthropoda	516 (124)	Crustacean	170 (53)	Behavior	1 (1)
		Chelicerate	10 (3)	Chemical	6 (2)
		Insect	336 (68)	Condition	49 (24)
Chordata	584 (226)	Amphibian	151(44)	Ecotype	5 (4)
		Bird	377 (148)	Genotype	10 (4)
		Fish	45 (27)	Phenology	1 (1)
		Mammal	2 (2)	Size	521 (191)
		Reptile	9 (5)	Structural	322 (76)
Ciliophora	5 (1)	Protist	5 (1)	Visual	156 (32)
Mollusca	10 (8)	Gastropod	10 (8)		

Note: N gives the number of raw values from the original studies and (in parentheses) the number of species-trait means. Detailed definitions of the trait categories are given in Table A2.

Table 1.1: Summary of database by taxon and trait category

Distribution of the strength of assortative mating

The distributions of assortative mating strength based on raw estimates and species-trait means are shown in Figure 1.1. The mean value of $\bar{r}$ is 0.28 with a 95% confidence interval of [0.25, 0.31], based on a random-effect model with no fixed effects and species-trait means as the unit of replication. The mean correlation between mated pairs in the raw dataset is 0.24. The test for heterogeneity is significant ($Q_E = 91275$, d.f. = 359, $P < .0001$), rejecting the hypothesis that all species exhibit a single shared strength of assortative mating. Rather, our random effects model estimates the variance of $\bar{r}$ is 0.0698 (standard deviation = 0.264).

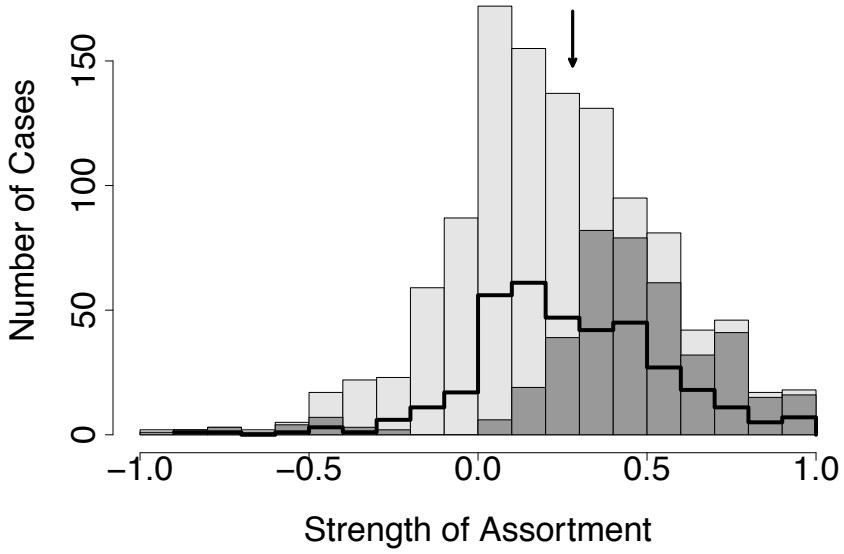

Figure 1.1: Histogram of the strengths of assortment for 1116 published empirical estimates

The dark grey and light grey areas indicate the number of significant and nonsignificant values, respectively, based on the raw correlation coefficients collected from the literature. The heavy black line shows the distribution of the strengths of assortment based on the species-trait means. The black arrow indicates the weighted mean strengths of those values (= 0.28).

Surprisingly, our simulations of sampling error indicate that the best-fit estimate for the underlying distribution of the strength of assortment has no negative assortative mating (Figure 1.2). This distribution of r has a minimum value and a mode at b = 0.02, and a long positive tail. The mean and variance of this distribution (m = 0.27, s2 = 0.047) are close to the values estimated from a random-effects model (mean = 0.28, variance = 0.0698). The moderate difference in the variance estimates may be due to different assumptions about the underlying distribution: the random-effects model assumes a normal distribution, while our simulations assumes the modified beta. Simulated datasets using the best-fit distribution of r are not significantly different from the observed

distribution of $\bar{r}$ (Kolmogorov-Smirnov test, $P \geq 0.40$ for all replicate simulations of the optimal parameter combinations).

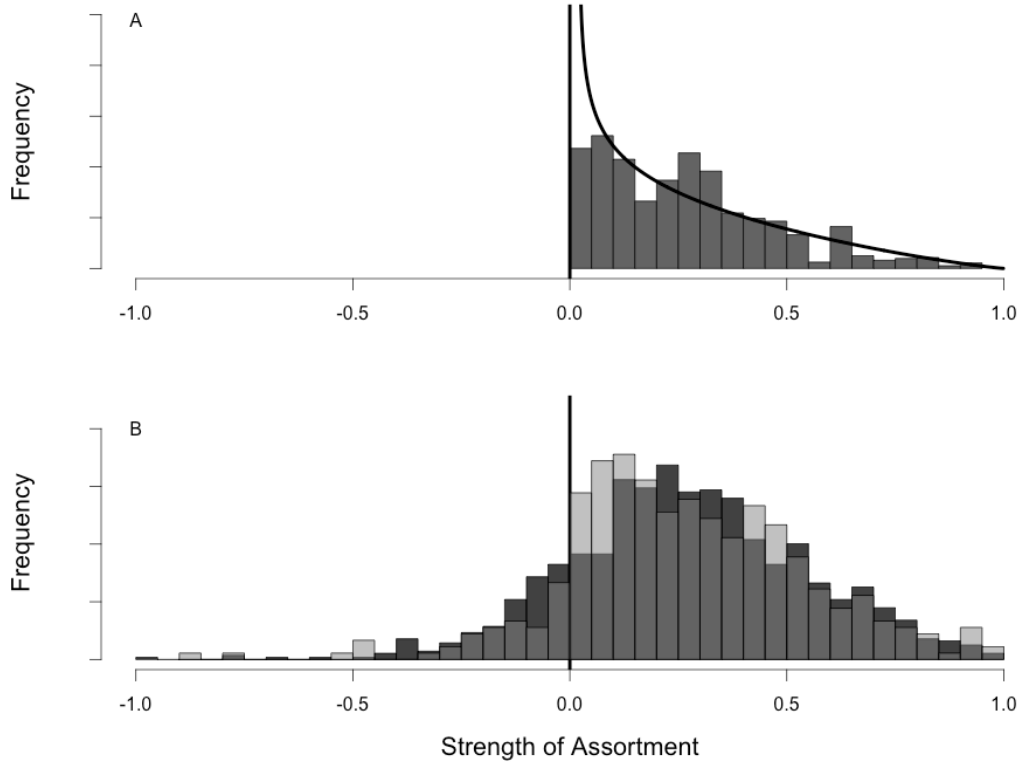

Figure 1.2: Estimate of the underlying distribution for the true strength of assortment for species-trait combinations.

In Panel A, the best-fit distribution for ϱ is shown by the curve, and a realization of 360 samples from this distribution is shown by the histogram. In Panel B, the simulated values for $\bar{r}$ (the species-trait means) that result are shown in dark grey, the observed distribution from our dataset are show in light grey, and overlapping regions in middle-grey. In this realization, the difference between the two distributions is not significant ($P = 0.673$, Kolmogorov-Smirnov test)

An important conclusion is that it is plausible that most or all published cases of negative assortment are a result of type I error, suggesting that negative assortment is absent or rare in the species included in our database. Two other observations highlight the remarkable goodness of fit between our optimal parameter values (no negative assortment) and our data. First, given a fixed mean = 0.28 as estimated in random-effects

model, best-fit parameter combination (b = 0, s2 = 0.05) generates 19.2% negative estimates, a value that is close to the 19% seen in the observed distribution of $\bar{r}$. Second, if type I error is indeed responsible for negative estimates, then studies reporting negative assortment should tend to have a smaller sample size (and so larger sampling error) than those reporting positive assortment. This is true in both the observed and simulated databases. In our database, studies reporting negative assortment averaged a sample size of 40, compared with 107 for studies reporting positive assortment (Wilcoxon rank sum test: $P < 0.0001$). In simulated datasets using the best-fit distribution of r, simulated negative and positive estimates of $\bar{r}$ averaged sample sizes of 49 and 112 respectively ($P < 0.0001$).

We are not, however, able to reject the hypothesis that the underlying distribution r includes negative values. Some distributions of r that include substantial frequencies of negative assortment ($b > -0.3$) yield estimate distributions that are not significantly different from the observed distribution (KS tests $P > 0.05$). However, distributions with negative b do not fit the data as well as our optimum. As discussed in the Appendix, limits to our method prevent us from putting confidence limits on b, and so it is difficult to be make a more quantitative conclusion. The best we can say at present is that there is no strong evidence for negative assortment, but that it may well occur.

Sources of heterogeneity in assortative mating strength

The average value for species-trait means based on categorical traits (= 0.11) is significantly smaller than that based on continuous traits (= 0.29) (Table 1.2). Since categorizing continuous data tends to decrease a correlation (Lipsey and Wilson 2001; Cooper et al. 2009), it is not clear whether there is an intrinsic difference between categorical vs. continuous traits, or whether this effect size difference is an artifact. To prevent this uncertainty from biasing our results, only continuous traits are included in

the following analysis. We also excluded annelids and protists in the following analysis because there is only one example of each.

Data subset or classification	r	CI	QM	QE
Positive assortmenta	0.33	(0.31, 0.36)	626	78047
Negative assortment	-0.18	(-0.26, -0.10)		
Categorical traitsb	0.11	(0.03, 0.21)	360	78650
Continuous traits	0.29	(0.26, 0.32)		
Among-Phyla variationc			401	31386
Among-taxa variationd			539	29367
Among-trait category variatione			395	36642

Note: All values for QM and QE are significant at $P < 0.0001$. CI stands for 95% confidence interval.
a. The Q statistics pertain to the difference between the absolute strength of positive and negative assortment.
b. The Q statistics pertain to the difference between categorical and continuous traits.
c. Excluding annelids and protists (due to small numbers of species-trait means).
d. Excluding annelids, protists, chelicerates, mammals, and reptiles (due to small numbers of species-trait means).
e. Excluding trait categories with insufficient data: behavior, ecotype and phenology. Also excluding annelids and protists.

Table 1.2: Strengths of assortment by different subsets and classifications of the data.

Mixed-model meta-analyses reveal that the strength of assortative mating differs significantly among phyla and lower taxa (Table 1.2). The difference among taxa remains robust after excluding underrepresented taxa (chelicerates, mammals, and reptiles). We arbitrarily consider an under-represented group to be any group with six or fewer species-trait means. Increasing the exclusion threshold to eight, and thus excluding gastropods,

has minimal impact on our results. The mean value of $\bar{r}$ is largest in fish (0.55), crustaceans (0.46), and chelicerates (0.40), and is smaller in amphibians (0.21) and insects (0.21) (Table B1). On average, assortative mating is significantly positive within all taxonomic groups ($P < 0.01$; Figure 1.3) except reptiles and mammals, which have small sample sizes.

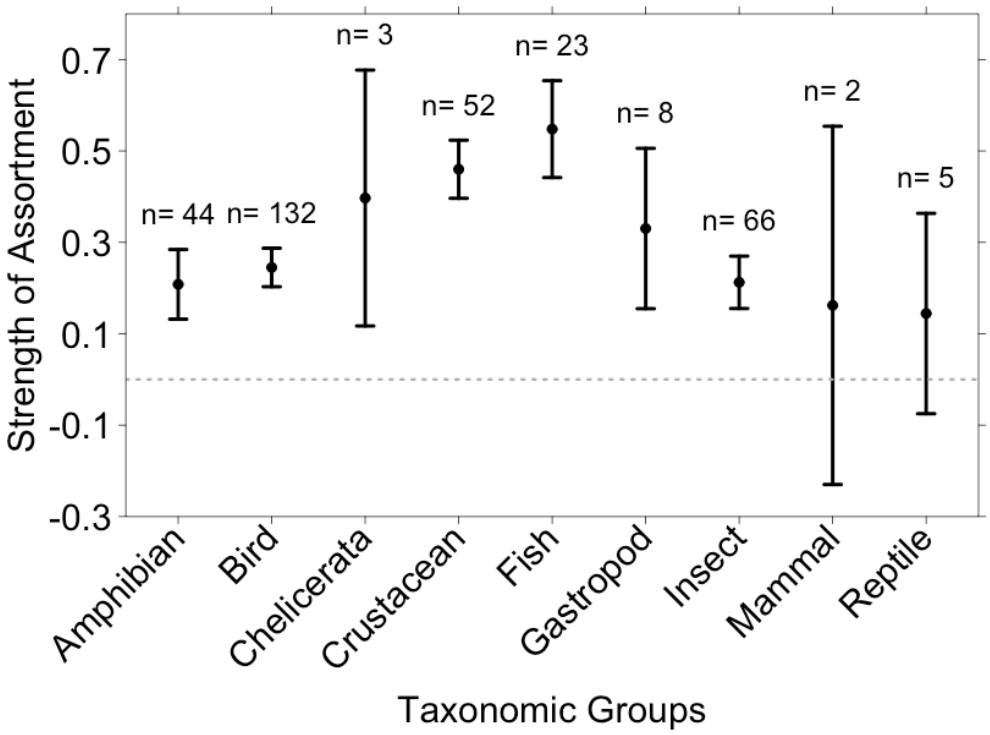

Figure 1.3: Comparison among taxa of the strengths of assortment, $\bar{r}$

Points show weighted means and horizontal bars show 95% confidence intervals (based on species-trait means). Sample sizes are the number of species-trait means.

The strength of assortative mating also differs significantly among trait categories. This result remains robust after excluding underrepresented categories, i.e. behavior, ecotype and phenology (Table 1.2). Assortative mating tends to be strongest on phenology and ecotype (mean $\bar{r}$ = 0.79 and 0.50 respectively). The species-trait means for visual signals, age, and size (mean $\bar{r}$ = 0.34, 0.34, and 0.31 respectively) tend to be larger than those for condition and structural characters (mean $\bar{r}$ = 0.26 and 0.21 respectively) (Table B2). The strength of assortment is significantly positive for all trait categories except behavior, chemical traits, and genotype ($P < 0.05$; Figure 1.4).

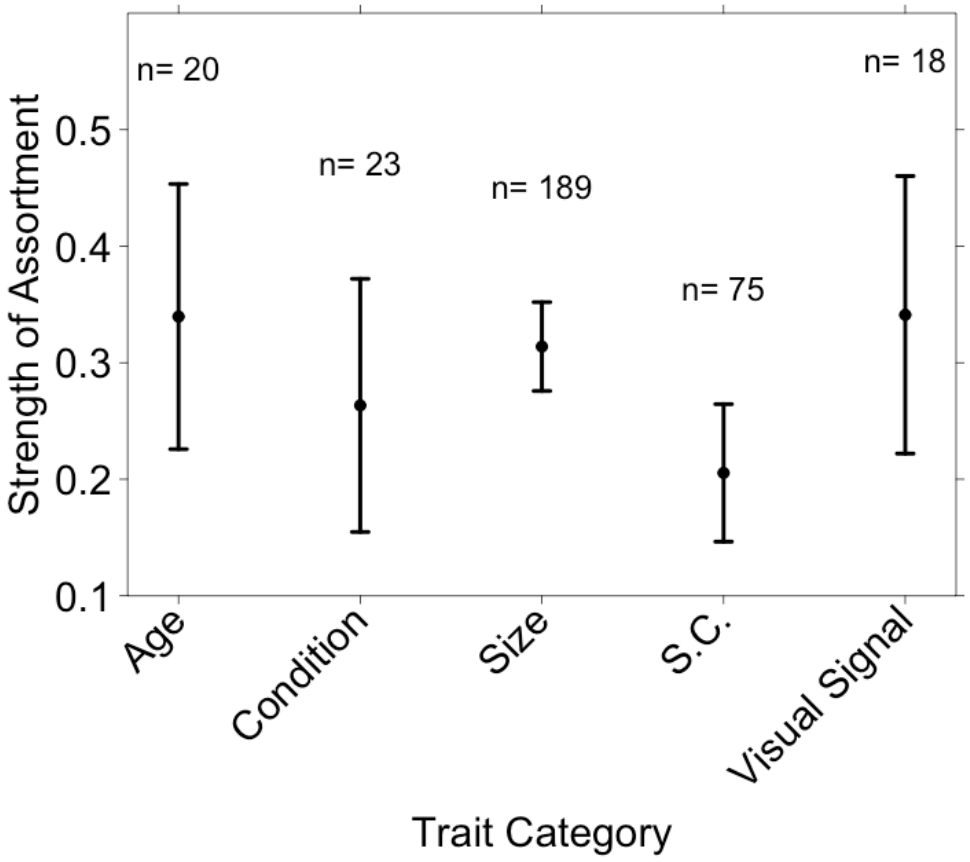

Figure 1.4: Comparison among trait categories of the strengths of assortment, $\bar{r}$. "S.C." refers to structural characters

Points show weighted means and horizontal bars show 95% confidence intervals (based on species-trait means). Sample sizes are the number of species-trait means.

The tests of between-taxon and between-trait heterogeneity were conducted in separate models. It is possible that biologists are more likely to measure assortment based on certain traits in certain taxa (e.g., chemical cues in insects). Such biases could confound the effects of trait category and taxon. Unfortunately, we are unable to separate the effects for these factors using a single multifactorial model, because of the uneven sample size across combinations of taxon and trait category. Only two taxa (birds and insects) contain sufficient (> 6) species-trait means within each of two or more trait

categories. Only two trait categories (size and structural characters) contain sufficient sample sizes for more than one taxon. Focusing on these subsets of our data, we found no evidence that trait and taxon have confounded effects. We continue to observe significant among-taxon heterogeneity within each of two widely measured traits, size and structural characters (Table B3). We also observe heterogeneity among trait categories within each of two taxa (birds, and insects) that have sufficient sample sizes to let us analyze multiple trait categories (Table B4).

Tests of publication bias

We detected no evidence of publication bias, towards either positive or negative assortative mating, in the complete set of 1116 correlation coefficients. The funnel plot is symmetric around mean effect size (linear regression test for asymmetry: $P = 0.16$, corrected for pseudoreplication) and there is no gap in the mouth of the funnel plot (Figure 5), suggesting that there is no appreciable publication bias against small effect or nonsignificant results. Furthermore, the fail-safe numbers calculated from original data and species-trait means are 15,639,977 and 8,171,541 respectively. Thus an implausibly large number of missing or unpublished studies with zero effect would be needed to reduce the mean strength of assortative mating to zero. We conclude that our dataset, while not necessarily an exhaustive compilation of case studies, is likely to yield an unbiased estimate of the typical strength and direction of assortative mating.

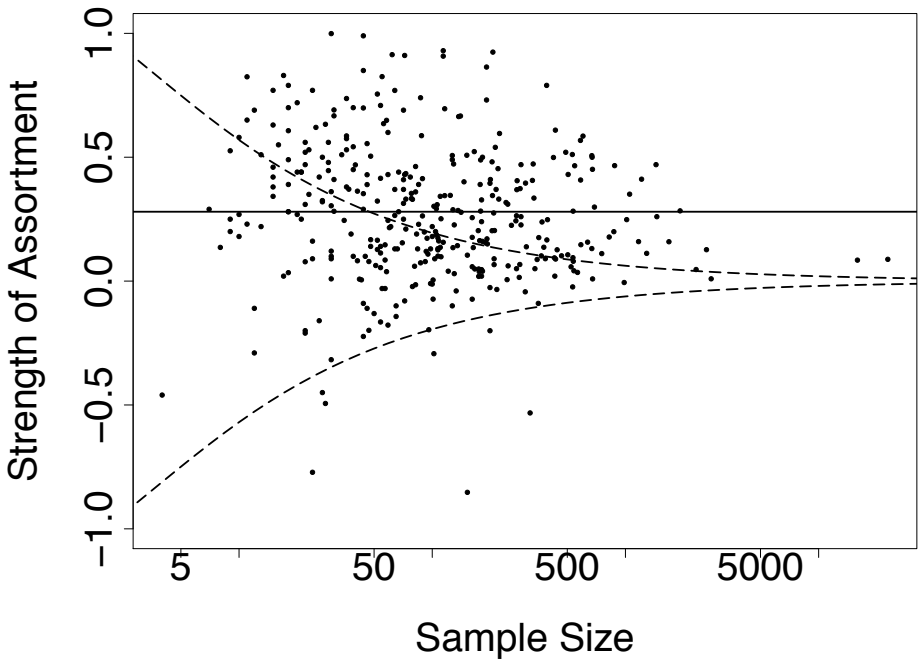

Figure 1.5: Funnel plot of species-trait mean, $\bar{r}$, versus sample size

The horizontal line indicates the grand mean (0.28). The two dashed curves show critical values for the correlation coefficient needed to achieve significance at $P < 0.05$ at a given sample size.

DISCUSSION

Our results provide three core insights. First, weak positive assortative mating is typical in animals: the mean strength of assortment is 0.28. Second, we found that positive assortative mating is observed far more frequently than negative assortative mating. Indeed, the rare cases of negative assortative mating are best explained as type I error arising from small sample size studies of species with random or weakly positive assortative mating. Third, the strength and frequency of assortment differs significantly among combinations of species and traits, among taxonomic groups, and among different

types of traits. These conclusions may have implications for adaptation, population genetic structure, and speciation.

Is negative assortative mating real?

Although assortative mating is predominantly positive, in roughly one tenth of species-trait combinations studies estimated negative assortment. Some reports of negative assortative mating are statistically significant. However, our simulations, which account for the sampling error in the database, suggest that negative assortative mating could be rare or absent. A beta distribution in which the true strength of assortment lies between 0 and 1 produces a distribution of simulated values for $\bar{r}$ that that closely matches our dataset (Figure 1.2). Furthermore, the simulations recapitulate, with remarkable quantitative accuracy, the proportion of negative values of $\bar{r}$ and the tendency for those values to come from studies with small sample sizes. Therefore, we conclude that the minority of case studies reporting negative assortative mating may be spurious, resulting from type I error occurring across many studies.

However, we emphasize that the analysis we employed does not prove that there is no negative assortment. Most importantly, distributions of r that have lower bounds as small as b = -0.3 give reasonable fits to the data, albeit not as good a fit as b = 0. As we discuss in Appendix C, it does not seem possible to obtain confidence intervals to quantify that statement further.

A number of studies have reported negative assortative mating based on the Major Histocompatibility Complex, or MHC (Mays and Hill 2004). These are not included in our meta-analysis because the strength of assortment cannot naturally be expressed as a correlation. It seems difficult to draw strong and general conclusions about assortment based on MHC at this time. While many existing studies give persuasive evidence of nonrandom mating, they often do not distinguish between mating based on genetic

similarity, which is a form of assortment, from that based on heterozygosity, which is not. Among the few studies that do distinguish between these two mechanisms, mixed conclusions were drawn (Bonneaud et al. 2006; Beltran et al. 2008). A further limitation to our current understanding of assortment based on MHC is that most studies are of just two species, mice and humans (Roberts and Petrie 2006).

How does assortative mating evolve?

Theory suggests that the strength of assortative mating can evolve adaptively in response to stabilizing or disruptive selection (Kondrashov and Shpak 1998; Kirkpatrick and Nuismer 2004), though empirical evidence for this claim remains scarce (Rice and Hostert 1993; Coyne and Orr 2004). Stabilizing selection is expected to favor negative assortment, while disruptive selection favors positive assortment. Many evolutionary biologists assume that stabilizing selection is more frequent than disruptive selection (Endler 1986). If that is true and if the strength of assortative mating evolves as an adaptation to indirect selection, we would then expect negative assortment to dominate. This expectation is not supported by our results, which suggest that negative assortment is rare or possibly absent. But is the common intuition about the prevalence of stabilizing selection correct? Kingsolver et al. (2001) reviewed over 2,500 estimates of the strength of phenotypic selection in natural populations, and concluded that disruptive selection and stabilizing selection occur at similar frequency and are of similar strength, though both are fairly weak. That result implies that positive and negative assortment should occur at about the same frequency and strength. Our results convincingly reject this expectation as well.

The results therefore suggest that indirect disruptive or stabilizing selection is not the primary force determining the evolution of assortative mating within populations. While indirect selection seems likely to drive the evolution of assortative mating in some

cases, our results suggest that it is not a general explanation. An adaptationist explanation of assortative mating would instead have to invoke direct selection favoring trait-matched mate pairs. Examples of such direct selection do exist (Dekkers 1994), but are less widely documented.

An alternative possibility is that assortative mating is typically not adaptive, but rather arises as an incidental consequence of other aspects of the mating system. There are at least three proximate mechanisms that could drive the evolution of the strength of assortative mating under the non-adaptive hypothesis (Crespi 1989; Arnqvist et al. 1996; Cézilly 2004). The first mechanism is allochronic isolation (Waser 1993; Helfenstein et al. 2004; Weis 2005; Weis et al. 2005). For example, temporal segregation caused by different arrival dates causes two populations of European blackcaps (Sylvia atricapilla) to mate assortatively at a sympatric breeding site (Bearhop et al. 2005). Two analogous processes can also generate assortment. In monogamous species with indeterminate growth, such as seahorses, if young and small individuals form pair bonds, and they grow larger together, there will tend to be a correlation in body size between mates (Jones et al. 2003). Similarly, a combination of age-specific access to reproduction and strong mate fidelity can generate age-assortative mating in socially monogamous bird species (but see Cézilly and Johnson 1995).

A second mechanism of incidental assortment arises from spatial segregation, when there is covariance between a phenotype and the habitat in which individuals mate, which increases the probability of encountering phenotypically similar candidate mates (Snowberg and Bolnick 2008). This results when individuals have matching habitat preferences (Edelaar et al. 2008), such as in insect host races that mate on their host plants (Drès and Mallet 2002; Malausa et al. 2005), or when there are phenotypic clines (Edelaar et al. 2008). Roulin (2004) pointed out that birds with similar plumage color

tend to occur in the same habitat, and this co-occurrence can cause assortative mating. Similarly, in insects and crustaceans that exhibit spatial variation in body size, assortment can simply arise as a side effect of spatial segregation of individuals with different phenotypes, even when mating within a patch is random (Birkhead and Clarkson 1980; Crespi 1989; Dick and Elwood 1996; Bollache et al. 2000; Bernstein and Bernstein 2003). Assortative mating arising from these causes, while not an adaptation in and of itself, may still be a key factor facilitating ecological speciation.

Third, assortment can arise as a by-product of intrasexual competition and intersexual conflict (Crespi 1989; Cézilly 2004; Henry 2008). For example, when larger females are more fecund, selection favors male preferences for larger partners (Salthe and Duellman 1973; Kuramoto 1978; Bastos and Haddad 1996). If large males are more successful in courting or defending these females, competitively inferior males end up mating with the remaining less favored females, resulting in positive size assortative mating (Arak 1983; Hume et al. 2002; Wogel et al. 2005). In other cases, large females are more able to resist aggressive male courtship attempts, and only the largest males are able to mate them (Arak 1983), resulting in positive assortment. When one sex exhibits mate choice, as when larger females prefer larger males, a positive correlation between mates can result even if smaller females mate randomly, a phenomenon some call "apparent assortative mating" (Arnqvist et al. 1996). Assortment resulting from intrasexual selection is commonly documented in anurans (Arak 1983; Wogel et al. 2005) and crustaceans (McLain and Boromisa 1987; Crespi 1989; Bollache and Cézilly 2004). Various intensities of intersexual competition and/or intersexual conflict among populations of the same species or in one population at different times may incidentally lead to substantial variation in the strength of assortment (McLain 1982; McLain and Boromisa 1987; Bernstein and Bernstein 1999; Harari et al. 1999). For example,

assortative mating is stronger under high population density in milkweed longhorn beetle Tetraopes tetraophthalmus because at high density large males are more likely to interfere with small males' copulation with large females (McLain and Boromisa 1987).

Clearly, assortative mating evolves adaptively in some cases. Examples include positive assortative mating based on heterozygosity in the lesser kestrel (Falco naumanni) and on MHC diversity in house sparrows (Passer domesticus, Bonneaud et al. 2006; Ortego et al. 2009), and disassortative mating in human and mice (Mus musculus) based on MHC alleles (Yamazaki et al. 1976; Wedekind et al. 1995). We do not suggest that disruptive or stabilizing selection never drives the evolution of assortative mating. Rather, our results suggest that this indirect selection for adaptive assortative mating may be the exception rather than the rule.

Assortative mating and speciation

Theory shows that assortative mating could be important to speciation in two contexts. It can cause a single population to split into two, resulting in sympatric speciation (Maynard Smith 1966; Udovic 1980; Felsenstein 1981, Doebeli 1996). Second, assortative mating can contribute to the genetic isolation of two populations that come into secondary contact and so prevent them from merging back into a single population (Kondrashov and Shpak 1998; Bolnick and Kirkpatrick 2012).

How do our results relate to the outcomes for speciation predicted by theory? Unfortunately, it is not easy to make a direct connection. Our data pertain to the strength assortment, but give no direct information about parameters that appear in models regarding mate choice behaviors. For example, models of sympatric speciation use mating preference functions that determine the probability a female accepts a potential mate. The width of the preference function is allowed to evolve in response to indirect selection, which then leads to assortative mating. In contrast, the phenotypic correlation

between mates (which we analyze here) depends both on mate preferences and the phenotype distribution of the population. The phenotypic variance is itself a dynamic variable, and how it evolves depends on the underlying genetics of the trait. Thus there is no simple and general set of predictions that can be made about the outcome of speciation based only on the correlation between mated pairs.

It is, however, possible to make inferences in the reverse direction. Given detailed assumptions about genetics and behavior, we can calculate the correlation that is expected from a particular model, then ask where that result falls in the empirical distribution shown in Figure 1.1. Consequently, speciation models making assumptions about mate choice parameters can in the future test whether their assumptions generate empirically reasonable levels of assortative mating (Bolnick and Kirkpatrick 2012). This may be particularly valuable in choosing starting conditions for theoretical models. There is ongoing debate over exactly what initial conditions are required for a given case of divergence to qualify as sympatric speciation (Fitzpatrick et al. 2008). Many sympatric speciation models assume random mating as a starting point. Our results imply that complete panmixia is not necessarily empirically appropriate initial condition for a speciation model, as many populations exhibit some weak positive assortative mating.

Yet one more factor clouds the relationship between the inter-mate correlation and the potential for sympatric speciation. If a population currently has a weak correlation, we might be tempted to conclude there is little opportunity for sympatric speciation. A population subject to disruptive selection, however, may evolve increased choosiness leading to stronger assortative mating and ultimately speciation (Dieckmann and Doebeli 1999; Gavrilets 2004; Bürger et al. 2006). Consequently, initially weak assortative mating is not necessarily a barrier to future speciation. Furthermore, immediately following sympatric speciation each nascent daughter species exhibits little

within-population phenotypic variance and thus little assortative mating, even though assortment was strong just before the single ancestral population split into two. This returns us to the definitional problem, discussed in the Introduction, of how to delineate populations when estimating the strength of assortment.

Differences between taxa and trait categories

The strength of assortative mating varies among closely related species (Arak 1983; Crespi 1989; Arnqvist et al. 1996; Bernstein and Bernstein 2003). Our analysis reveals heterogeneity at other levels as well: the strength of assortative mating differs significantly among higher taxa and among trait categories. For example, assortative mating is particularly strong in fish (which are well represented among putative cases of sympatric speciation) but weak in birds (which do not appear to undergo sympatric speciation; Coyne and Price 2000). Assortment on phenology is strong, but weak for structural characters.

It is not clear why higher taxa and trait categories should on average exhibit stronger or weaker assortative mating. We speculate that this variation may reflect differences in mean levels of allochrony, microhabitat segregation, sensory modality, sexual selection, or in life history or mating system. The intensities of intrasexual competition and intersexual conflict are known to play a role in explaining the different strength of size-assortative mating among some arthropods and anuran amphibians (Arak 1983; Crespi 1989). We endeavored to test whether assortative mating differed by life history or mating system features, but were unable to find sufficiently clear-cut categorizations for species in our dataset.

A confounding factor in any meta-analysis of assortative mating is nonindependence (or "pseudoreplication") in the data. There are several possible sources. The most obvious comes from multiple studies of the same trait in the same species. We

controlled for this source of nonindependence by analyzing the mean values across studies for species-trait combinations. A second source of pseudoreplication can arise from using separate estimates of assortment for multiple traits in the same species. These estimates will not be independent when the traits are phenotypically correlated. We were unable to correct for this effect because we lack data on correlations between traits tested for assortment. Further, most studies in our database include results for only a single trait. A third source of pseudoreplication comes from phylogenetic relationships. Clearly two sibling species that have recently diverged are likely to share similar patterns of assortative mating for purely historical reasons. The same effect occurs to different degrees at all levels of phylogenetic relationship. In principle, it is possible to correct for phylogenetic dependencies using a phylogeny for all species in the database and a plausible null model for how assortative mating evolves (Adams 2008). Since we lack both of those ingredients, we treated species as independent observations. In any event, we know of no reason why these possible causes of nonindependence in our data might bias our general conclusions.

Future directions

Our results raise many further questions. These include the need to identify the proximate mechanisms that generate assortment, the underlying evolutionary forces that lead to weak positive assortment, its population genetic consequences, and the potential effects of such non-random mating on evolutionary and genetic inferences (e.g., Redden and Allison 2006). A key question is if assortment is adaptive, how often does it result from selection directly favoring trait-matched mate pairs, versus selection act indirectly on the parents' mating behavior in response to the fitness of their offspring? Our results favor direct selection or by-products as explanations for positive assortment, but the mechanisms and frequency of direct selection remain unclear. Alternatively, if assortment

is often incidental and non-adaptive, what ecological and evolutionary conditions can explain variation in the strength and direction of the trait correlations? Uncovering the evolutionary cause of positive assortative mating will require a combination of new theory, laboratory evolution experiments, detailed behavioural studies of mate choice and mating competition (Rowe and Arnqvist 1996) and comparative analyses of the strength of assortment across populations subject to different selective pressures or genetic architectures.

The population genetic consequences of assortment in natural populations are not widely considered. To what extent does positive assortative mating inflate the phenotypic variance of quantitative traits, linkage disequilibrium among loci, and drive deviations from Hardy-Weinberg equilibrium? Can assortative mating within populations be extrapolated to explain levels of reproductive isolation among phenotypically divergent populations or closely related species in sympatry (Bernstein and Bernstein 1999; Bolnick and Kirkpatrick 2012)? Answers to such evolutionary questions can be provided by some existing theory, but merit more extensive empirical investigation as well. The results in any given case will doubtless depend on the heritability and genetic architecture of the traits subject to assortment. The correlation between mates that we study here is mostly phenotypic, and gives minimal direct information about the correlation between the underlying genotypes. Thus, an important early step in future research on this topic is to distinguish between phenotypic and genotypic assortment. If indeed there is a substantial genetic component to this assortative mating, then random mating is not a default feature of animal populations, at least with respect to genes linked to traits subject to assortative mating. Moreover, there is an increasing amount of literature on genotypic assortment that was not included in our database due to the lack of suitable statistical metrics. Future studies on genotypic assortment are highly recommended to provide

suitable metrics to facilitate the comparison between genotypic and phenotypic assortment.

In conclusion, we have shown that natural populations vary dramatically in the strength of assortative mating. Positive assortative mating appears to be dominant (and perhaps even exclusive), although the strength of this assortment varies between taxa and among traits for unclear reasons. We believe that these results can be valuable in designing more empirically informed models of adaptive speciation, and to explain standing levels of phenotypic and genetic variation in natural populations.

Chapter 2: Divergent Rheotaxis Contributes to Divergent Habitat Uses Between Parapatric Lake and Stream Threespine Stickleback

ABSTRACT

Adaptive divergence among populations is a major driver of evolutionary change. Adaptive divergence is often explained as a balance between the diversifying effect of divergent selection and the homogenizing effect of migration. However, adaptive divergence can also be explained by divergent habitat uses. Divergent habitat uses can reduce the actual rate of migration among contrasting habitats, promoting adaptive divergence. Increased adaptive divergence can in turn promote stronger divergent habitat uses. For example, divergent habitat uses documented between parapatric lake and stream stickleback can bring about a several-fold increase in the extent of adaptive divergence between the two populations. Here, we evaluate a behavioral mechanism that might underlie the divergent habitat uses. We found that inlet stream stickleback exhibited significantly more positive rheotaxis than lake fish did during the breeding season. Inlet stream fish were better at holding their positions in currents, spent a larger percentage of time facing towards currents and spent more time in low-current boundary areas. As a result, we infer that lake fish expended significantly more energy in flowing water. Divergent rheotaxis likely explains the divergent habitat uses between the two populations and promotes parapatric diversification between lake and stream stickleback. We did not find divergent rheotaxis between lab-reared common garden inlet lake and stream stickleback that were lab-reared in a common garden experiment and never exposed to currents. Therefore, rheotaxis may not be heritable.

INTRODUCTION

Dispersal is one of the most fundamental components of ecology and evolutionary biology (Clobert 2001; Coyne and Orr 2004; Ronce 2007; Scheiner and Willig 2011; Clobert et al. 2012). There is increasing evidence that dispersal is often non-random with respect to individuals' phenotype or genotype (Edelaar et al. 2008; Edelaar and Bolnick 2012). Habitat preference can lead to biased movement of individuals within a heterogeneous environment (Jaenike and Holt 1991; Armsworth and Roughgarden 2005; Odling-Smee et al. 2013). Habitat use determines the regime of natural selection that individuals experience, thus can have profound evolutionary consequences (Thorpe 1945; Jones and Probert 1980; Rice and Salt 1988). When habitat use differ between or within populations (divergent habitat uses), it can facilitate the maintenance of polymorphism (Jones and Probert 1980; Garcia-Dorado 1986; De Meeus et al. 1993; Ravigné et al. 2004) and impact the degree and rate of local adaptation (Holt and Barfield 2008; Ravigne et al. 2009; Bolnick and Otto 2013). Divergent habitat uses also reduce the actual rate of dispersal among contrasting habitats compared to what would be expected based on individual's dispersal capacity. Thus, divergent habitat uses can promote habitat-based assortative mating both in sympatry and in parapatry, if mating takes place preferentially within habitats (Smith 1966; Rice 1987; Beltman and Metz 2005; Taborsky et al. 2014).

Most empirical studies on divergent habitat uses come from host preference studies in phytophagous insects (reviewed by Thompson and Pellmyr 1991, Gripenberg et al. 2010 and Dres and Mallet 2002; examples see Singer and Thomas 1996 and Via 1999). The proximate behavioral mechanisms of host uses in phytophagous insects can be quite different from habitat uses in vertebrates (Jiggins et al. 2005). Thus, knowledge

of the proximate behavioral mechanisms in vertebrates is needed to understand the evolution of divergent habitat uses beyond phytophagous insect systems (Nosil 2012).

Parapatric lake and stream populations of threespine stickleback (*Gasterosteus aculeatus*) are an excellent system to study the behavioral mechanisms of divergent habitat uses. Multiple pairs of lake/stream stickleback populations have independently evolved morphological, ecological and genetic divergence (Hendry and Taylor 2004; Moore et al. 2007; Berner et al. 2008; 2009). Fish in inlet streams (where water flows into a lake) tend to show far more abrupt morphological clines from lake to stream phenotypes compared to fish in outlet streams (water flowing from the lake) (Hendry and Taylor 2004; Moore et al. 2007; Berner et al. 2008; 2009). This divergence is driven by divergent natural selection in the lake and stream environment and constrained by the homogenizing effect of gene flow, which is mainly from the relatively large lake population into the outlet stream (Hendry et al. 2002; Hendry and Taylor 2004; Moore and Hendry 2005; Garant et al. 2007). However, phenotypic and genetic divergence between inlet stream and lake stickleback is often too abrupt (over a few meters) to be plausibly explained by migration-selection balance alone, but are instead best explained by divergent habitat uses (Bolnick et al. 2009).

A recent transplant experiment demonstrated strong divergent habitat uses between parapatric lake and stream stickleback from Blackwater Lake and its inlet stream on Vancouver Island, B.C., Canada (Bolnick et al. 2009). Lake and stream fish were caught, marked and released at the intersection of the lake and stream where they experienced equal opportunities to disperse into lake and stream habitats. At recapture four days later, a majority (90%) of displaced fish had returned to their original habitat. Notably, those individuals that switched habitats were morphologically predisposed to do so, as they more closely resembled residents of their non-native habitat. A simple model

showed that such divergent habitat uses could increase phenotypic and genetic divergence several fold relative to the expectations with random movement (Bolnick et al. 2009; Bolnick and Otto 2013). The behavioral mechanism of the divergent habitat uses was unknown (Bolnick et al. 2009).

The presence of currents in stream environments is one of the major characteristics that distinguish streams from lake environments. Thus rheotaxis can be a promising candidate mechanism to explain the divergent habitat uses documented in lake and stream stickleback. Rheotaxis is the behavioral orientation to water currents (Lyon 1904; Arnold 1974; Montgomery et al. 1997). Individuals with positive rheotaxis orient or move upstream, while those with negative rheotaxis orient or move downstream (Montgomery et al. 1997; Pavlov et al. 2010).

Rheotaxis is known to be important in spawning fish, guiding fry from separate incubation areas to a mixed rearing area (Hartman et al. 1962; Raleigh 1967; Brannon and Commission 1972; Kaya 1989; Kaya and Jeanes 1995; Hensleigh and Hendry 1998; Caiger et al. 2012). For example in sockeye salmon (*Oncorhynchus nerka*), juveniles usually live in lakes, but adults may spawn in inlet streams, outlet streams or within lakes, depending on the population (Hartman et al. 1962; Hensleigh and Hendry 1998; Lohmann et al. 2008). Newly emerged fry in the stream must migrate back to the lake, and this migration is known to be partly guided by rheotaxis (Hensleigh and Hendry 1998). Fry from inlet stream populations typically swim downstream while their counterparts from outlet stream populations swim upstream, both towards the direction of their rearing lake (Hartman et al. 1962; Raleigh 1967). These rheotactic responses are known to be genetically based (Raleigh 1967; Brannon and Commission 1972). Similar differences in rheotaxis were found between inlet and outlet spawning Arctic grayling fry (*Thymallus arcticus*) (Kaya 1989; Kaya and Jeanes 1995).

The primary aim of this study is to test if rheotaxis could contribute to the habitat uses of lake and stream stickleback populations. We tested rheotaxis of wild-caught lake and stream stickleback during their breeding season. Having found divergent rheotaxis, we then conducted the same behavioral assays on lab-reared common-garden stickleback to test whether divergent rheotaxis is heritable. To test whether wild-caught lake and stream stickleback exhibit corresponding differences in movement in the field, we examining the dispersal of the two ecotypes in Blackwater inlet stream and evaluated the rheotaxis of recaptured individuals in laboratory.

METHODS

Study Site

Blackwater Lake is a medium-sized (37.2 hectare) long and narrow mesotrophic lake on Northern Vancouver Island, B.C., Canada. Water drains from the upstream Amor Lake, through a 1.2km-long stream (inlet stream), into the southern end of Blackwater Lake. The outlet stream drains the northern end of Blackwater Lake, for 1.2 km into Farewell Lake. The inlet stream has in general more rapid flow rates almost throughout all its length (0.135 to 0.513 m/s) compared to the outlet stream (mostly <0.1m/s).

We sampled threespine stickleback from the following four sites using unbaited minnow traps: 1) the inlet stream of Blackwater Lake (UTM: between 10N 556079mN, 314904mE and 5560088mN, 314910mE); 2) the south end of Blackwater Lake near the inlet (‘inlet lake fish’ UTM: 5560392mN, 315048mE); 3) Blackwater Lake near the outlet (‘outlet lake fish’ UTM: 5562436mN, 315323mE); 4) the outlet stream of Blackwater Lake ('UTM: 5562436mN, 315323mE). All fish were collected with permission from the British Columbia Ministry of Forests, Lands and Natural Resources

Operations (NA11-7031 and NA13-85103). All the collection, transportation and experimental procedures were approved by the University of Texas Institutional Animal Care and Use Committee (#AUP-2010-00059 and #AUP-2013-00027).

Circular Flow Tank Design

We used a circular flow tank to quantify individuals' rheotaxis [Figure 2.1]. This tank was designed to allow each test individual to swim freely upstream or downstream indefinitely. The flow tank was made of white smooth FRP plastic sheeting (outside diameter: 80cm; inside diameter: 50cm; tank height: 25cm, water depth: 16cm), equipped with two aquarium pumps that generated uni-directional circular flow (clockwise or counter-clockwise, alternated across different test individuals) with minimal turbulence. The flow rate was set to be within the natural range of Blackwater inlet stream. Flow was the highest at the outermost part of the tank (0.24 m/s), and steadily decreased to 0.10 m/s at the innermost part of the tank. We estimated the flow rate at the innermost part of the tank by recording the speed of food coloring diffusing in water, because the flow rate was below the minimal accuracy of our water velocity instrument. We measured the flow rates of the other three parts via a flow probe (FP111, Global Water, College Station, U.S.). Flow rates of each part of the tank were measured at four equidistant spots within the part and then averaged.

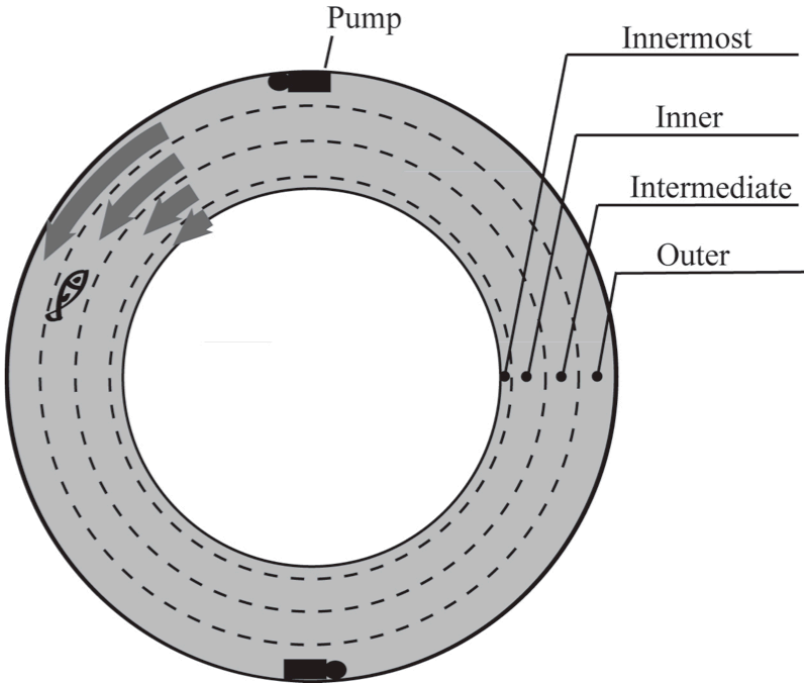

Figure 2.1: An overhead schematic of the circular flow tank illustrated with counter-clockwise currents

Shaded area indicated the test area, which was divided into four parts during the video quantification, as indicated by dashed concentric circles and labeled respectively. The innermost part was defined as a two-centimeter-wide ring against the inner wall of the flow tank where flow rate was minimal (0.01m/s). The rest of the test area was equally divided into three concentric rings with equal widths (9.3 cm), including the low-flow-rate inner part (0.12 m/s), medium-flow-rate intermediate part (0.16 m/s) and the high-flow-rate outer part (0.20 m/s).

Each individual was tested separately in the flow tank to measure rheotaxis without schooling effects that might arise in groups. Each test individual was first given fifteen minutes to acclimate in the tank in still water, then videotaped by an overhead webcam, in two consecutive five-minute trials with still water or current. The test order of lake- or stream-origin fish was randomized. The order of still-water and current trials was randomized for each test individual, as was the direction of flow (clockwise /counter-clockwise).

Prior to video analysis, one researcher named all the videos after a random number, then a second researcher (blind to fish identity) tracked all fish movements using the same computer and same zoom level (150%). This blind scoring design avoided subjective biases during the tracking process. Frames were extracted from each trial video at a rate of 3.4 frames/second, and in each frame the test individual's anterior end and posterior end (caudal peduncle) were manually tracked using ImageJ analysis software (http://rsb.info.nih.gov/ij/) with MtrackJ plugin.

In each frame, the x and y coordinates of the focal fish's anterior end and posterior end were determined and then averaged to obtain the mid-point of the individual. We quantified four measures of rheotactic behavior:

Net displacement: the difference between the individuals' ending versus starting locations, accounting for any full circuits of the tank upstream or downstream. The net displacement value is positive when the ending location is upstream to the starting location, indicating positive rheotaxis. Larger values of net displacement indicate more positive rheotaxis.

Cumulative upstream movement: total upstream path length that each test individual swam during each five-minute trial. This differs from net displacement because the cumulative movement includes the length of multiple upstream swimming bursts that, because of intervening downstream movement, could result in little or no net displacement. Energy expenditure increases with cumulative movement, so fish with high cumulative movement are exerting substantial swimming effort. If this high effort results in little net displacement, fish exhibit poor energetic efficiency in the current.

Upstream orientation: the proportion of time each test individual faced upstream into currents (±45º relative to the tangent of circular flow at the midpoint of the fish). A higher proportion of upstream orientation is indicative of more positive rheotaxis. In still

water, upstream orientation was calculated relative to the same tangents as in the current trial for that individual. A randomly oriented fish is expected to face upstream and downstream (±45° relative to opposite direction of the tangent of circular flow at the midpoint of the fish) for equal proportions of time.

Flow regime: In each frame video frame, the test individual was scored as being in the innermost, inner, middle, and outermost part of the tank channel (scored as 0, 1, 2, 3), corresponding to increasing flow rates (Fig. 1). These scores were averaged across all frames to obtain a single mean flow regime score per individual. Higher flow scores indicate use of higher velocity locations in the tank; lower scores reflect a preference for boundary areas where flow is slower. Because of the relative of surface areas of each of the four regions, a fish distributed randomly across flow regimes is expected to have a flow score of 1.98.

Experiment I: Rheotaxis of wild-caught stickleback

We evaluated the rheotaxis of wild-caught stickleback from lake and stream sites, at both the inlet and outlet of Blackwater Lake. Our field study was during the peak breeding season (June), as was that of Bolnick et al. (2009). Using unbaited minnow traps set overnight, we captured 18 inlet lake fish, 18 inlet stream fish, 12 outlet lake fish and 14 outlet stream fish. All individuals were temporarily housed in large coolers (66 liters) in a shaded area, with aeration and regular water changes, and tested between 2-10 hours after capture, using the flow tank experiment described above.

We tested for significant pairwise differences among all four groups of stickleback, for each of the four measures described above, both in still water and in currents. We used Wilcoxon rank-sum tests to compare pairwise differences among four groups of fish in currents, and pairwise differences among four groups of fish in still water. We used Wilcoxon signed-rank tests to compare the swimming behavior of each

group of test individuals in currents versus still water. We also tested the upstream orientation and flow regime of each group of test individuals against the null hypotheses of random orientation and random distribution across flow regimes using Wilcoxon signed-rank tests and one-sample Wilcoxon signed-rank tests respectively.

We applied a heuristic method to approximate the energetic expenditure of inlet lake and stream fish in currents. Boisclair and Tang 1993 measured the energetic costs of swimming and fish weight, swimming speed under different swimming patterns (Boisclair and Tang 1993). Our experiment mostly resembled their "forced swimming" pattern where fish were forced to swim against a unidirectional current of constant velocity at any given time. We calculated the upstream swimming speed against currents (relative to the average current speed of the zone where the individuals were at in that moment) at each tracked frame using cumulative upstream movement. We then used the upstream swimming speed at each tracked frame and the body weight of the test individual to calculate the transient energetic expenditure of the focal individual at each tracked frame following Boisclair and Tang 1993. Note that there are no significant correlations between body weight and cumulative upstream movement in either inlet lake or inlet stream stickleback [details see Chapter 3]. Then we calculated the average energetic expenditure of each test individual during the five-minute flow water trial.

Experiment II: Rheotaxis of common-garden stickleback

To test whether divergent rheotaxis of lake and stream stickleback is heritable, we reared offspring of inlet lake stickleback, and offspring of inlet stream stickleback, in laboratory aquaria. Fish were reared from eggs to adulthood (~1 year old). We then evaluated individuals' rheotaxis using the circular flow tank assay described above.

We performed in vitro crosses between 14 pairs of wild-caught Blackwater inlet stream stickleback and between 34 pairs of inlet lake stickleback in early June 2010 (11

and 30 clutches, respectively, developed to maturity). Fertilized eggs were shipped back to the University of Texas at Austin within six days after fertilization. Families were kept in separate aquarium tanks and reared in standardized conditions (more details on transportation and rearing see Appendix D). Due to aquarium space limitations, as the fish grew we pooled families to generate one outbred population of lake fish (2 fish from each of 11 surviving families) and one population of stream fish (2 fish from each of 30 surviving families). In November 2011, we sampled adult stickleback from each pooled population (N=15 each) for rheotaxis assays, as described above. All test individuals were naïve (never exposed to currents) prior to the behavioral assay.

We tested for significant heritable differences between lab-reared inlet lake and stream fish, for each of the four behavior measures described above, both in still water and in currents. We used Wilcoxon rank-sum tests to compare lake versus stream fish in currents, and lake versus stream fish in still water. We used Wilcoxon signed-rank tests to compare the swimming behavior of fish in currents versus still water. We also tested the upstream orientation and flow regime of lake and stream fish against the null hypothesis of random orientation and random distribution across flow regimes using Wilcoxon signed-rank tests and one-sample Wilcoxon signed-rank tests respectively.

Experiment III: Rheotaxis and dispersal in a semi-natural setting

This experiment was designed to test whether wild-caught lake and stream stickleback exhibit differences in movement in a semi-natural setting in Blackwater inlet stream. We transferred wild-caught inlet lake and stream stickleback to an enclosed mesh dispersal tunnel [Figure 2.2] at the lake-inlet stream intersection, then recorded their choices as going up against or going down with currents. We subsequently measured rheotaxis of some of these same fish using the circular flow tank.

The enclosed tunnel was composed of three chambers connected to each other, submerged in the inlet stream ten meters above the lake stream intersection and parallel to the direction of current. Fish were placed into a release chamber (3m long and 1m in diameter), whose upstream and downstream ends formed uni-directional funnels into collection chambers (1.25m long and 1m in diameter each) [Figure 2.2]. This dispersal tunnel allowed us to count how many fish chose to disperse up- versus downstream once they were released.

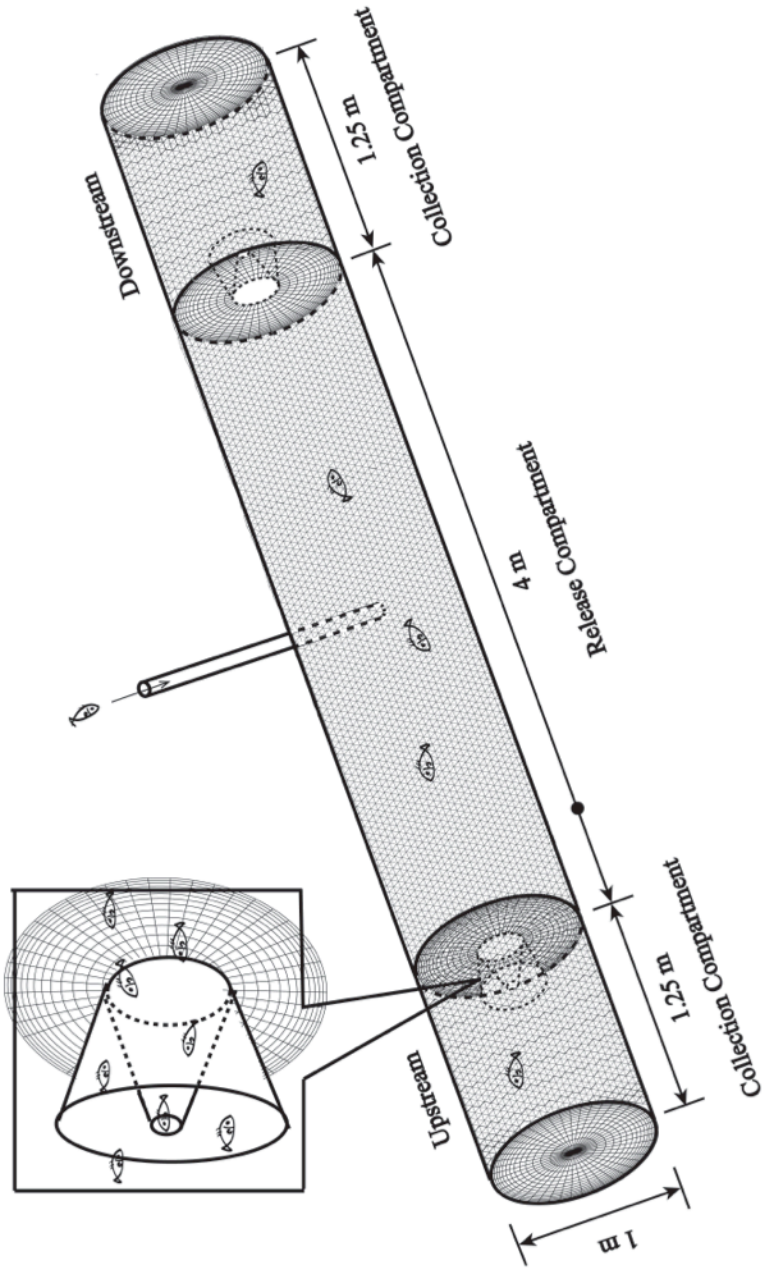

Figure 2.2: An isometric schematic of the dispersal tunnel

We captured 191 inlet stream stickleback at the inlet stream sample site and 206 inlet lake stickleback at the inlet lake sample site using unbaited minnow traps on April 17 2013. This was before the breeding season began. We marked stream individuals and

lake individuals by clipping the first and second dorsal spine respectively All stickleback were released individually with random sequence of lake and stream fish, to avoid confounding effects of schooling behavior [Figure 2.2]. Twenty hours after all fish were released, we retrieved the tunnel and recorded the number of lake and stream individuals in the upstream, downstream, and central chambers (positive, negative, and no rheotaxis, respectively). We used Fisher's exact tests to test for differences between inlet lake and stream stickleback with respect to the number of individuals in each group that exhibited up-/downstream movements.

Experiment IV: Rheotaxis of wild-caught non-breeding stickleback

All individuals from the tunnel experiment were brought to the Fred Hutchinson Cancer Research Center in Seattle for further rheotactic behavioral assays (see Appendix D for transportation and animal care details). These tests were intended to replicate Experiment I, albeit using fish collected prior to the breeding season, which began in June. Fish were transported rather than measured on Vancouver Island, because we also measured lateral line morphology on these individuals (which required a fluorescent dissecting scope that was not available in the field), the results of which are reported in a separate paper. Using the circular flow tank described above, we tested randomly selected inlet stream and inlet lake stickleback for divergent rheotaxis, with equal sample sizes of 22 in both groups. We omitted the still water trial, because prior assays had found divergent rheotaxis and swimming behavior only in current. We used Wilcoxon rank-sum tests to check for differences between lake and stream fish in each of the four measures of rheotactic behavior. We also tested the upstream orientation and flow regime of lake fish and stream fish against the null hypotheses of random orientation and random distribution across flow regimes. We used Wilcoxon signed-rank tests and one-sample Wilcoxon signed-rank tests, respectively.

All analyses were done using the R statistical language (Venables and Ripley 2002; R Core Team 2013; Legendre et al. n.d.).

RESULTS

Experiment I: Rheotaxis of wild-caught stickleback

In currents, pairwise comparisons showed that wild-caught inlet stream fish and inlet lake fish differed in all four measures of rheotaxis [Figures 3-6, Table 2.1]. First, stream fish exhibited more positive rheotaxis than lake fish. Neither ecotype exhibited strongly positive rheotaxis, but stream fish were displaced less far downstream (-6 meters) than their lake counterparts (-18 meters) [Figure 2.3, Table 2.1]. Second, despite maintaining their position better, stream fish actually swam significantly shorter cumulative upstream movements compared to their lake counterparts (4.6 meters versus 7.4 meters on average) [Figure 2.4, Table 2.1]. Lake fish repeatedly swam downstream (or were displaced downstream) and then swam up against currents to compensate. Third, stream fish faced into the current more often (84% of the time) than lake fish (65%) [Figure 2.5, Table 2.1]. Both ecotypes spent significantly more time facing upstream, compared with null expectations of random orientation [Figure 2.5, Table 2.2]. Fourth, stream fish spent significantly more time in the slower-current part of the tank, compared to their lake counterparts [Figure 2.6, Table 2.1]. Both ecotypes disproportionately stayed within lower-flow inner parts of the tank compared with null expectations predicted [Figure 2.6, Table 2.2]. No other pairwise behavioral differences in currents were found among inlet and outlet lake and stream stickleback [Table 2.1], except that inlet stream fish also showed significantly shorter cumulative upstream movement than outlet lake fish [Figure 2.4, Table 2.1].

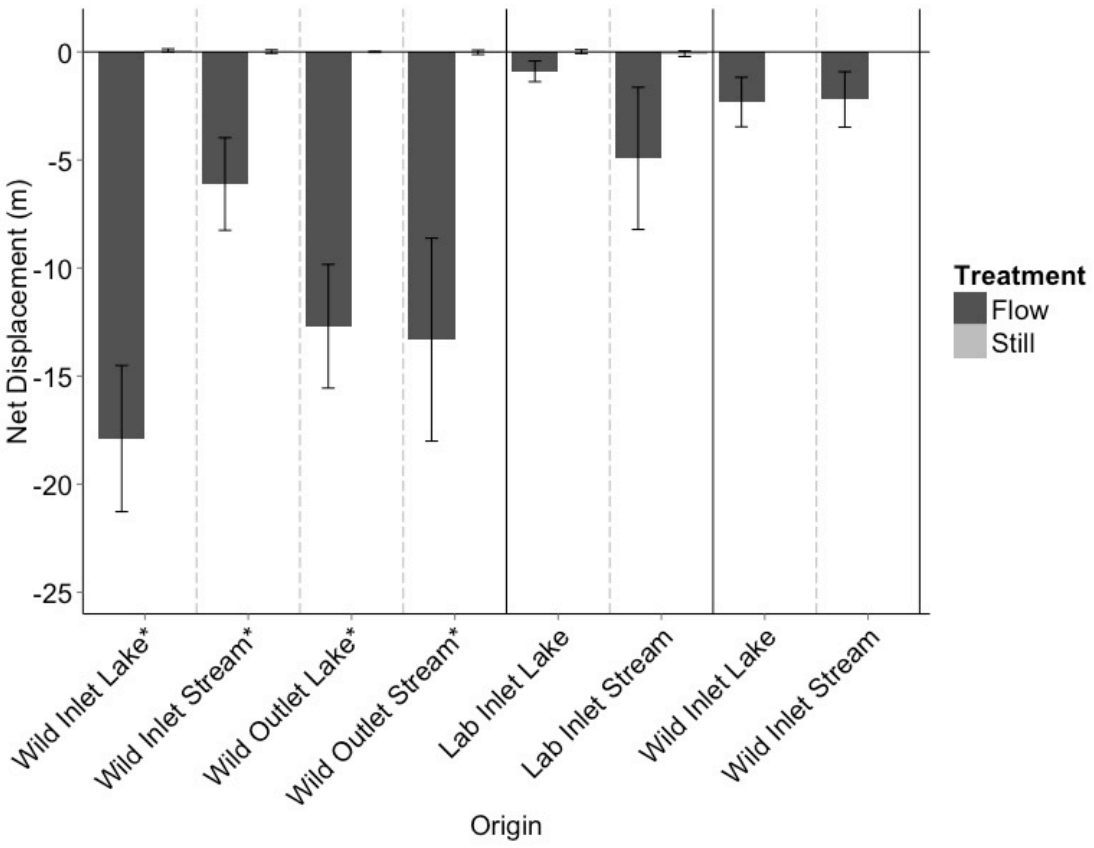

Figure 2.3: A side-by-side comparison of the net displacement (in meter) of all study populations in current trials and still-water trials

The height of each bar indicates the group mean and the error bars are the standard errors. Note that since the Experiment IV did not contain still-water trials, light grey bar is not applicable in panel C and the spaces are only for place-holding. At the end of the five-minute current trials, given all groups of individuals exhibited negative values in net displacement, more positive rheotaxis is visualized as better position holding with less net downstream displacement (shorter dark grey bars). Populations in breeding status are indicated by asterisks.

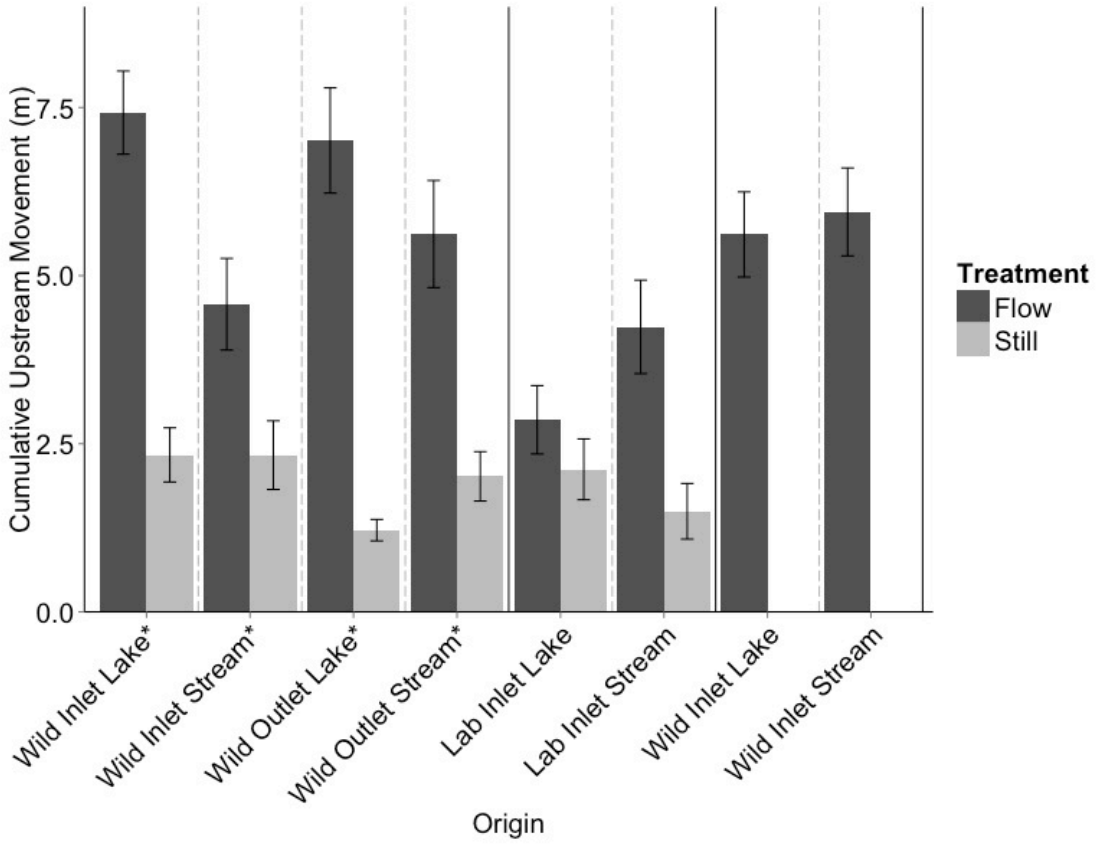

Figure 2.4: A side-by-side comparison of the cumulative upstream movement (in meter) of all study populations in current trials and still-water trials

The height of each bar indicates the group mean and the error bars are the standard errors. Again still-water trials (light grey bars) are not applicable for Experiment IV (panel C). Populations in breeding status are indicated by asterisks.

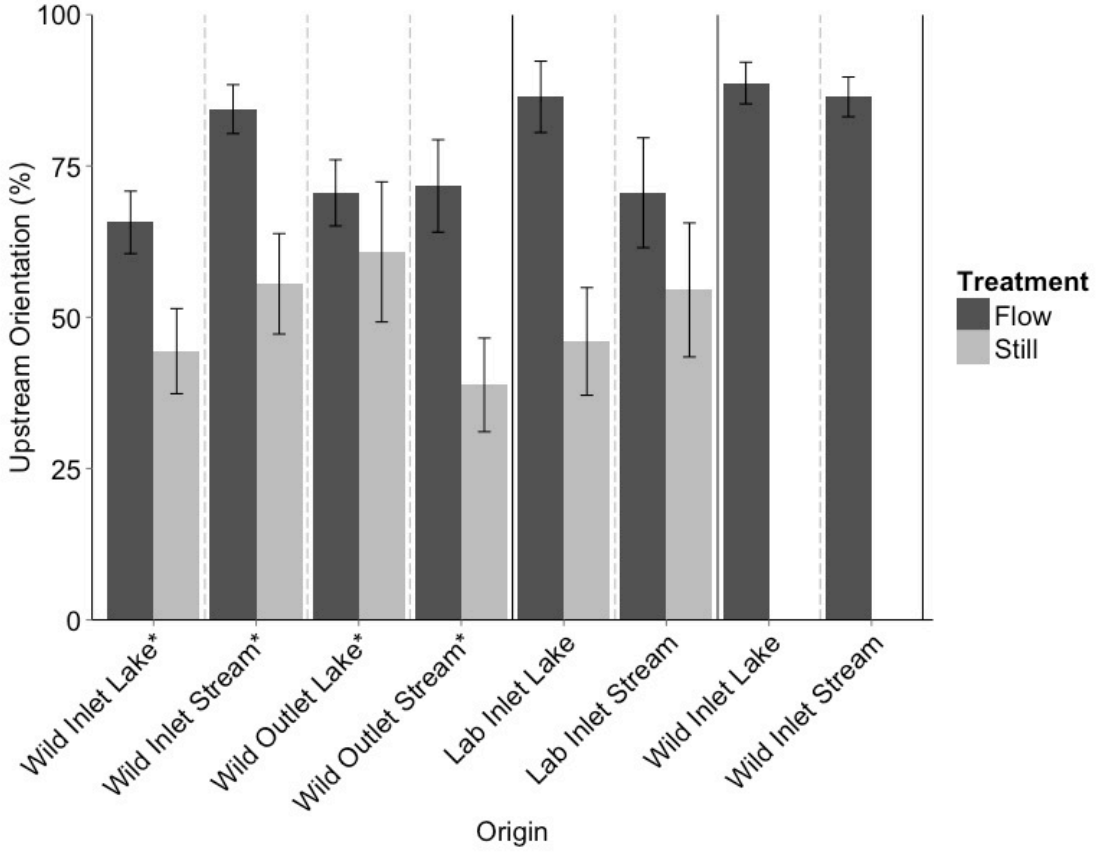

Figure 2.5: A side-by-side comparison of the upstream orientation of all study populations in current trials and still-water trials

The height of each bar indicates the group mean and the error bars are the standard errors. Again still-water trials (light grey bars) are not applicable for Experiment IV (panel C). The Y-axis indicates the percentage of time individual spend facing upstream during the five-minute trial period. Populations in breeding status are indicated by asterisks.

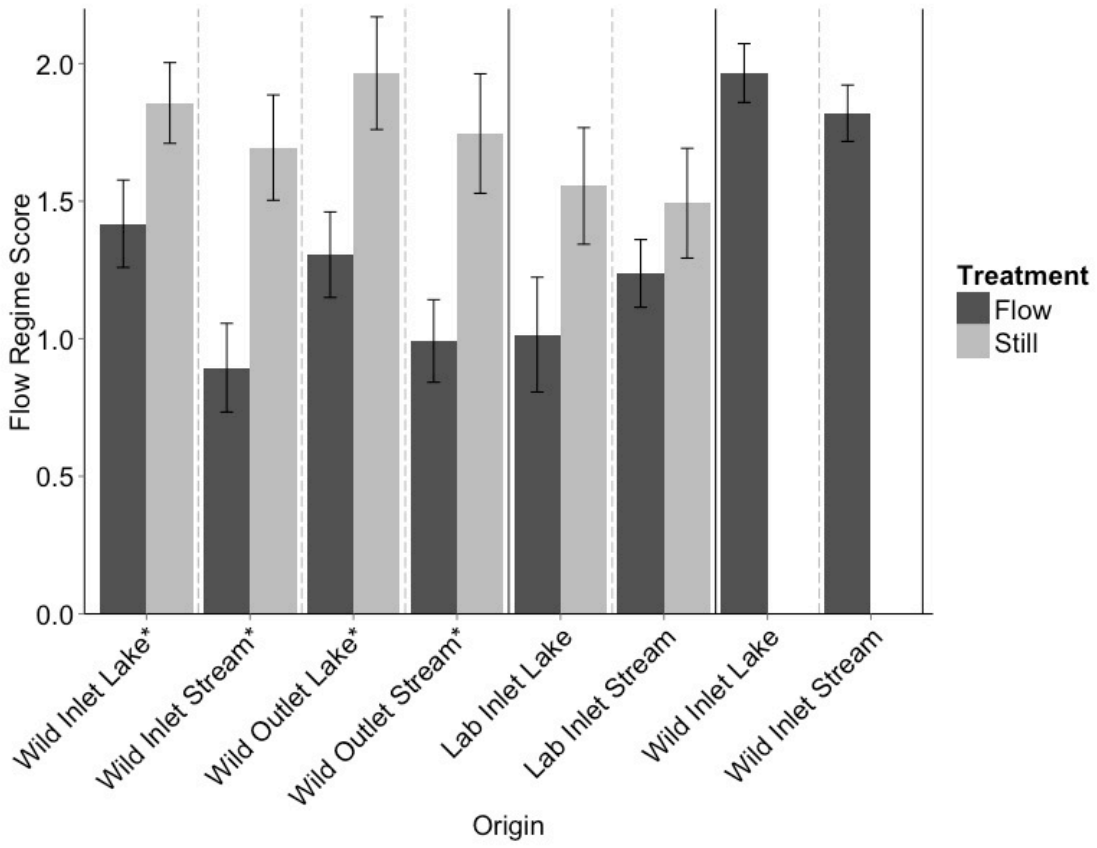

Figure 2.6: A side-by-side comparison of the flow regime of all study populations in current trials and still-water trials

The height of each bar indicates the group mean and the error bars are the standard errors. Again still-water trials (light grey bars) are not applicable for Experiment IV (panel C). The higher the scores, the higher velocity locations in the tank individuals used. Random distribution across flow regimes is indicated by Y = 1.98. Populations in breeding status are indicated by asterisks.

The average energy expenditure of wild-caught inlet lake fish was 0.14 mg O_2 per hour, twice as much as that of wild-caught inlet stream fish 0.074 mg O_2 per hour (Wilcoxon rank sum test $p<0.05$). Again, the qualitative aspect of this result is more meaningful than the precise quantitative values, because the exact amount of energy consumption of lake and stream fish depends many other factors aside from fish weight

and swimming speed. Taking all possible factors into account would require specific experimental design (Boisclair and Tang 1993), which is beyond the scope of this study.

In still water, we found no differences in any measures of swimming behavior between any groups of wild-caught stickleback [Figure 2.3-2.6, Table 2.1]. Thus, the significant behavioral differences between lake and stream stickleback in currents did not result from flow-independent differences in swimming behavior. In addition, we found that in still water all four groups of fish exhibited random orientation (except for inlet stream fish) with no location preferences, in contrast with their behavior in currents described above [Table 2.2].

			Net displacement		Cumulative upstream movement		Upstream orientation		Flow regime	
	Populations \ Trials		Flow	Still	Flow	Still	Flow	Still	Flow	Still
Exp. I	Wild Inlet Lake	Wild Inlet Stream	**79 [0.008]**	178 [0.63]	**249 [0.005]**	181 [0.56]	**78 [0.007]**	137.5 [0.44]	**236.5 [0.019]**	177 [0.65]
	Wild Inlet Lake	Wild Outlet Lake	93 [0.55]	127 0.44]	119 [0.66]	147 [0.10]	99 [0.72]	81.5 [0.27]	122 [0.57]	98 [0.69]
	Wild Inlet Lake	Wild Outlet Stream	96 [0.27]	141[0.59]	169 [0.11]	130 [0.89]	102 [0.37]	129 [0.92]	**177 [0.054]**	134.5 [0.76]
	Wild Inlet Stream	Wild Outlet Lake	153 [0.06]	119[0.66]	**52 [0.02]**	136 [0.24]	154 [0.053]	100 [0.75]	**62 [0.053]**	91 [0.48]
	Wild Inlet Stream	Wild Outlet Stream	149 [0.40]	127 [0.99]	100 [0.33]	123 [0.92]	148 [0.42]	164.5 [0.15]	109 [0.54]	126.5 [1]
	Wild Outlet Lake	Wild Outlet Stream	73 [0.60]	73 [0.60]	107 [0.25]	57 [0.18]	72 [0.56]	112.5 [0.15]	112 [0.16]	95 [0.60]
Exp. II	Lab Inlet Lake	Lab Inlet Stream	134 [0.39]	104 [0.74]	82 [0.22]	147 [0.16]	146 [0.17]	98 [0.56]	88 [0.32]	119.5 [0.79]
Exp. III	Wild Inlet Lake	Wild Inlet Stream	230 [0.79]	-	231 [0.81]	-	280 [0.38]	-	283 [0.34]	-

Significant results in bold.

Table 2.1: Wilcoxon rank-sum test statistics and P values of pairwise comparisons between populations for four measures of rheotaxis

		Net displacement	Cumulative upstream movement	Upstream orientation			Flow regime		
		Flow vs. Still	Flow vs. Still	Flow vs. Still	Flow vs. Random	Still vs. Random	Flow vs. Still	Flow vs. Random	Still vs. Random
Exp. I	Wild Inlet Lake	168 [<0.001]	3 [<0.001]	29[0.012]	300 [<0.001]	203 [0.20]	149 [0.004]	20 [0.003]	78 [0.77]
	Wild Inlet Stream	164 [<0.001]	1[<0.001]	18[<0.002]	323 [<0.001]	225 [0.048]	159 [<0.001]	4 [<0.001]	59 [0.26]
	Wild Outlet Lake	76 [0.0014]	0 [<0.001]	29[0.47]	144 [<0.001]	94.5 [0.20]	67 [0.027]	1 [<0.001]	42 [0.85]
	Wild Outlet Stream	100 [0.0012]	1 [<0.001]	20[0.041]	181 [<0.001]	107 [0.25]	100 [0.012]	0 [<0.001]	38 [0.39]
Exp. II	Lab Inlet Lake	86 [0.15]	35 [0.17]	7 [0.004]	223 [<0.001]	125.5 [0.60]	99 [0.026]	8 [0.0034]	30 [0.094]
	Lab Inlet Stream	99 [0.026]	16 [0.01]	30 [0.17]	189.5 [0.0014]	149 [0.13]	79 [0.30]	2 [<0.001]	20 [0.043]
Exp. III	Wild Inlet Lake	-	-	-	484 [<0.001]	-	-	129 [0.95]	-
	Wild Inlet Stream	-	-	-	483 [<0.001]	-	-	100 [0.41]	-

Table 2.2: Within each population, test statistics and P values for comparing each of the four measures of rheotaxis between currents versus still water or a random expectation

Wilcoxon signed-rank test statistics are shown for currents versus still water comparisons, and for current/still water versus random comparisons for upstream orientation. One-sample Wilcoxon signed-rank test statistics are shown for current/still water versus random comparisons for flow regime.

Comparing the swimming behavior of each group of test individuals in currents versus still water, we found that in currents, all fish exhibited significantly more cumulative movement than in still water [Table 2.2]. Second, in currents, all but outlet lake fish spent more time facing upstream than in still water [Table 2.2]. Third, in

currents, all four groups of fish disproportionately stayed within lower-flow inner parts of the tank than in still water [Table 2.2].

Experiment II: Rheotaxis of common-garden stickleback

We did not find significant differences between lab-reared common garden inlet lake and stream fish, for any behavioral measure, in current or still water [Figure 2.3-2.6, Table 2.1]. Statistical results of comparing the behavioral measures of both groups in currents versus still water are shown in Table 2.2.

Experiment III: Rheotaxis and dispersal in a semi-natural setting

In the dispersal tunnel experiment, 96% of the lake fish and 94% of the stream fish exhibited positive initial rheotactic responses and swam against the current, ending up in the upstream collection chamber. Only 6 lake fish and 7 stream fish exhibited negative initial rheotactic response, ending up in the downstream collection chamber. Another 5 individuals (1 lake fish and 4 stream fish) did not make any choice and stayed in the release chamber. Thus, we found that, prior to the breeding season, wild-caught inlet lake and stream stickleback did not differ in rheotaxis ($P = 0.78$). Both predominantly show positive rheotactic responses compared to random choices ($P<10\text{-}15$, with no choice individuals excluded).

Experiment IV: Rheotaxis of wild-caught non-breeding stickleback

The results of a follow-up circular flow tank behavioral essay were consistent with the dispersal tunnel experiment results. In currents, no significant difference was found in any measure of rheotaxis between inlet lake and stream fish prior to the breeding season [Table 2.1]. In fact, both groups' rheotaxis was comparable to that of inlet stream fish during the breeding season in Experiment I. After five minutes in currents, net displacement of inlet stream fish and inlet lake fish was only -2.2 and -2.3 meters

downstream on average [Figure 2.3]. In currents, stream fish and lake fish faced upstream for 86% and 88% percentage of time (84%) respectively [Figure 2.5].

DISCUSSION

This study tested for divergent rheotaxis between wild-caught and lab-reared parapatric lake and stream stickleback. We found strong divergent rheotaxis in multiple aspects between wild-caught inlet lake and stream stickleback during their breeding season. These behavioral differences should facilitate the upstream movements of stream fish and downstream movements of lake fish, returning them to their respective habitats. These results are consistent with the habitat uses between the same parapatric populations studied by Bolnick et al. (2009). Divergent rheotaxis may not be heritable, as no differences were found in naïve lab-reared common garden inlet lake and stream stickleback with no prior experience with currents. A replicate experiment between the same parapatric populations prior to the breeding season found no difference in rheotaxis, suggesting possible seasonal variations in the strength of divergent rheotaxis. The results of the dispersal tunnel experiment supported the assumption that the measures of rheotaxis in the circular flow tank can be extrapolated to infer fish's movements between natural lake and stream habitats.

During the breeding season, wild-caught inlet stream fish exhibited more positive rheotaxis, three-fold less net downstream displacement, and shorter cumulative upstream movement compared to their lake counterparts. Inlet stream fish were also more often found in slower current compared to their lake counterparts. This pattern may suggest differences in preferred flow regime between the two ecotypes, and may also be an incidental consequence of stream fish, especially inlet stream fish, being better at maneuvering to facilitate their position-holding in currents, perhaps through having more

prior experience. Divergence in rheotaxis, regardless of its cause, might cause reduced energy expenditure for stream fish in the stream environment than that for lake fish, which can serve as a target for natural selection (McCormick et al. 1998; Mohammed et al. 2012).

We emphasize that the behavioral divergence between wild-caught inlet lake and stream fish did not result from flow-independent differences in swimming behavior between them, because no differences were detected in any behavioral measures between the two ecotypes in still water. The fact that the presence of current triggered significant behavioral differences in all aspects in both inlet lake and stream stickleback further confirmed that the divergent rheotaxis we detected arose in response to the currents.

During the breeding season, outlet lake and stream fish did not differ in any measures of rheotaxis, though the presence of current triggered significant rheotactic responses in all four measures in both ecotypes. Although outlet lake fish showed no significant differences in upstream orientation in currents versus still water, in currents they faced upstream for significantly longer periods of time compared with null expectations of random orientation, contrasting with their random orientation in still water. Given that in still water the "upstream" orientation is artificially defined to be consistent with the upstream orientation in the current trial for each test individuals, deviation from random orientation is a more appropriate way to evaluate the effect of current on individuals' orientation. The lack of divergent rheotaxis is consistent with smaller differences in flow rates between the lake the outlet stream than between the lake and the inlet stream, as well as less morphological and genetic divergence between lake fish and outlet stream fish (Hendry et al. 2002; Moore et al. 2007).

Naïve common-garden inlet lake and stream fish that were never exposed to current did not differ in any measures of rheotaxis in current or still water. There are

three possible explanations for the lack of divergence. First, divergent rheotaxis may not be heritable. Second, all lab-reared test individuals were raised in still water with no prior exposure to currents, and the full development of rheotactic behavior may require prior exposure to currents, especially in inlet stream fish. For example, there could be a genotype by environment interaction that we did not measure because we could not recreate biologically realistic flow rates in the lab. Third, all lab-reared test individuals were non-breeding individuals. If divergent rheotaxis is specific to breeding individuals, no differences in would be observed between non-breeding lab-reared inlet lake and stream stickleback even if rheotaxis is heritable.

The dispersal tunnel experiment was a semi-natural setting similar to that study by Bolnick et al. (2009). Bolnick et al. 2009 found 90% of the lake and stream stickleback went back to their original habitat four days after being released at the intersection of the lake and the inlet stream. Our results showed that almost all wild-caught inlet lake and stream stickleback swam upstream against the currents after being released into the dispersal tunnel (positive rheotactic responses). Nevertheless, the dispersal tunnel results were consistent with the results of the flow tank behavioral assay on recaptured individuals of the dispersal tunnel experiment. Prior to the breeding season, wild-caught inlet lake and stream stickleback did not differ in any of the four measures of rheotaxis in currents measured by the same flow tank behavioral assay as in Experiment I. Moreover, wild-caught inlet lake and stream stickleback prior to the breeding season both exhibited comparable level of positive rheotaxis to the wild-caught inlet stream stickleback during the breeding season in the circular flow tank. These results supported that measures of rheotaxis in the circular flow tank can be extrapolated to natural habitats.

Contrasting the flow tank behavioral assay results of Experiment I and III (which differ regarding breeding status), we speculate that divergent rheotaxis may be breeding-

season specific. Seasonal variations of rheotaxis have been documented. Schmitz (1992) examined the annual variations in rheotaxis in a population of Arctic char (*Salvelinus alpinus*) that has been landlocked for about 6000 years and discovered seasonal changes in rheotaxis (including rheotaxis reversal) which were directionally consistent with smolting, and even coupled with physiological changes in seawater adaptability (Schmitz 1992). This can be relevant to our study given that the freshwater threespine stickleback populations are derived from marine ancestors, including fresh-water breeding anadromous populations (McPhail 1994; Taylor and McPhail 2000; McKinnon and Rundle 2002; Hendry et al. 2009). Moreover, the sexual maturation of threespine stickleback during the breeding season is known to be initiated by changes in thyroid hormone pathway in response to photoperiod (O'Brien et al. 2012). Thyroid hormone pathway mediates many important physiological and behavioral functions including rheotaxis (Edeline et al. 2005; Kitano and Lema 2013).

Habitat choice is a complex process that involves sequential stages from departure, through transience, to settlement (Clobert 2001; Clobert et al. 2012). In our study system, divergent rheotaxis directly affects all three stages of the habitat choice process. Compared to stream fish, lake fishes' greater downstream orientation, farther downstream displacement and higher energetic cost in currents may directly result in higher departure rate from stream to lake habitat (upon their entering of stream environment). Lake fish may also be selected against when they do enter a stream, due to their higher energetic costs when swimming in flowing water. The pattern of divergent habitat choice between lake and stream fish could be generated by divergent rheotaxis alone. Thus, the divergent rheotaxis between wild-caught inlet lake and stream stickleback we revealed in can nevertheless be an important component of divergent

habitat uses, and play an important role in driving the diversification between these two parapatric populations.

Chapter 3: Phenotype-dependent Rheotactic Behavior in Lake and Stream Threespine Stickleback

ABSTRACT

Gene flow is widely thought to homogenize spatially separate populations, eroding the effects of divergent selection. This belief rests on the assumption that all genotypes are equally likely to disperse. However, there is growing realization that gene flow may not be random: certain phenotypes may be disproportionately likely to migrate. When these phenotypes are heritable, phenotype-dependent dispersal generates non-random gene flow between populations. This biased migration can promote rather than hinder local adaption and population divergence. Here, we present an example of phenotype-dependent dispersal in parapatric lake and stream stickleback. In each of many watersheds, lake and stream stickleback exhibit extensive morphological and genetic divergence. Such divergence often occurs over a scale of a few meters, too abrupt to be plausibly explained by migration-selection balance alone. A previous study showed that non-random dispersal maintains these microgeographic clines. Another study identified a possible mechanism for non-random dispersal: divergent rheotaxis. Here, we examine a possible phenotypic basis for divergent rheotaxis. We first confirmed that the lateral line system is necessary for lake and stream stickleback to exhibit typical rheotactic behavior. We then showed that lateral line size (the number of neuromasts) and pectoral fin morphology are correlated with rheotactic behavior, in both wild-caught and lab-reared individuals. Lab-reared lake stickleback have more superficial neuromasts than stream individuals, suggesting that neuromast number is heritable. In summary, we established a case of phenotype-dependent rheotactic behavior based on a peripheral sensory trait that has a genetic basis.

INTRODUCTION

Gene flow plays a crucial role in population divergence (Endler 1973; Slatkin 1985; 1987; Garcia-Ramos and Kirkpatrick 1997; Lenormand 2002). Gene flow typically acts against divergent selection and homogenizes the populations, hindering adaptive divergence (Ehrlich and Raven 1969; Endler 1973; Slatkin 1987; Bolnick and Nosil 2007; Räsänen and Hendry 2008). Occasionally, random gene flow may facilitate divergence by bringing in novel foreign mutations that are adaptive to the local population (Rieseberg and Burke 2001; Morjan and Rieseberg 2004). However, the generally constraining effect of gene flow rests on an assumption, that gene flow is random with respect to migrants' phenotypes or genotypes (Endler 1973; Slatkin 1987; Garcia-Ramos and Kirkpatrick 1997; Lenormand 2002). There is growing realization that gene flow might often be non-random, meaning that certain genotypes are disproportionately likely to disperse (Gilbert and Singer 1973; Haag et al. 2005; Phillips et al. 2010; Shine et al. 2011), or to settle in particular habitats (Thomas and Singer 1987; Edelaar et al. 2008). Thus, gene flow may play a far more complex role in evolution than has been previously assumed (Edelaar et al. 2008; Edelaar and Bolnick 2012). Non-random gene flow can promote adaptive divergence and increase the likelihood of speciation with gene flow (Armsworth and Roughgarden 2005; Garant et al. 2005; Postma and van Noordwijk 2005; Edelaar et al. 2008; Shine et al. 2011; Edelaar and Bolnick 2012; Bolnick and Otto 2013).

Non-random gene flow results when a component of individual's dispersal behavior depends on a phenotypic trait. Phenotype-dependent dispersal can cause migration rates between habitats to deviate from expectations based on individuals' movement abilities (Thomas and Singer 1987; Bolnick et al. 2009). Phenotype-dependent dispersal can also alter the genetic composition of migrants, determine the natural

selection regime migrants experience, and affect the genetic variation of populations that natural selection acts on (Thomas and Singer 1987; Garant et al. 2005; Postma and van Noordwijk 2005). Phenotype-dependent dispersal may promote population divergence and exaggerate genetic clines over small spatial scales via improving the matching between individuals to their environment (Postma and van Noordwijk 2005; Armsworth and Roughgarden 2005; Bolnick et al. 2009). The behavioral, physiological, or genetic causes of phenotype-dependent dispersal are well understood in a few systems (examples see Thomas and Singer 1987; Duckworth and Badyaev 2007; Hanski 2011; reviewed in Jaenike and Holt 1991; Clobert 2001; Edelaar et al. 2008; Clobert et al. 2012), while they remain largely unknown for most organisms (Clobert et al. 2012). Here, we use three-spine stickleback (*Gasterosteus aculeatus*) as a model system to examine the phenotypic basis for variation in locomotion ability, which may give rise to phenotype- and genotype-dependent dispersal.

Study system

Parapatric lake and stream stickleback populations exhibit extensive ecological, morphological and genetic divergence on Vancouver Island, B.C., Canada (Reimchen et al. 1985; Lavin and McPhail 1993; Hendry and Taylor 2004; Moore et al. 2007; Berner et al. 2008; Hendry et al. 2009; Berner et al. 2009). Divergence often occurs over a much finer scale (a few meters) relative to individuals' dispersal ability (up to 150 meters in four days). This divergence is too abrupt to be plausibly explained by migration-selection balance alone, suggesting a possible contribution by habitat use and non-random dispersal (Bolnick et al. 2009; Bolnick and Otto 2013). Indeed, a mark-recapture study in Blackwater Lake and its inlet stream (where water flows into a lake) confirmed that most displaced individuals returned to their native habitat (Bolnick et al. 2009). The exceptions were fish that were already morphologically pre-adapted to the adjoining foreign habitat.

Such non-random dispersal can maintain steep morphological clines between lake and stream habitats across a few meters (Bolnick et al. 2009).

This divergent habitat use may arise from any of several mechanisms, but one obvious possibility is rheotaxis. Rheotaxis is the behavioral orientation towards currents (Lyon 1904; Arnold 1974; Montgomery et al. 1997; Pavlov et al. 2010). In many fish species, rheotaxis plays an important role in guiding fish to spawning locations, and in the subsequent dispersal of fry into nursery habitats (Hartman et al. 1962; Raleigh 1967; Brannon and Commission 1972; Kaya 1989; Kaya and Jeanes 1995; Hensleigh and Hendry 1998; Caiger et al. 2012). Many aspects of rheotactic behavior are important. These include 1) position-holding in currents, 2) reducing the energetic expenditure in currents, 3) orienting towards currents, and 4) finding low-flow-rate refuge from currents (Mohammed et al. 2012; Coombs et al. 2013). Each of these can have strong influences on individuals' fitness (Mohammed et al. 2012).

We recently tested whether parapatric lake and stream stickleback differed in rheotaxis. During the breeding season, wild-caught inlet stream fish exhibited more positive rheotaxis than lake fish. This rheotactic difference would tend to promote dispersal of lake and stream fish back into their respective native habitats (Chapter II). Stickleback that more frequently orient towards currents, that hold their position better in currents with less energetic expenditure, and that utilize low-flow-rate refuge from currents are more likely to enter and/or remain in a stream habitat, while individuals that do the opposite are more likely to end up in the lake habitat. Although this result is consistent with the hypothesized role of rheotaxis in explaining divergent dispersal behavior and reduced gene flow between lake and stream stickleback, the mechanistic basis of rheotactic differences remained unclear. Here, we examine the role of stickleback morphology, including sensory and locomotor traits, in generating variation in rheotaxis.

Candidate phenotypes

The lateral line system is a major sensory modality unique to aquatic vertebrates (Bleckmann 1986; Münz 1989; Bleckmann and Bullock 1989). The lateral line mediates rheotactic behavior in a variety of fish (Montgomery et al. 1997; Baker and Montgomery 1999a; Suli et al. 2012; Coombs et al. 2013). Each lateral line is composed of neuromasts, clusters of hair cells that detect local water displacement over the body surface (Dijkgraaf 1963; Coombs et al. 2013). There are two types of neuromasts, superficial neuromasts that reside on the surface of the skin and canal neuromasts that reside in fluid-filled enclosed canals below the skin surface (Coombs and Montgomery 1994; Coombs et al. 2013). Superficial neuromasts are known to detect water velocity along the body surface (Coombs and Montgomery 1994; Braun and Grande 2008; Coombs et al. 2013), while canal neuromasts detect the acceleration and deceleration of water flow by responding to pressure differences in canal pores that reflect fluid movements (Münz 1989; Braun and Grande 2008; Coombs et al. 2013). Threespine stickleback have a total of twelve lateral lines, which are composed solely of superficial neuromasts [Figure 3.1] (Wark and Peichel 2010).

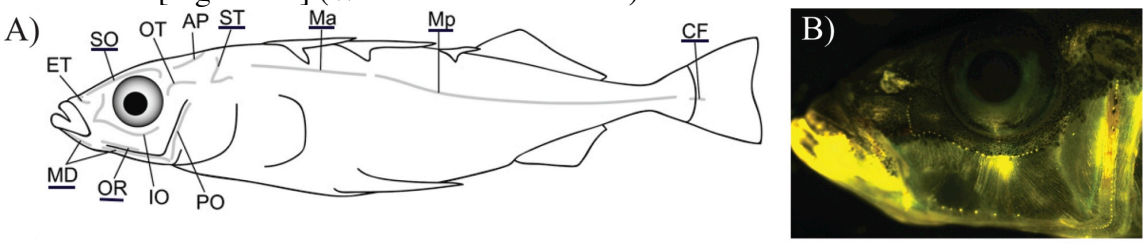

Figure 3.1: The lateral lines of threespine stickleback.

A) A schematic of the twelve lateral lines of threespine stickleback, reproduced from Wark and Peichel 2010 with modifications. These lateral lines include the infraorbital (IO), oral (OR), mandibular (MD), preopercular (PO), otic (OT), supratemporal (ST), main trunk line anterior (Ma), main trunk line posterior (Mp), caudal fin (CF), ethmoid (ET), supraorbital (SO) and anterior pit (AP) lateral lines. Lateral lines with known QTLs

affecting the numbers of lateral line neuromasts are indicated by underscore. B) An image of DASPEI-labeled neuromasts.

Superficial neuromasts are the only type of neuromasts which mediate rheotaxis (Montgomery et al. 1997; Baker and Montgomery 1999a). Pharmacological blocking of lateral lines is shown to drastically increase the flow rate threshold needed to induce rheotactic response in many fish species (Montgomery et al. 1997; Baker and Montgomery 1999a,b; Suli et al. 2012; for a counter-example see Van Trump and McHenry 2013). Proliferation of superficial neuromasts increases fishes' sensitivity to water disturbance (Engelmann et al. 2000; 2002; Coombs et al. 2013), thus is hypothesized to be associated with fish species that are less active swimmers or live in slower moving water (Montgomery et al. 1995; Coombs et al. 2013).

Extensive variation in the lateral line system, especially the number (Vischer 1990; Wark and Peichel 2010; Beckmann et al. 2010; Vanderpham et al. 2012) and the type (Dijkgraaf 1963; Wark and Peichel 2010; Coombs et al. 2013) of neuromasts have been found among fishes and has been hypothesized to play a role in adaption to different environments (Dijkgraaf 1963; Wark and Peichel 2010; Trokovic et al. 2011; Coombs et al. 2013). Variation in neuromast number has been shown to be heritable. At least in stickleback, different lateral lines are controlled independently by different regions of the genome (Wark et al. 2012). However, little is known on intraspecific variation in lateral lines, as most of the existing knowledge on the lateral line system has come from interspecific comparisons, with few exceptions (Wark and Peichel 2010; Trokovic et al. 2011; Vanderpham et al. 2012). Specifically, to our knowledge no studies have examined the covariation, within a species, between the number of neuromasts and individual's rheotactic behavior.

In contrast, many studies have examined intraspecific variation in fish body shape, in relation to flow regime (Blake 1983; Videler 1993; Vogel 1996; Langerhans 2008). Morphological traits that are associated with swimming performance can play a role in rheotactic behavior. For example, shallower body (i.e. more streamlining) and longer pectoral fins relative to body size often favor prolonged swimming in open water, while deeper body and shorter pectoral fin relative to body size often favor maneuverability in currents (Webb 1982; Walker 1997; Hendry et al. 2011). Lake stickleback typically have shallower body and similar pectoral fin sizes compared to inlet stream stickleback.

In this study we 1) carried out a lateral line ablation experiment and showed that the lateral lines were necessary for typical rheotactic behavior of stickleback; 2) tested for correlations between neuromast number and individuals' rheotactic behavior using both wild-caught and lab-reared common garden lake and stream stickleback; 3) tested for heritable differences in lateral line structure between the two ecotypes; and 4) examined the correlations between rheotaxis and three other sets of morphological traits (body sizes, body shapes, and pectoral fin morphology) for phenotype-dependent rheotactic behavior.

METHODS

Study System

Blackwater Lake is a medium-sized mesotrophic lake on Northern Vancouver Island, B.C., Canada. We chose Blackwater lake because its lake and stream stickleback populatons have previously been shown to exhibit nonrandom dispersal behavior (Bolnick 2009), and divergent rheotaxis (Chapter 2). All wild-caught parapatric stream and lake stickleback used in this study were sampled from the inlet stream of Blackwater

Lake (between 50º9'50''N, 125º35'30''W and 50º9'51''N, 125º35'30''W) and from Blackwater Lake near the inlet (50º10'1''N, 125º35'23''W; see Bolnick 2009 for a map of the study site). Fish were captured using unbaited minnow traps. All collection, transportation and experimental procedures were approved [Appendix E].

Circular Flow Tank Design

We used a circular flow tank (Figure 3.2) to quantify individuals' rheotactic behavior, as described in Chapter II. Each test individual can swim freely upstream/downstream indefinitely in the flow tank, without encountering upstream or downstream barriers typical of linear flow tank designs. The flow tank was made of white smooth FRP plastic sheeting and equipped with two aquarium pumps (Maxi-jet 1200, Marineland, Blacksburg, VA) that generated uni-directional circular flow (clockwise or counter-clockwise, alternated across different test individuals). The flow rates were within the natural range of Blackwater inlet stream. The outermost part of the tank has the highest flow rate and the inner most part of the tank has the slowest flow rate (Figure 3.2). The concentric variation in flow rate gave test individuals a choice of flow regimes. The direction of flow (clockwise or counterclockwise) was randomly alternated across trials.

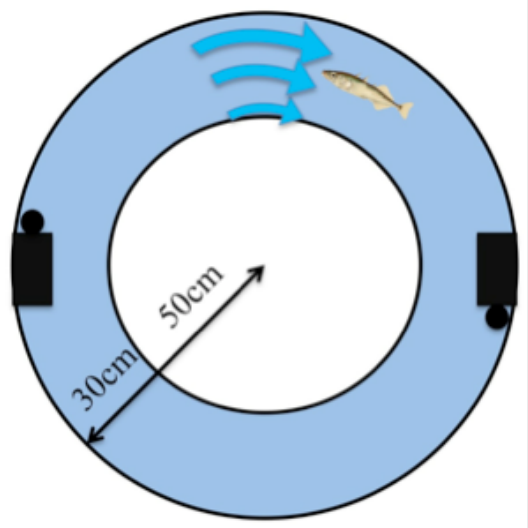

Figure 3.2: An overhead schematic of the circular flow tank

The innermost part of the tank (a two-centimeter-wide ring against the inner wall of the flow tank) has minimal flow rate (0.01m/s). The rest of the test area was equally divided into three concentric rings with equal widths (9.3 cm), including the low-flow-rate inner part (0.12 m/s), medium-flow-rate intermediate part (0.16 m/s) and the high-flow-rate outer part (0.20 m/s).

We tested each individual separately in the flow tank to avoid schooling effects. We gave each test individual fifteen minutes to acclimate in the tank in still water, then an overhead webcam videotaped the rheotactic behavior of each test individual in a five-minute trial with current. We randomized the test order of lake- or stream-origin fish.

We followed the video analysis protocol as in Chapter II. A researcher who was blind to fish identity tracked all fish movements using a constant zoom level (150%). The researcher extracted frames from each trial video at a rate of 3.4 frames/second, and manually tracked the test individual's anterior end and posterior end (caudal peduncle) using ImageJ analysis software (http://rsb.info.nih.gov/ij/) with MtrackJ plugin. In each frame, we averaged the coordinates of the focal fish's anterior end and posterior end to obtain the mid-point of the individual as an indicator of the focal individual's location in

the flow tank. All distance-related measures were converted from pixels into physical distances. We quantified the following four measures of rheotaxis:

1) Net displacement: the distance between the test individual's location at the beginning and the end of the current trial, including any full circuits of the tank upstream or downstream. The net displacement is positive when the ending location is upstream to the starting location, and negative when the fish ended downstream of its start. Positive net displacement would tend to cause dispersal farther upstream (away from the lake). Negative net displacement would tend to move fish downstream (into the lake).

2) Cumulative upstream movement: the total upstream path length that each test individual swam during the five-minute current trial. This differs from net displacement because the cumulative movement includes the length of multiple upstream swimming bursts that could be interspersed with downstream movements that result in little or no net displacement. Energy expenditure increases with cumulative movement depending on swimming speed and body mass (Boisclair and Tang 1993), so fish with high cumulative movement are exerting substantial swimming effort. If this high effort results in little net displacement, fish exhibit poor energetic efficiency in the current.

3) Upstream orientation: the fraction of time each test individual faced upstream into currents (±45º relative to the tangent of circular flow at the midpoint of the fish). A higher frequency of upstream orientation is indicative of more positive rheotaxis (facing into currents). A randomly oriented fish is expected to face upstream and downstream (±45º relative to opposite direction of the tangent of circular flow at the midpoint of the fish) with equal frequency. A prior study showed wild-caught stickleback during the breeding season both faced upstream more often than random expectations, but lake fish faced upstream less than inlet fish (Chapter II).

4) Flow regime: we scored the location of the test individual in each frame (innermost = 0, inner = 1, middle = 2, and outermost = 3), corresponding to increasing flow rates. We averaged the scores of all frames to obtain a single average flow regime score for each individual. The higher the average flow score is, the more often individuals use the high velocity locations in the tank. A fish distributed randomly across flow regimes is expected to have a flow score of 1.98, taking into account the relative surface areas of each of the four regions.

Question I: Does the lateral line system mediate rheotactic behavior in lake and stream stickleback?

If the lateral line system is necessary for the normal rheotactic, the ablation should result in altered behavior in both populations. We pharmacologically ablated the lateral line system of 15 wild-caught lake fish and 15 wild-caught stream fish, and tested their rheotactic behavior. We then compared the rheotactic behavior of the manipulated individuals with the measured rheotactic behavior of their wild-caught non-manipulated counterparts (22 lake fish and 22 stream fish) (Chapter II).

Both the control and the manipulated lake and stream fish were sampled in April 2013, two months prior to the breeding season of these two parapatric populations (Bolnick, pers. obs.). We clipped the first or second dorsal spine to mark stream or lake individuals respectively. Upon capture, all individuals were immediately transported to the Peichel lab at Fred Hutchingson Cancer Research Center, kept in 125 gallon fish tanks under standardized conditions with 16 hours light at a temperature of 16-18 C. Fish were fed twice per day on frozen Mysis shrimp. The rheotaxis of individuals was tested prior to daily feedings.

We used neomycin (Sigma) to ablate the lateral line system of wild-caught stickleback. Mechanoreceptive lateral line hair cells are sensitive to neomycin exposure.

Neomycin exposure of sufficient dosage induces lateral line hair cell death and temporarily inhibits lateral line function removing sensitivity to water vibration. However, lateral line hair cells regenerate quickly once neomycin is removed. Hair cell proliferation starts (accompanied with lateral line sensitivity recovery) within 12 hours after neomycin exposure (Harris et. al 2003, Kaus 1987). In stickleback, the recovery of lateral line hair cells and the lateral line function after pharmacological ablation happens within 3-4 days (Catherine Peichel, pers. comm.).

We held individual fish overnight (10 hours) in an aquarium with 5mM neomycin. This treatment ensured full ablation. We rinsed fish with fresh water for one minute, then returned them to their original aquarium for at least one hour, to acclimate. All behavior assays were carried out within six hours after neomycin exposure to ensure neuromasts had not yet regrown. In a random sample of 20% of the test, we confirmed via DASPEI staining that no neuromasts regrew within this time-frame.

We used Wilcoxon rank-sum tests to evaluate differences between manipulated and control lake fish, and between manipulated and control stream fish, for each of the four measures of rheotaxis. We also tested whether individuals' use of flow regimes deviated from random distribution (in proportion to the relative areas of the define flow regimes). We tested for divergence in rheotactic behavior between lake fish and their stream counterparts for both manipulated and control groups using Wilcoxon rank-sum tests.

Question II: Is there a quantitative relationship between the number of neuromasts and rheotaxis in lake and stream stickleback?

To test for the quantitative relationship between the number of neuromasts and rheotaxis, we visualized the functioning neuromasts of the wild-caught control fish (22 lake and 22 stream individuals) described in Question I. Since the rheotactic behavior of

these individuals appears in a previous study (Chapter II), we used these data again here. All lateral line data were collected within 12 hours after each individual's rheotactic behavioral assay. We stained live individuals with a fluorescent live dye 2-[4-(dimethylamino) styryl]-N-ethylpyridinium iodide (DASPEI; VWR International, Radnor, PA, USA) using a protocol adapted from Wark and Peichel (2010). We first transferred each individual fish from its temporary aquarium to a same-size aquarium filled with 0.025% DASPEI staining solution. We allowed each individual to swim freely in the staining aquarium for twenty minutes and kept the water oxygenized all the time via portable aerators. Then each individual was euthanized via a two-minute immersion bath in MS-222 (500mg/L) after two brief rinses with fresh aquarium water. Immediately after euthanasia, we counted the number of neuromasts in each of the 12 lateral lines on the left side of the fish, using a Leica fluorescent dissecting scope with a FITC filter set (Leica Microsystems Inc., Bannockburn, IL, USA). All euthanized fish were fixed in 10% formalin, then rinsed and preserved in 70% isopropanol for morphometric analysis.

We conducted a canonical correlation analysis (CCA) to test whether there is a multivariate correlation between neuromast numbers and rheotactic behaviors. This CCA contained both lake and stream fish. The neuromsat matrix contains the number of neuromasts at each of the twelve lateral lines. The criterion variable matrix contained three measures of rheotaxis (net displacement, cumulative upstream movement, upstream orientation). We did not include flow regime as one of the criterion variables, because lateral line ablation had no significant effect on the flow regime choice (see Results). CCA reduced lateral line variables and rheotactic behavior variables to three sets of paired canonical variates (CVs). Each pair of CVs are the linear combinations of the criterion variable set and the predictor variable set that have maximized correlations to each other. Each canonical function comes with a canonical correlation coefficient (R_c),

the square of which represents the proportion of variance shared by the CV pair, i.e. squared canonical correlation$\left(R_c^2\right)$. Each subsequent R_c^2represents the amount of remaining variation explained by a CV pair, after accounting for the effects of the previous CV pair(s). We used permutation tests (10000 runs) to test the statistical significance of each pair of CVs using Wilks' λ as the test statistic. For each significantly correlated CV pair, variables are commonly regarded to be important when the absolute values of their corresponding structure coefficients(r_s) are larger than 0.3. We used r_s to identify important dependent and predictor variables, and used the standardized canonical function coefficients (coef) to infer the direction of correlation between each of the important criterion variables and predictor variables in each pair of CV. For each pair of significantly correlated CVs, we used ANCOVA to verify the effect of neuromasts (a covariate) on rheotaxis, while controlling for origin (lake vs. stream, a fixed effect), and to test for significant origin effect and significant interaction effect between lateral line and origin.

We also tested for lateral-line mediated rheotaxis using lab-reared common-garden lake and stickleback with no prior experience to currents. We performed in vitro crosses between wild-caught inlet stream stickleback and between wild-caught inlet lake stickleback from Blackwater Lake. We obtained 11 stream fish clutches and 30 lake fish clutches that developed to maturity (see Chapter II for details of shipping and rearing). We pooled families to generate one outbred population of lake fish (2 fish from each of 11 surviving families) and one population of stream fish (2 fish from each of 30 surviving families).

We sampled 15 lake and 15 stream stickleback from each pooled lab-reared population and evaluated their rheotactic behavior. We test whether rheotaxis covaries with individuals' neuromast numbers. After the rheotaxis and lateral line assays, all

individuals were euthanized and all specimens were fixed in 10% formalin, then rinsed and preserved in 70% isopropanol for morphometric analysis. As described above for the wild-caught fish, we used (CCA) to test whether the number of neuromasts covaries with rheotactic behavior in lab-reared fish. For each pair of significantly correlated CVs, we used ANCOVA to verify the effect of neuromasts on rheotaxis controlling for origin (lake vs. stream), and to test for significant origin effect and significant interaction effect between lateral line and origin.

Question III: Does lateral line structure diverge between lake and stream stickleback?

We tested whether lake and stream stickleback differ in the number of neuromasts in each of the twelve lateral lines. We used Wilcoxon rank-sum tests to test for differences in neuromast numbers, separately for each lateral line, between wild-caught and lab-reared fish. We used the same wild-caught lake and stream stickleback (22 for each group). Lab-reared fish were randomly sampled 22 stream fish and 27 lake fish, including the individuals in Question II. Because of violations of MANOVA assumptions, we used a weighted Z test (Whitlock 2005) to combine the independent tests from each of the twelve lateral lines into a single test.

For each lateral line, we also combined all samples (wild and lab-reared) and then used two-way ANOVAs to test whether neuromast numbers exhibit significant effects of ecotype (lake vs. stream), origin (wild-caught vs. lab-reared) and their interactions (Legendre and Anderson 1999; Anderson and Legendre 1999). We used permutation tests to calculate p-values when the two-way ANOVA residual normality assumption was violated. We also tested for significant correlations between total neuromasts numbers and standard length in both lab-reared and wild-caught individuals

using Spearman's rank correlation. Because we found no correlation, we did not use body size as a covariate in the preceding analyses.

Question IV: Is rheotaxis morphology-dependent in lake and stream stickleback?

To test for morphological traits that are correlated with rheotaxis, we measured body shapes, body sizes and pectoral fin sizes on preserved specimens from individuals used for Questions I-III. We also examined wild-caught lake and stream stickleback captured during the breeding season in a previous year (Chapter II), lacking neuromast data. To obtain metrics representing the body shape of each individual, we took photographs of the right side of all individuals and digitized 20 homologous landmarks using the software tpsDIG2 (Rohlf 2007). We adopted the homologous landmark configuration Berner et. al. 2009, and added the caudal tip of the posterior process of the pelvic girdle, the posterior tip of the ectocoracoid, the anterior edge of the eye, the base of the last pectoral fin ray, and the tip of the pelvic spine. We used tpsUtil to remove the effects of specimen bending owing to preservation, and calculated relative warps (RWs, principal components of shape variables) of all specimens using tpsRelw (Rohlf 2007). We generated RWs separately for non-breeding wild-caught, breeding wild-caught, and non-breeding lab-reared animals. We retained RWs that contributed more than 5% of the total shape variation (five RWs for each group) for further analysis.

We obtained body sizes by measuring five variables, including body mass, standard length, pelvic width, body width at the pectoral fin and body width at preoperculum. We obtained pectoral fin sizes by taking photographs of the pectoral fin on the left side of each specimen and measuring the length and area using Image J.

We conducted CCAs to test whether each of the three sets of morphological traits is a significant predictor of rheotactic behavior, in each of the four groups of stickleback separately. In each group, we used the morphological traits of both lake and stream fish

as predictor variables, and used all four measures of rheotaxis of both lab-reared lake and stream fish as criterion variables. For each pair of significantly correlated CVs in each CCA, we used ANCOVA to verify the effect of morphological trait on rheotaxis controlling for origin (lake vs. stream), and to test for significant origin effect and significant interaction effect between morphological trait and origin. We also used Wilcoxon rank-sum tests to test for differences in each morphological trait between wild-caught and lab-reared fish.

All analyses were done using the R statistical language (Venables and Ripley 2002; R Core Team 2013; Legendre et al. 2014).

RESULTS

I: The lateral line system mediates rheotactic behavior in stickleback

We found that lateral line ablation altered the rheotactic behavior of both lake and stream stickleback in three out of four behavior measures. First, lateral line ablation significantly increased the net displacement in inlet lake fish ($W=95$, $p=0.03$) and showed similar but not significant effects in stream fish ($W=148$, $p=0.86$) [Figure 3.3A]. Control fish typically exhibited significant negative rheotaxis (net displacement down-current), whereas ablated fish remained essentially stationary in the current. Second, lateral line ablation significantly reduced cumulative distance upstream in both lake and stream fish ($W=295$, $p<0.0001$ and $W=263$, $p=0.0002$ respectively) [Figure 3.3B]. The negative net displacement of control fish was the result of a mixture of extensive up- and down-stream movements, which was not observed as much in ablated fish. Third, lateral line ablation significantly increased the frequency of upstream orientation in lake fish ($W=69.5$, $p=0.003$) and showed similar but not significant effect in stream fish ($W=111$, $p=0.16$) [Figure 3.3C]. Lateral line ablation had no significant effect on which flow regime(s) fish

used (W=175 and 121, P = 0.77 and 0.29 respectively) [Figure 3.3D]. All pre-breeding wild-caught lake and stream fish distributed randomly in currents. This was true for control fish with lateral lines (V= 62 and 56, P = 0.93 and 0.85 for lake and stream fish respectively) or ablated fish without functional lateral lines (V= 129 and 100, P = 0.94 and 0.41 for lake and stream fish respectively).

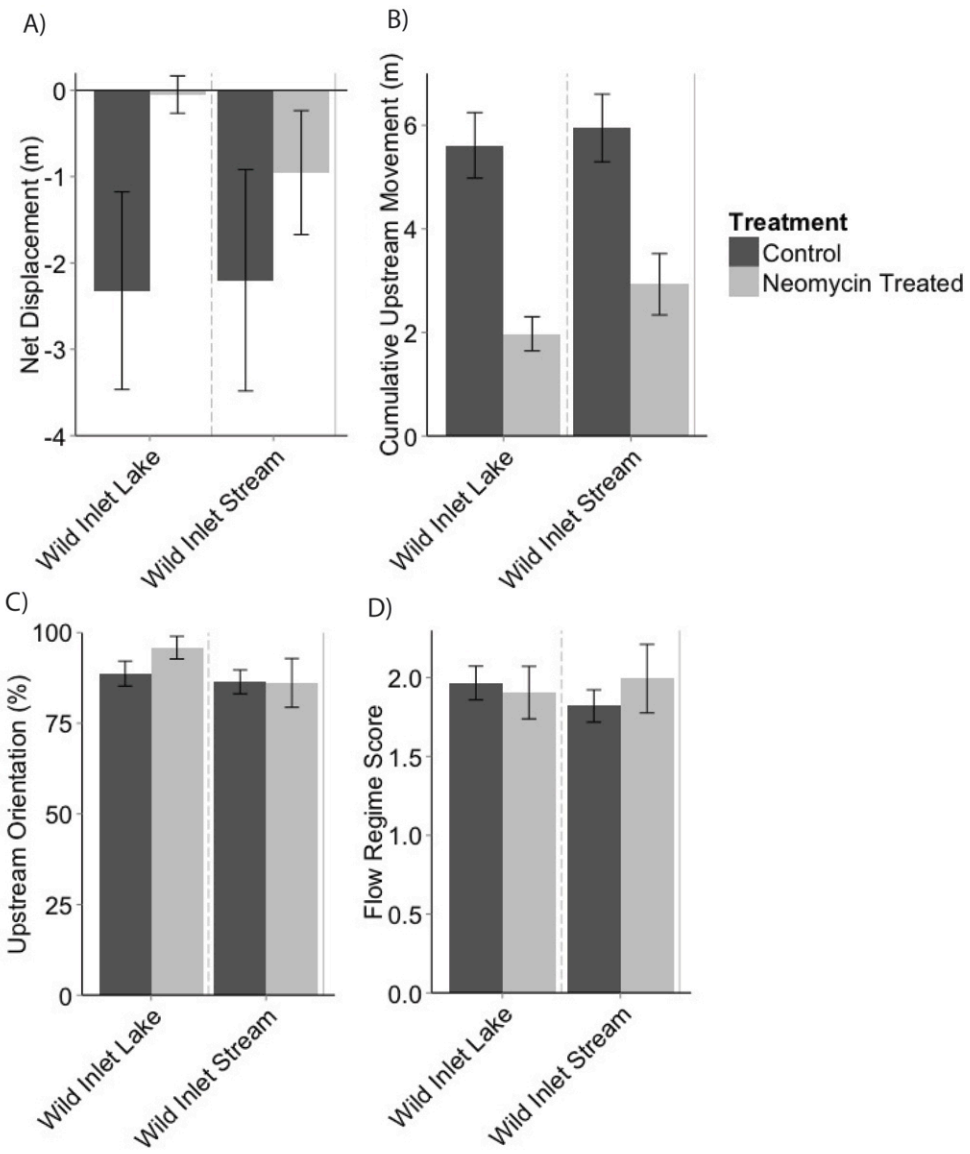

Figure 3.3: A side-by-side comparison of the rheotactic behavior of wild-caught non-breeding lake and stream stickleback and their counterparts with lateral-line ablated

A) net displacement (in meter), B) cumulative upstream movement (in meter), C) upstream orientation and D) flow regime. The height of each bar is the group mean and the error bars are the standard errors.

We found no difference in any of the four measures of rheotaxis between lake and stream stickleback in the control group (see Chapter II for details), or in the manipulated group (net displacement: W = 230 and 145, P = 0.79 and 0.08 for lake and stream fish respectively, the same below; cumulative upstream movement: W = 231 and 69, P =0.81 and 0.12; upstream orientation: W = 280 and 135, P = 0.80 and 0.20; flow regime: W= 283 and 99.5, P = 0.35 and 0.83).

II: The number of neuromasts correlates with rheotactic behavior

In wild-caught non-breeding lake and stream stickleback, we found significant correlations between rheotaxis and neuromast number for the first canonical variate (CV1) (full model) (R_c = 0.83, Wilks's λ = 0.18, P= 0.018) [Figure 3.4A, Table E1]. A substantial amount (69.1%) of the variation in rheotaxis in CV1 can be explained by variation in neuromast number, though CV1 explained a small (10.7%) but yet significant amount of the total variation in rheotaxis. CV1 showed that the numbers of neuromasts (especially more neuromasts along MP and CF) are significantly correlated with more cumulative upstream movement and more upstream orientation [Table 3.1]. CV1 has minimal effect on up or downstream dispersal, since net displacement only explain 2% variation in CV1. The CV1 of neuromasts number remains a significant predictor of the CV1 of rheotaxis after controlling for fish origin (P < 0.001). Lake and stream fish do not differ for the CV1 of neuromast number (P = 0.53). No interaction effect between the CV1 of neuromast number and fish origin and were found (P = 0.45).

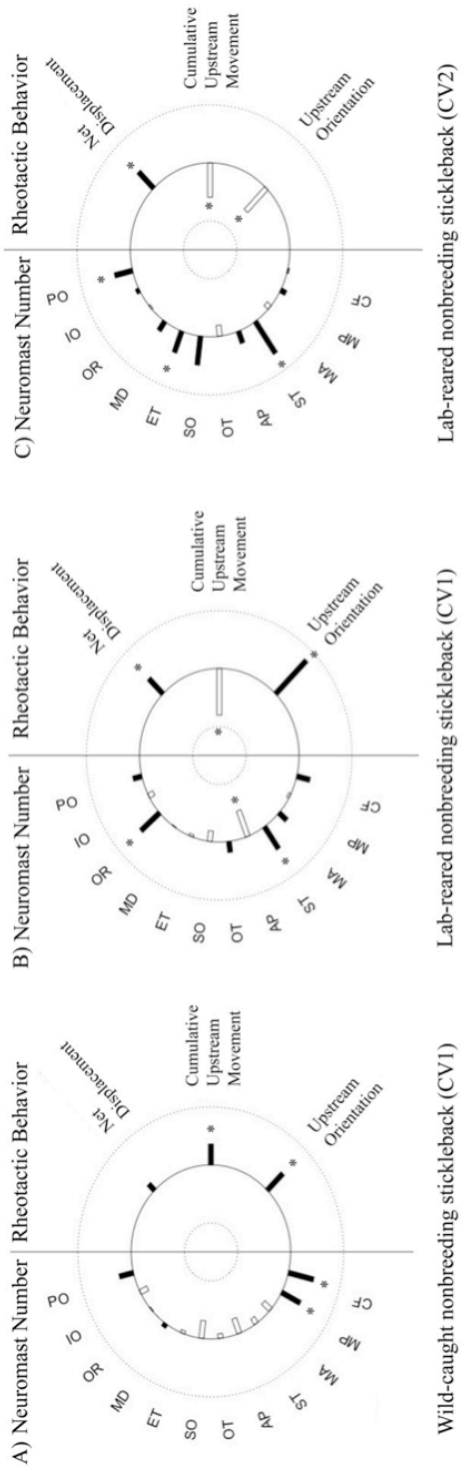

Figure 3.4: Helio plots of structural coefficients

Figure 3.4. Helio plots of structural coefficients of A) the first canonical function predicting rheotactic behavior using neuromast numbers in non-breeding wild-caught fish; B) the first canonical function and C) the second canonical function predicting rheotactic behavior using neuromast numbers in non-breeding lab-reared fish. Data are displayed in radial bars, with larger positive values orienting outward and smaller negative values pointing inward. The length of the bar reflects the importance of the variable. Important variates (structural coefficient >0.3) are indicated by asterisks.

Population	Predictor Trait	Net Displacement	Cumulative Upstream Movement	Upstream Orientation	Flow Regime	Behavioral Implication
Wild; breeding	Fin length (+), fin Area (-)	-	n.s.	-	-	Net downstream displacement in areas with slow currents
Wild; non-breeding	MP (+), CF (+)	n.s.	+	+	n.s.	High energy expenditure in currents
Lab-reared; non-breeding	AP (-), OR (+), ST (+)	+	-	+	NA	Net upstream displacement with low energy expenditure
	PO (+), ET (+), ST (+)	-	-	-	NA	Net downstream displacement with low energy expenditure
	Fin length (+), fin Area (-)	n.s.	+	-	-	High energy expenditure in currents, more downstream facing, often in areas with slow currents

Table 3.1: A summary of major contributing variables of each of the significant CCA results with interpretations

In lab-reared non-breeding lake and stream stickleback, neuromasts could not be counted for a few lateral lines on a few individuals, because of insufficient DASPEI staining intensity or high background fluorescence. These individuals were excluded from our analyses, leaving 13 lake and 12 stream fish. We found significant correlations between rheotaxis and neuromast number for both CV1 (full model) ($R_c = 0.94$, Wilks's $\lambda = 0.021$, $P = 0.04$) [Figure 3.4B, Table E2] and the second canonical variates (CV2) (reduced model) ($R_c = 0.83$, Wilks's $\lambda = 0.18$, $P = 0.043$) [Figure 3.4C, Table E2]. Neuromast number has a substantial contribution to rheotaxis. CV1 explained 48.2% of the total variation in rheotaxis, and a majority of the explained variation (88.2%) can be

explained by variation in neuromast number. CV1 showed that the neuromast number is significantly correlated with more net displacement, less cumulative upstream movement, more frequent upstream orientation [Table 3.1 and A2]. There were strong effects with decreasing neuromast number along AP and increasing neuromast number along OR and ST, ranked by degrees of contribution to CV1. There were weak effects on decreasing neuromast number along CF. CV2 explained 27.1% of the residual variation in rheotaxis that were not explained by CV1, and a majority of the explained residual variation (68.8%) could be explained variation in neuromast number [Table E2]. CV2 showed that the neuromast number is significantly correlated with more net displacement downstream, less cumulative upstream movement and more frequent upstream orientation [Table 3.1 and A2]. Both CV1 and CV2 of neuromasts number remain significant predictors of the corresponding CVs of rheotaxis after controlling for fish origin (both $P < 0.001$). Lake and stream fish do not differ for both CV1 and CV2 of neuromast number ($P = 0.22$ and 0.74 respectively). No interaction effects were found between CV1 or CV2 of neuromast number and fish origin ($P = 0.32$ and 0.81 respectively). The structure coefficients for lateral lines do not differ significantly between wild-caught and lab-reared individuals ($P = 0.668$). This p-value was obtained by calculating CCAs between lateral lines and rheotaxis, separately for lab- and wild-fish. For a test statistic, we calculated the correlation between CCA coefficients of wild- and lab fish. We then generated 10,000 null correlations by shuffling wild/lab identity and recalculating this correlation.

III: Heritable difference in the lateral line structure between lake and stream stickleback

We found heritable differences in lateral line structure between lake and stream stickleback. The means and standard deviations of the number of neuromasts along each

of the 12 lateral lines for both wild-caught and lab reared lake and stickleback are given in Table 3. Neuromasts numbers are size-independent, as there is no correlation between the number of total neuromasts and standard length in both wild-caught (Spearman's rho = 0.005, 0.13 and -0.057, p= 0.98, 0.57 and 0.71 for lake fish, stream fish and pooled lake and stream fish respectively) and lab-reared individuals (Spearman's rho = -0.16, 0.11 and -0.011, p= 0.43, 0.61 and 0.94 for lake fish, stream fish and pooled lake and stream fish respectively).

Lab-reared lake stickleback tended to have more neuromasts than their lab-reared stream counterparts along all twelve lateral lines. This difference was significant for the IO, MD and OT lines [Table 3.2]. Wild-caught non-breeding lake stickleback also tended to have more neuromasts than their stream counterparts along ten out of the total of twelve lateral lines, among which the differences along only the MP line was significant [Table 3.2]. We combine the probability tests from individual lateral lines using weighted-Z method and found that lake stickleback has significantly more neuromasts than stream stickleback, both in wild-caught individuals (combined two-sided $P = 0.025$) and in lab-reared common-garden individuals (combined two-sided $P < 0.001$).

	Wild Lake (N=29)	Wild Stream (N=29)	P	Lab Lake (N=27)	Lab Stream (N=22)	P
IO	20.8(6.3)	23.7(8.5)	0.2	37(6)	33.8(4.9)	0.0082*
OR	5.6(4.1)	4.7(3.6)	0.45	7.3(1.7)	6.6(1.5)	0.26
MD	16.5(7.4)	18.5(9.3)	0.50	43(4.7)	39.7(4.7)	0.021*
PO	9.4(4.8)	8.6(4.2)	0.51	21.4(6)	20.4(3.6)	0.78
OT	6.4(3.6)	5.8(3.6)	0.58	12.2(2.1)	10.7(1.8)	0.022*
ST	9.5(3.7)	8.0(3.4)	0.14	18.6(4.1)	16.8(3.1)	0.11
Ma	18.7(8.7)	17(4.8)	0.35	25.7(6.8)	23.2(5.8)	0.20
Mp	60.2(23.3)	45(25.9)	0.036*	91.9(26.8)	81.8(29.6)	0.14
CF	3.3(2.3)	2.5(2.2)	0.29	6.4(3.3)	5.6 (2.5)	0.47
ET	5.9(2.6)	5.5(3.1)	0.56	7(1.2)	6.7(1.4)	0.27
SO	18.6(7.3)	17.5(6.8)	0.63	32.3(4.8)	30.5(5.7)	0.22
AP	8.5(4.4)	6.8(2.6)	0.13	10.9(2.1)	10.7(2.8)	0.224

Table 3.2: Number of Neuromasts in all 12 lateral lines for both wild-caught and lab-reared common-garden lake and stream stickleback and the two-tailed p values of Wilcoxon rank-sum tests for lake-stream comparisons

Mean (standard deviation) number of neuromasts is shown for each of the lateral line for each population. Neuromasts numbers that significant differ between lake and stream stickleback are indicated by asterisks.

Two-way ANOVAs on Mp, MD, OT and IO lines showed significant ecotype effect on the number of neuromasts along Mp line but not the others (ecotype effect: P = 0.025, 0.33, 0.077 for Mp, MD, OT lines respectively; P = 0.55 for IO lines, based on 999 permutations). Lab-reared individuals consistently had more neuromasts along Mp, MD, OT and IO lines compared to their wild-caught counterparts (origin effect: all P < 0.001; the p value for IO line was based on 999 permutations). There was no significant interaction effect between ecotype (lake versus stream) and origin (lab-reared and wild-caught) in any of the lines, except for the IO line (ecotype*origin: P = 0.64, 0.066 and 0.45 for MP, MD and OT lines respectively; P = 0.015 for IO line, based on 999

permutations), suggesting that the effect sizes did not differ between wild-caught and lab-reared fish along MP, MD and OT lines. Mp accounts for 32.8% and 27.5% of the total number of neuromasts in wild-caught lake and stream fish respectively. IO, MD and OT together accounts for 29.4% and 29.6% of the total number of neuromasts in lab-reared lake and stream stickleback respectively.

IV: Rheotactic behavior is morphology-dependent

In wild-caught non-breeding lake and stream stickleback with intact lateral lines, we found no significant canonical correlations between rheotaxis and body size (full model: $R_c = 0.42$, Wilks's $\lambda = 0.60$, $P = 0.63$) [Table E3], pectoral fin morphology (full model: $R_c = 0.40$, Wilks's $\lambda = 0.82$, $P = 0.48$) [Table E4] or body shape (full model: $R_c = 0.59$, Wilks's $\lambda = 0.52$, $P = 0.21$) [Table E5].

In lateral-line-ablated wild-caught lake and stream stickleback (caught alongside the wild-caught non-breeding fish with intact lateral lines), body shape is significantly correlated with rheotaxis for both CV1 (full model) ($R_c = 0.72$, Wilks's $\lambda = 0.21$, $P= 0.045$) and CV2 (reduced model) ($R_c = 0.70$, Wilks's $\lambda = 0.45$, $P= 0. 015$) [Table E6]. However, both of the significant results were driven by one individual. Removing the individual removed the significant relationship between body shape and rheotaxis along both CV1 ($R_c = 0.73$, Wilks's $\lambda = 0.35$, $P= 0.30$) and CV2 ($R_c = 0.45$, Wilks's $\lambda = 0.76$, $P= 0.72$). We found no significant canonical correlations between rheotaxis and body size (full model: $R_c = 0.63$, Wilks's $\lambda = 0.35$, $P = 0.27$) [Table E7], pectoral fin size (full model: $R_c = 0.50$, Wilks's $\lambda = 0.69$, $P = 0.34$) [Table E8].

In lab-reared lake and stream stickleback, we found significant correlations between rheotaxis and pectoral fin size for CV1 (full model) ($R_c = 0.72$, Wilks's $\lambda = 0.46$, $P = 0.044$) [Figure 3.5A, Table E9]. Pectoral fin size and neuromasts numbers are not correlated ($R_c = 0.81$, Wilks's $\lambda = 0.22$, $P = 0.47$). CV1 explains 19.8% of the total

variation in rheotaxis, and a substantial part (51.1%) of the explained variation in rheotaxis can be explained by variation in pectoral fin size. CV1 showed that higher aspect ratio of pectoral fin (longer fin, less fin area) is a significant predictor of longer cumulative distance upstream, less frequent upstream orientation, and tendency to stay in areas with slower flow regimes [Table 3.1]. We found no significant correlations between rheotaxis and body size (full model: $R_c = 0.73$, Wilks's $\lambda = 0.40$, $P = 0.62$) [Table E10] or body shape (full model: $R_c = 0.61$, Wilks's $\lambda = 0.46$, $P = 0.78$) [Table E11] in lab-reared lake and stream fish. The CV1 of pectoral fin morphology remains a significant predictor of the CV1 of rheotaxis after controlling for fish origin ($P = 0.002$). Lake and stream fish do not differ for the CV1 of neuromasts numbers ($P = 0.37$). No interaction effect between the CV1 of neuromasts and fish origin and were found ($P = 0.95$). No divergence in pectoral fin length ($W = 76$, $P = 0.94$) or area ($W = 78.5$, $p= 1$) was found between lake and stream fish.

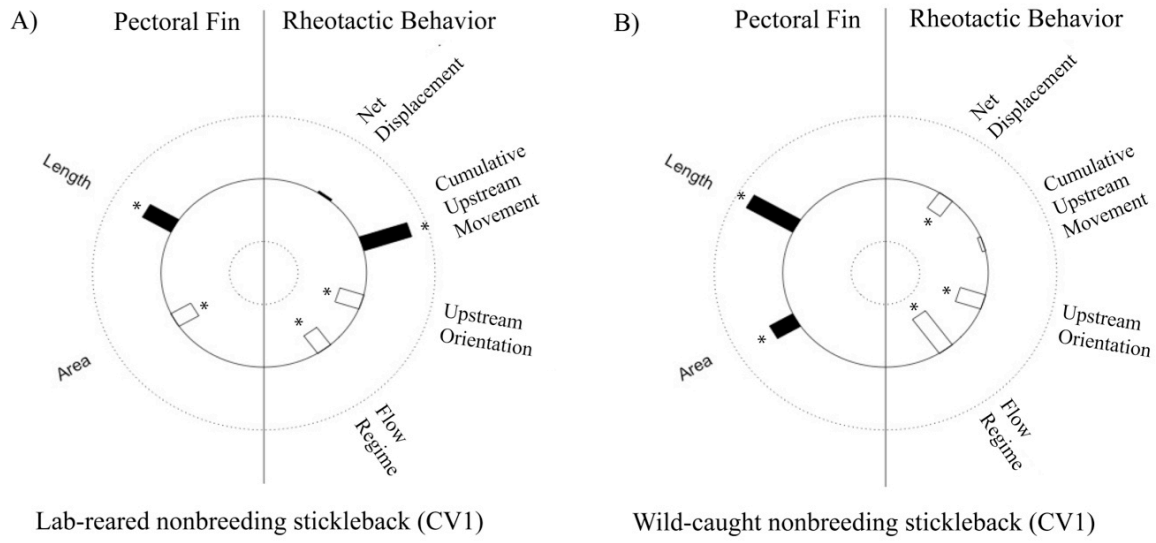

Figure 3.5: Helio plot of structural coefficients of the first canonical function predicting rheotactic behavior using pectoral fin size in A) non-breeding lab-reared fish and B) wild-caught breeding fish

Data are displayed in radial bars, with larger positive values orienting outward and smaller negative values pointing inward. The length of the bar reflects the importance of the variable. Important variates (structural coefficient >0.3) are indicated by asterisks.

In wild-caught breeding lake and stream stickleback, we found significant canonical correlations between rheotaxis and pectoral fin morphology for both CV1 (full model) (R_c = 0.56, Wilks's λ = 0.59, P= 0.041) and CV2 (reduced model) (R_c = 0.36, Wilks's λ = 0.60, P= 0. 041) [Figure 3.5B, Table E12]. CV1 explained 17.4 % of the total variation in rheotaxis, and a third (31.6%) of the explained could be explained by variation in pectoral fin size. CV2 explained 61.8 % of the remaining variation in rheotaxis that were not explained by CV1, though only a small percentage (12.6%) of the explained variation could be explained by variation in pectoral fin size. Thus we focus on the interpretation of CV1, in which pectoral fin size has a substantial contribution to rheotaxis. CV1 showed that higher aspect ratios of pectoral fins (longer fin and smaller fin area) were correlated with less net displacement, less frequent upstream orientation and slower flow regime, consistent with what we found in lab-reared fish [Table 3.1 and A12]. The CV1 of pectoral fin morphology remains a significant predictor of the CV1 of rheotaxis after controlling for fish origin (P = 0.01). Lake and stream fish do not differ for the CV1 of pectoral fin measures (P = 0.91). No interaction effect between the CV1 of neuromasts and fish origin and were found (P = 0.82). We did not find divergence in pectoral fin length (W = 138, P = 0.46) or area (W = 133, p= 0.37) between lake and stream fish. Moreover, neither body size (full model: R_c = 0.52, Wilks's λ = 0.57, P = 0.67) [Table E13] nor body shape (full model: R_c = 0.26, Wilks's λ = 0.59, P = 0.72) [Table E14] were significant correlated with rheotaxis in wild-caught breeding lake and stream stickleback.

DISCUSSION

This study identified a case of phenotype-dependent dispersal propensity. We showed that both superficial neuromast number and pectoral fin morphology are correlated with both rheotactic behavior in lab-reared and wild-caught individuals. We showed that the lateral line was necessary for stickleback to exhibit typical rheotactic behavior. We also showed that lake stickleback have more neuromasts than stream stickleback in the wild. This difference is heritable as it persists in common-garden lab-reared fish, consistent with prior evidence for heritable neuromast numbers (Wark et al. 2012). Collectively, these results suggest that heritable variation in neuromast numbers may lead to heritable variation in rheotaxis within and among lake and stream stickleback. To the extent that rheotaxis affects dispersal behavior in the wild, this would tend to generate genotype-dependent dispersal that could accentuate adaptive divergence.

The lateral line system, vision and tactile senses are the three major sensory modalities fish employ to orient and respond to currents (Montgomery et al. 1997; Coombs et al. 2013). We showed that the lateral line is essential for the typical rheotactic behavior of stickleback. Previous studies on rheotactic behavior following lateral line ablation mainly measured the minimal flow rate inducing upstream orientation in linear flow tanks with constant current speed (Montgomery et al. 1997; Baker and Montgomery 1999a; Suli et al. 2012). Superficial neuromasts have repeatedly been shown to alter rheotactic behavior (for counter examples, see (Brown et al. 2011; Coombs et al. 2013; Van Trump and McHenry 2013). Ablating neuromasts reduces sensitivity to currents and drastically increases the minimal threshold current speed to induce rheotaxis (Montgomery et al. 1997; Baker and Montgomery 1999a; Suli et al. 2012). In this study, we measured multiple facets of rheotactic behavior, using a circular flow tank, to provide

a more multivariate view of rheotaxis in a heterogeneous fluid environment than has been done to date.

Lateral line ablation removes an important sensory modality for fish to make movement decisions in a heterogeneous flow regime (Liao 2007; Bleckmann et al. 2012).We showed that lateral-line ablated stickleback exhibited stronger position-holding behavior in currents. Ablated fish remained essentially stationary on average, whereas control fish tended to move down-current. Compared to control fish, ablated individuals covered smaller cumulative distances and faced upstream more frequently. This behavior pattern is similar to the behaviors that breeding stream fish use to remain in place in the current (Chapter II). In contrast, non-breeding and lab-reared stickleback all exhibited negative rheotaxis as seen in the control fish. To explain this result, we hypothesize that in lake and non-breeding stream stickleback, lateral lines generate information about current that fish use to orient downstream. Ablating the neuromasts removes this source of information, eliminating fish's ability to detect (and thereby follow) a current. In the absence of neuromast-derived information about the current, fish can use vision to generate an external reference frame of the surroundings, and hold their position as a response to stabilize the images of the surroundings (Coombs et al. 2013). Thus, ablation may have forced fish to rely on visual cues, inducing place-holding behavior rather than the typical negative rheotaxis. This explanation could be tested by repeating these experiments in the dark, with the expectation that place-holding is eliminated in ablated fish who also lack visual cues. It is not clear, at present, why breeding-season stream stickleback exhibit the slight positive rheotaxis seen in ablated fish. It may be that their neuromasts are either damaged, lost, or no longer used for sensory information.

Previous studies, which mostly focused on among species comparisons, have associated the reduction of superficial neuromast number with high flow environments

and more active swimmers (Dijkgraaf 1963; Vischer 1990; Guarnieri et al. 1993; Coombs et al. 2013); for a counter-example, see (Beckmann et al. 2010). This association is supported by studies showing that superficial neuromasts are always stimulated in running water or when fish are swimming (Engelmann et al. 2000; 2002), thus the reduction in superficial neuromast number reduces the sensitivity of fish to hydrodynamic noise (Engelmann et al. 2000; 2002; Coombs et al. 2013). As superficial neuromasts mediate rheotactic behavior (Montgomery et al. 1997; Baker and Montgomery 1999a; Suli et al. 2012), the association among superficial neuromasts, hydrodynamic environments and rheotactic behavior was implied, though never explicitly tested.

This is the first study to have examined a quantitative relationship between superficial neuromast number and rheotactic behavior. We found significant correlations between the number of superficial neuromasts and rheotactic behavior in both lab-reared and wild-caught fish. Superficial neuromast number better explained rheotactic behavior in lab-reared individuals than wild-caught individuals. CCAs identified different lateral lines as having major contributions to rheotactic behavior between wild-caught and lab-reared fish, although simulation tests showed that the differences in the relative contribution of each lateral line to rheotactic behavior was not significant between wild-caught and lab-reared individuals. In wild-caught individuals, having more posterior neuromasts (MP and CF) was consistently associated with high energy expenditure in currents (high cumulative distance traveled for a given net displacement) and facing upstream more often. In lab-reared individuals, we found that having more anterior neuromasts (e.g. along PO, ET, AP lines) was often associated with more net downstream displacement and more downstream orientation (though this was not the case for the OR line). Prior experience with currents, developmental plasticity and environmental effects

may all play a role in explaining the differences between lab and wild fish. The relationship between neuromast number and rheotactic behavior also appears to be complex within each group, as various lateral line regions jointly contributed to various measures of rheotactic behavior. It is clear that further studies are needed to determine the behavioral effects of individual lateral lines, and combinatorial interactions among lateral lines.

We also found significant divergence in superficial neuromast number between lake and stream stickleback. In both samples, lake fish typically had more neuromasts than stream fish. These results are consistent with the general pattern of superficial neuromast number and hydrodynamic environment association among species described in other studies (Dijkgraaf 1963; Vischer 1990; Guarnieri et al. 1993; Coombs et al. 2013)). However, univariate tests showed inconsistent differences in lateral line structure between lake and stream fish. Lab-reared lake fish had more neuromasts along three anterior lateral lines (IO, MD and OT) than their stream counterparts, while wild-caught lake fish had more neuromasts along the major posterior (MP) lateral line than their stream counterparts. Compared to lab-reared individuals, which typically have fully developed superficial neuromasts along the entire length of all lateral lines, wild caught individuals showed a significant reduction in superficial neuromast number along all lateral lines. Thus, developmental and environmental plasticity in superficial neuromast number as well as our limited statistical power may all contribute to the inconsistency. Despite the significant neuromast differences between wild- and lab-fish, the consistently higher neuromast counts in lake fish in the lab and wild is consistent with heritable differences in neuromast numbers, between lake and stream populations. The heritable basis in neuromast number is consistent with genetic mapping studies from other stickleback populations, which found QTLs for multiple lateral lines (Wark et al. 2012).

Given that neuromast numbers are heritable, and are correlated with rheotactic behavior in both lab-reared naïve individuals and wild-caught individuals, we posit that rheotaxis variation is heritable as well. However, we cannot.definitively infer heritability of rheotaxis from our data, because the heritability data relies on a lake/stream contrast, whereas the neuromast-rheotaxis correlation is based on all individuals regardless of their ancestry. A full quantitative genetic study would be necessary to establish the genetic correlation between neuromast and rheotaxis traits.

The few studies that have examined the among population variation in lateral line structures all found associations between habitat types and the number of superficial neuromasts (Wark and Peichel 2010; Trokovic et al. 2011) and/or canal neuromasts (Trokovic et al. 2011; Vanderpham et al. 2012). However, the directions of these associations were inconsistent. For example, Walk and Peichel 2010 examined the lateral line structure of 16 threespine stickleback populations, including a lake population and parapatric populations in the lake's inlet and outlet streams. They did not find lateral line divergence between parapatric lake and stream populations, but they did find that those stream stickleback had more neuromasts than marine stickleback. They also found that benthic species of stickleback had more neuromasts than sympatric limnetic species (Wark and Peichel 2010). Both among- and within-species comparisons suggested that the lateral line sensory system may experience different selection regimes in alternative habitats (Dijkgraaf 1963; Vischer 1990; Guarnieri et al. 1993; Wark and Peichel 2010; Trokovic et al. 2011; Vanderpham et al. 2012; Coombs et al. 2013). The hydrodynamic environment and other factors, such as behavioral adaptation or sociality, may jointly shape the variation in the lateral line system (Wark and Peichel 2010; Greenwood et al. 2013; Coombs et al. 2013). In our study, divergence in the lateral line system occurred between the lake and stream sampling sites that are only 350 meters away from each

other with no physical barrier to dispersal. We speculate that lateral line mediated non-random dispersal, as well as adaptation to lake and stream hydrodynamic environments may both play a role in shaping the divergent lateral line structure between parapatric lake and stream stickleback.

Rheotactic behavior is also correlated with pectoral fin morphology, but not with body size or body shape. Longer pectoral fins with small areas were associated with rheotactic behaviors that mostly facilitate downstream dispersal (i.e. less net displacement, more cumulative upstream, more upstream facing). The association between pectoral fin morphology and rheotactic behavior is consistent with the prior knowledge that longer pectoral fins with small areas favor prolonged swimming (Walker and Westneat 2002), and is important in lake habitat (Hendry et al. 2011). Shorter pectoral fins with small areas have been suggested to favor maneuvering (Walker and Westneat 2002), which is important in stream habitat (Hendry et al. 2011). However, the correlation between pectoral morphology and rheotactic behavior only exists when the lateral line is functional. The correlation is removed in lateral-line ablated fish. Thus, variance in rheotactic behavior was not merely a result of sensory system variation, but also reflected variation in swimming ability influenced by locomotor traits.

In summary, we have shown that lake and stream stickleback differ in sensory morphology, locomotor traits, and swimming behavior in flowing water. Variation in the sensory and fin traits is at least partly heritable, and is correlated with swimming behavior. Consequently, our data provide evidence for phenotype-dependent dispersal. To the extent that rheotaxis traits influence individuals' dispersal into lake versus stream habitats (as yet unproven), we speculate that this phenotype-dependent dispersal behavior may also give rise to non-random gene flow. Such non-random gene flow may facilitate

the special sorting of individuals to the matching environment and explain the fine-scale cline, promoting instead of hindering adaptive divergence.

Appendices

APPENDIX A

Search methods and trait categories

Assortative mating has been studied throughout the history of evolutionary biology. Consequently, a very large body of literature has accumulated on the subject. As of November 10th, 2011, a Google Scholar search returned 327,000 and 174,000 publications containing the phrase "non random mating" and "non random pairing", respectively, in their main text or references; 29,700 and 14,800 publications contained "assortative mating" and "assortative pairing", respectively. However, only a small number of these matches are relevant to our study, in the sense of providing quantitative measures of assortative mating within a single conspecific population.

We employed a three-step search strategy to generate a representative and sufficient dataset for the meta-analysis:

Step 1: We first went through a complete list of all literature containing the words "assortative mating" or "disassortative mating" in their title, as identified by title searches in "Google Scholar", "JSTOR" and "Web of Science".

Step 2: We conducted keyword searches for papers whose full text contains at least one of the listed combinations of keywords using "Google Scholar" in subject area of "Biology, Life Sciences, and Environmental Science". The full list of key words is given in Table A1. Whenever possible, we also search for antonyms of search terms in order to reduce the possibility of bias either towards positive or negative results. Note that although some authors may distinguish "assortative pairing" from "assortative mating" in a way that the previous does not necessarily result in procreation, most authors use these two terms as semantics. Thus we did not attempt to distinguish these two terms in our database due to insufficient information.

Keyword combination	Number of publications
"assortative pairing" and "correlation"	282
"assortative pairing" and "coefficient of correlation	4
"assortative pairing" and "correlation coefficient"	51
“disassortative pairing”	562
"disassortative pairing" and "correlation"	15
"disassortative pairing" and "coefficient of correlation"	4
"disassortative pairing" and "correlation coefficient”	51
"disassortative mating" and "correlation"	370
"disassortative mating" and "correlation coefficient"	44
"disassortative mating" and "coefficient of correlation"	2
“assortative mating” and “correlation”	5070
“assortative mating” and “coefficient of correlation”	56
"assortative mating" and "correlation coefficient"	687
amplexus size assortative	223
amplexus size disassortative	6

Table A1: Keyword combinations used in literature search and the number of relevant publications identified

Step 3: We noted potentially relevant citations from the text of papers we read based on the descriptions in text and titles in the citation section. To reduce publication bias arising from the possibility that prominent journals may favor significant results, we were careful to survey journals regardless of their stature. We were also careful not to restrict the year of publication: some publications in our database date back as far as 1906, although most of the references in our database were published after 1970.

Our most recent search was conducted on November 10, 2011. Table A1 reports the number of relevant publications returned by search engines for different search terms. Table A2 shows how traits were classified into trait categories.

Category	Traits included
Age	Age
Behavior	Feeding rate
Chemical	Pheromone, pheromone response, triiodothyronine (an avian thyroid hormone also called T3), testosterone
Condition	Condition, ectoparasite loads, hematocrit (volume percentage of blood composed of red blood cells), cloacal microbial abundance, molt score, parasite incidence, parasite load, time left to molt, wing wear
Ecotype	Hatching site, ecotype, diet
Genotype	MHC alleles, heterozygosity
Phenology	Arrival date
Size	Body length, body weight
Structural	Ninth primary length, asymmetric wings incidence, bill depth, bill length, bill size, bill width, chela size, cheliped length, claw size, culmen length, degree of asymmetry between two tail streamers, elytron length, femur length, first primary length, flag area, flipper length, foretibial length, forewing length, gape length, gonys length, head length, head width, head-bill length, longest tail streamer length, lower mandible length, lower mandible width, mouth-opening direction, outer tarsus length, pectoral band, pectoral spots, pronotal width, prosoma width, prothorax width, racket area, rectrix length, second tail streamer length, sternopleural chaeta numbers, tail height, tail length, tarsometatarsus length, tarsus length, thorax length, tibia length, toe length, upper mandible depth, upper mandible length, upper mandible width, wing length
Visual	Bill color, body color, breast stripe width, color morph, color phase, crest size, crown plumage brightness, crown plumage chroma, elytral spot length, head color, immaculateness, long-wave brightness, mid-wave brightness, ornament, plumage brightness, plumage color, plumage hue, prothorax spot length, prothorax spot width, UV angular breadth (a measure of UV reflectance), UV brightness, UV chroma, UV hue, yellow chroma, yellow hue

Table A2: Trait categories used in the meta-analysis, giving the specific traits included in each category

APPENDIX B

Additional analyses

Taxon	r	N	CI
Amphibians	0.21***	44	(0.13, 0.28)
Birds	0.25***	132	(0.20, 0.29)
Chelicerates	0.40**	3	(0.12, 0.68)
Crustaceans	0.46***	52	(0.40, 0.52)
Fish	0.55***	23	(0.44, 0.65)
Gastropods	0.33***	8	(0.15, 0.51)
Insects	0.21***	66	(0.16, 0.27)
Mammals	0.16	2	(-0.23, 0.55)
Reptiles	0.14	5	(-0.07, 0.36)
Arthropods	0.32***	121	(0.28, 0.37)
Chordates	0.27***	206	(0.23, 0.30)
Molluscs	0.33***	8	(0.14, 0.52)

** $P < 0.01$ when testing whether the mean correlation is different from a null hypothesis of zero correlation.
*** $P < 0.001$

Table B1: The strength of assortative mating by taxon

Trait category	r	N	CI
Age	0.34***	20	(0.23, 0.45)
Behavior	0.35	1	(-0.25, 0.95)
Chemical	0.22	2	(-0.14, 0.59)
Condition	0.26***	23	(0.16, 0.37)
Ecotype	0.50*	2	(0.11, 0.89)
Genotype	0.23	4	(-0.03, 0.48)
Phenology	0.79**	1	(0.29, 1.29)
Size	0.31***	191	(0.28, 0.35)
Structural	0.21***	75	(0.15, 0.26)
Visual	0.34***	18	(0.22, 0.46)

*** $P < 0.001$
** $P < 0.01$
* $P < 0.05$

Table B2: The strength of assortment by trait category

The sample sizes (N) are the numbers of species-trait mean effects.

Trait category	Taxon	r	N	CI
Size				
	Amphibians	0.20***	40	(0.12, 0.29)
	Birds	0.16***	34	(0.07, 0.25)
	Crustaceans	0.47***	45	(0.40, 0.54)
	Fish	0.55***	21	(0.44, 0.66)
	Gastropods	0.33***	8	(0.15, 0.51)
	Insects	0.22***	34	(0.14, 0.30)
Structure				
	Birds	0.18***	44	(0.12, 0.23)
	Insects	0.19***	23	(0.11, 0.27)

*** $P < 0.001$

Table B3: Strengths of assortment for size and structural characters by taxon

Differences between taxa for both size (QM = 351, QE = 12411) and structure (QM = 59, QE = 485) are statistically significant at $P < 0.0001$.

Taxon	Trait category	r	N	CI
Birds				
	Age	0.38***	18	(0.28, 0.48)
	Condition	0.28***	14	(0.17, 0.40)
	Size	0.16***	34	(0.09, 0.24)
	Structural	0.18***	44	(0.11, 0.24)
	Visual	0.37***	14	(0.26, 0.48)
Insects				
	Size	0.22***	34	(0.14, 0.30)
	Structural	0.18***	23	(0.08, 0.28)

*** $P < 0.001$

Table B4: The strength of assortative mating by trait category within the two major taxa

Variation in r across trait categories within birds ($QM = 171$, $QE = 1796$) and insects ($QM = 44$, $QE = 5336$) is significant at $P < 0.0001$.

APPENDIX C

Estimating the distribution of ϱ

This appendix describes the simulation procedure that we used to estimate the underlying distribution of r, the strength of assortment. The basic strategy is to sample values of r from an underlying distribution, then add sampling error comparable to that in the original studies, and then to average the data in same way that we did in meta-analyses. The result is a simulated distribution of $\bar{r}$, the species-trait means that we calculate from our dataset. We compare this simulated distribution with the observed data and find the parameter values for the underlying distribution that generate the best fit.

This approach is similar in spirit to Approximate Bayesian Computation (ABC) (Beaumont 2011). A full ABC analysis offers the possibility of estimating confidence regions for the parameters. Of particular interest here is the lower bound b of the distribution of r, specifically whether it admits negative values. We chose not to undertake a full ABC analysis for three reasons. First, we make an assumption about the form of the underlying distribution (a modified beta distribution) for reasons of convenience. While that seems a reasonable choice, we have no strong theoretical justification for it. Second, the data include not only the sampling error that we model, but also other unknown sources of error (e.g. due to measurement). Third, the estimated values for assortment that make up our data are not independent: correlations between the values result from phylogenetic relations between species and from phenotypic correlations between traits in the same species. We are not able to determine how violations of our assumptions, additional sources of error, or nonindependence in the data would affect the ABC analysis.

In short, we view the following as a heuristic exercise. It seems to be the limit of what is possible with meta-analyses that are based on a highly heterogeneous set of data.

Modified beta distribution

Correlation coefficients are constrained to lie between -1 and 1, and therefore are described by a bounded probability density function. We chose to work with the beta distribution both because it is bounded and because it is quite flexible. However, the beta distribution is bounded between 0 and 1. We therefore generalized the beta distribution to have the range [b, 1]. Parameterizing this distribution in terms of the lower bound b, the mean m, and the variance s2, the density function is

$$P(\rho \mid m, s^2, b) = \frac{C}{(1-\rho)}\left(\frac{b-\rho}{b-1}\right)^{\left(\frac{1-2b+m+\frac{(b-m)^2(-1+m)}{s^2}}{-1+b}\right)}\left(\frac{\rho-1}{b-1}\right)^{\left(\frac{(1-m)\left(b-bm-m+m^2+s^2\right)}{(b-1)s^2}\right)}$$

(Eq C1)

where C is the constant

$$C = \frac{\Gamma\left(-\frac{b-m-bm+m^2+s^2}{s^2}\right)}{\Gamma\left(\frac{(b-m)\left((b-m)(m-1)-s^2\right)}{(b-1)s^2}\right)\Gamma\left(\frac{(1-m)\left(b-bm+(m-1)m+s^2\right)}{(b-1)s^2}\right)}$$

(Eq C2)

A Mathematica Notebook with the derivation of this result is available from the authors upon request.

Using this distribution for r, we will seek the parameter combinations (b, m, s2) that best explain the observed data.

Fitting the distribution of r to the data

We searched for the combination of b, m, and s2 values that gave the best fit. We iterated through combinations of of m (0.2 to 0.4 in increments of 0.02), b (-0.3 to 0.1 in increments of 0.02) and s2 (0.03 to 0.075 in increments of 0.005). Given a set of parameter values, we sampled 360 values of r and generated 1116 estimates of assortative

mating using study sample sizes from actual studies in our dataset. These were then averaged to generate 360 species-trait mean correlations, and this simulated distribution was compared with the empirical distribution. Five replicate distributions were simulated for each parameter set.

To evaluate the fit of the simulated and observed distributions, we used a modified c2 statistic that we denote as K. (We emphasize that we are using K as a simple descriptive statistic, and not to test significance. K plays a role here analogous to that of a summary statistic in an ABC analysis). We divided the values of $\bar{r}$ into 20 equal-width bins between -1 and 1 and determined the number of observed (Oi) and simulated (Si) values in each bin i. We then calculated

$$K = 2\sum_{i=1}^{20}\frac{(O_i - S_i)^2}{(O_i + S_i)} \qquad \text{(Eq. C3)}$$

Larger values of K imply a worse fit between the simulated and empirical distributions.

Figure C1 shows how K varies as a function of the parameters of the beta distribution. The best-fit parameters were m = 0.27, b = 0.02, and s2 = 0.047. This result suggests that assortative mating may be rare or absent in animals (Figure 2B). That conclusion must be accompanied by a several caveats. Most importantly, distributions for r that have lower bounds as small as b = -0.3 can yield simulated distributions of $\bar{r}$ that are not significantly different from the dataset (Kolmogorov-Smirnov test, $P > 0.05$), albeit with larger K than our best-fit model. Thus we cannot reject the hypothesis that weak negative assortment occurs at moderately low frequency. Second, our simulations assume that r follows our modified beta distribution. The true distribution may violate that assumption, which would have unknown consequences for our estimation procedure.

The modified beta distribution is, however, quite flexible and so we suspect that this concern is less important than the first caveat.

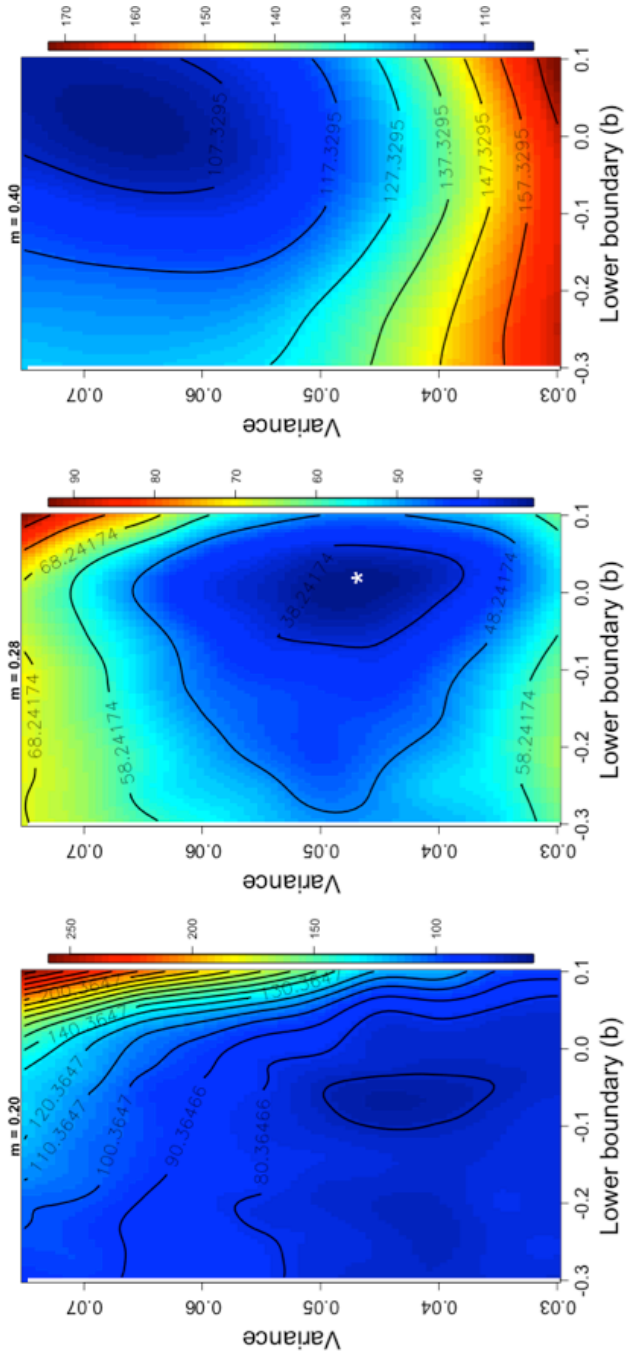

Figure C1: Spline contour plots of the deviation (measured by K) between the simulated and empirical distributions of $\hat{r}$ estimates

Figure C1: Spline contour plots of the deviation (measured by K) between the simulated and empirical distributions of estimates, as a function of the lower boundary of the beta distribution (b) and the variance of the beta distribution (s2) for three different m values. Hotter colors (red) indicate worse fit, bluer colors represent a better fit. A white star marks the combination of parameters that generate the best fit to the empirical data.

APPENDIX D

Live Fish transportation to the Peichel Lab and Animal Care

On April 17th 2013, we captured 191 inlet stream fish at site one and 206 lake fish at site one and two using unbaited minnow traps, and mark-release-recapture experiment were performed immediately afterwords. On April 18th, all recaptured individuals were placed in six sturdy 70 qt coolers filled with lake water (which these fish were accustomed to) and immediately travel to the Peichel lab at Fred Hutchingson Cancer Research Center in a pickup truck with sealed cargo area. Fish health was checked once every two hours during transportation. Portable aerators and air stones were used to keep the water oxygenized all the time during the transportation. Ice bags were wrapped in clean towels and then sealed in a Ziploc bag (so neither the ice nor the towel are in direct contact with fish) and placed along the bottom of each cooler to keep the water cool during transportation. Upon arrival, fish were placed into lab aquarium tanks using an aquarium net.

The Peichel Lab was within seven-hour driving distance from our field collection site on Vancouver Island in comparison to the five-day driving distance to the Bolnick Lab; has a quarantine room to host wild-caught sticklebacks, plus the cost of overnight internationally shipping of live fish is prohibitive (not to mention our terrible experiences with trying to get UPS customs to clear live fish in acceptably quick time), and have the facility of maintaining a health colony of wild-caught stickleback throughout the testing period (in field experiment, stickleback were trapped the night before, retrieved early in the morning and behavioral tests were performed during the same day before dawn), and thus a more suitable place to carry out behavioral test of wild-caught stickleback.

All stickleback were kept in four large fish tanks (125 gallons) measuring 72" long under summer condition with 16 hours light at a temperature of 16-18 C. Fish were

fed twice per day on frozen Mysis shrimp regularly. On behavioral test day individuals were fed after all behavioral test were finished to maintain the same standard as the Bolnick lab behavioral test and the field behavioral test.

Fertilized Eggs Transportation to the Bolnick Lab and Animal Care

Fertilized eggs were shipped back to the Bolnick Lab aquarium room at the University of Texas at Austin within six days after fertilization in coolers with chilled ice packs inside falcon tubes. On arrival, fertilized eggs were kept in petri dishes with 0.5 cm of water and daily water changes until hatching, at which point they were transferred into 100 ml beakers. After swim-up, fry were fed twice daily on freshly hatched brine shrimp nauplii until they reach 1.0 cm standard length, at which point they were transitioned onto a combination of pelleted trout chow and freeze-dried blood worms and transferred into 6 L tanks in the aquarium room (water temperature 16-17°C with 16 hours of light). These tanks were all connected to a recirculating temperature-controlled water supply system, with recirculated water sterilized by a manifold of ultraviolet lights. Individuals from different families were always kept in separate tanks during the rearing process in order to keep track of their family identities. Once individuals reached adulthood a year old, individuals were briefly transitioned into artificial winter conditions, held at 13 degrees C and 8 hours of light per day. Adults were fed twice daily on freeze-dried bloodworms. Also, to maintain a health density of individuals in the aquarium room, some randomly selected F1 lake and F1 stream families were transferred to a second aquarium room of the Bolnick Lab in 40-L tanks with a flow-through system. All other rearing conditions were kept exactly the same between the two aquarium rooms.

Dispersal Tunnel Design

The enclosed tunnel was composed of four metal mesh cylinders (1.25m length *1m diameter) connected to each other via three 50mm wide seine net sections, submerged in the inlet stream ten meters above the lake stream intersection and parallel to the direction of current. The two central metal cylinders formed a release chamber (3m length * 1m diameter). The two cylinders on each end were the collection chambers: one upstream to the release chamber and the other downstream to the release chamber. The two collection chambers were separated from the release chamber by a seine net barrier. At the center of each seine net barrier there was a metal mesh cone (half of a minnow trap) with its base opening towards the release chamber and its pointed opening (2.54 cm in diameter) facing towards the collection chamber [Figure 2.2]. With this arrangement, stickleback can easily swim from the central release chamber into either of the side collection chambers (depending on its rheotactic response). Once in the traps at the end of the release chamber, fish are unlikely to find the small opening that would allow them to return to the release area.

APPENDIX E

All animals used in this study were collected with permission from the British Columbia Ministry of Forests, Lands and Natural Resources Operations (NA11-7031 and NA13-85103). Wild-caught fish used for behavioral assays at the laboratory were transferred to the Peichel Lab at Fred Hutchinson Cancer Research Center with the permission from the British Columbia Ministry of Forests, Lands and Natural Resources Operations (VI13-86478). All collection, transportation and experimental procedures were approved by the University of Texas Institutional Animal Care and Use Committee (#AUP-2010-00059 and #AUP-2013-00027).

	Function 1			Function 2			Function 3		
Variable	Coef	r_s	r_s^2	Coef	r_s	r_s^2	Coef	r_s	r_s^2
PO	0.33	0.27	0.07	-0.82	-0.63	0.39	-0.16	-0.32	0.1
IO	0.08	-0.15	0.02	0.64	0.00	0	0.12	-0.19	0.03
OR	-0.36	-0.02	0	-0.08	-0.03	0	-0.73	-0.23	0.05
MD	0.12	0.07	0.01	-0.19	-0.14	0.02	0.03	-0.20	0.04
ET	0.10	-0.05	0	-0.13	-0.21	0.04	0.68	0.21	0.04
SO	-0.61	-0.31	0.1	0.25	0.01	0	-0.23	-0.10	0.01
OT	-0.32	-0.08	0.01	0.21	-0.30	0.09	-0.74	-0.49	0.24
AP	-0.44	-0.27	0.08	-0.27	-0.22	0.05	0.22	-0.05	0
ST	0.11	-0.12	0.01	-0.50	-0.54	0.29	-0.32	-0.14	0.02
MA	-0.16	-0.18	0.03	-0.33	-0.40	0.16	0.74	-0.09	0.01
MP	0.71	0.39	0.16	0.43	0.06	0	-0.47	-0.43	0.18
CF	0.58	0.49	0.24	0.11	0.00	0	0.60	0.19	0.04
Net Displacement	-0.07	0.15	0.02	-1.86	-0.69	0.48	-0.38	0.71	0.5
Cumulative Upstream Distance	1.26	0.36	0.13	-0.41	0.18	0.03	-0.68	-0.92	0.84
Upstream Orientation	1.35	0.41	0.17	1.05	-0.2	0.04	0.73	0.89	0.79

CV1 (full model): $R_c = 0.83$, Wilks's $\lambda = 0.18$, P= 0.018; CV2: $R_c = 0.56$, Wilks's $\lambda = 0.60$, P= 0. 33; CV3: $R_c = 0.36$, Wilks's $\lambda = 0.87$, P= 0.56

Table E1: Canonical solution for lateral lines predicting rheotactic behavior for each of the three canonical functions separately in control group wild-caught lake and stream fish prior to the breeding season

Standardized canonical function coefficients ($\boldsymbol{coef}$), structure coefficients($\boldsymbol{r_s}$), squared structure coefficients ($\boldsymbol{r_s^2}$) are given for each of the canonical functions. Important variates (structural coefficient >0.3) are indicated in bold for significant CVs.

	Function 1			Function 2			Function 3		
Variable	Coef	r_s	r_s^2	Coef	r_s	r_s^2	Coef	r_s	r_s^2
PO	0.15	0.17	0.03	0.11	0.34	0.12	0.17	0.2	0.04
IO	0.08	-0.11	0.01	0.45	0.06	0	-0.23	0.13	0.02
OR	0.47	0.47	0.22	-0.16	-0.03	0	0.06	0.07	0
MD	0.55	-0.01	0	-0.53	0.19	0.04	0.71	0.42	0.18
ET	-0.90	-0.04	0	0.11	0.39	0.15	0.43	0.44	0.2
SO	-0.50	-0.18	0.03	0.41	0.5	0.25	-0.32	-0.19	0.04
OT	0.21	0.19	0.04	-0.90	-0.19	0.04	-0.09	-0.09	0.01
AP	-0.49	-0.48	0.23	0.14	0.22	0.05	-0.13	0.09	0.01
ST	0.88	0.46	0.21	0.83	0.67	0.44	-0.30	-0.05	0
MA	-0.17	0.19	0.04	-0.18	-0.1	0.01	-0.62	-0.6	0.35
MP	-0.07	-0.04	0	0.09	0.11	0.01	-0.40	-0.08	0.01
CF	-0.02	0.25	0.06	0.27	0.02	0	0.31	0.11	0.01
Net displacement	0.16	0.37	0.14	-0.18	0.40	0.16	1.21	0.84	0.7
Cumulative upstream distance	-0.5	-0.80	0.65	-0.98	-0.59	0.35	0.69	0.02	0
Upstream Orientation	0.66	0.81	0.66	-0.89	-0.55	0.3	0.13	-0.19	0.04

CV1 (full model): $R_c = 0.94$, Wilks's $\lambda = 0.021$, $P = 0.04$; CV2: $R_c = 0.83$, Wilks's $\lambda = 0.18$, $P = 0.043$; CV3: $R_c = 0.65$, Wilks's $\lambda = 0.58$, $P = 0.08$

Table E2: Canonical solution for lateral lines predicting rheotactic behavior for each of the three canonical functions separately in lab-reared lake and stream fish prior to the breeding season

Standardized canonical function coefficients ($\boldsymbol{coef}$), structure coefficients($\boldsymbol{r_s}$), squared structure coefficients ($\boldsymbol{r_s^2}$) are given for each of the canonical functions. Important variates (structural coefficient >0.3) are indicated in bold for significant CVs.

	Function 1			Function 2			Function 3			Function 4		
Variable	Coef	r_s	r_s^2	Coef	r_s	r_s^2	Coef	r_s	r_s^2	Coef	r_s	r_s^2
Body mass	0.06	-0.47	0.22	-3.03	-0.1	0.01	-2.95	-0.46	0.21	-2.81	0.33	0.11
Standard length	-0.77	-0.31	0.09	-0.53	-0.22	0.05	2.44	-0.13	0.02	1.42	0.5	0.25
Pelvic width	0.91	-0.4	0.16	1.81	0.22	0.05	0.19	-0.31	0.1	-0.88	0.21	0.04
Width at pectoral fin	-1.89	-0.57	0.32	1.56	0.12	0.02	0.9	-0.47	0.22	2.27	0.43	0.18
Width at preoperculum	1.44	0.06	0	0.34	-0.09	0.01	-0.96	-0.47	0.23	0.64	0.69	0.47
Net displacement	-1.75	-0.28	0.08	-0.12	-0.52	0.27	0.46	-0.04	0	0.57	0.8	0.65
Cumulative distance upstream	-0.72	-0.23	0.05	-1.05	0.04	0	0.04	0.28	0.08	-0.93	-0.93	0.87
Upstream orientation	1.16	0.23	0.05	-1.38	-0.73	0.53	-0.53	-0.09	0.01	-0.51	0.64	0.41
Flow regime	0.25	0.26	0.07	0.17	-0.15	0.02	1.01	0.95	0.91	0.25	0.01	0

Table E3: Canonical solution for body size predicting rheotactic behavior for each of the four canonical functions in non-breeding wild-caught lake and stream fish

Standardized canonical function coefficients ($\boldsymbol{coef}$), structure coefficients($\boldsymbol{r_s}$), squared structure coefficients ($\boldsymbol{r_s^2}$) are given for each of the canonical functions.

	Function 1			Function 2		
Variable	Coef	r_s	r_s^2	Coef	r_s	r_s^2
Fin Length	1.52	0.63	0.39	0.06	0.78	0.61
Fin Area	-1.18	-0.04	0	0.95	1	1
Net displacement	0.442	0.77	0.59	1.183	0.421	0.18
Cumulative distance upstream	-0.977	-0.967	0.94	0.55	0.144	0.02
Upstream orientation	-0.44	0.619	0.38	-0.422	0.236	0.06
Flow regime	0.078	-0.169	0.03	0.705	0.742	0.55

Table E4: Canonical solution for pectoral fin size predicting rheotactic behavior for both canonical functions in non-breeding wild-caught lake and stream fish

Standardized canonical function coefficients ($\boldsymbol{coef}$), structure coefficients($\boldsymbol{r_s}$), squared structure coefficients ($\boldsymbol{r_s^2}$) are given for each of the canonical functions

	Function 1			Function 2			Function 3			Function 4		
Variable	Coef	r_s	r_s^2	Coef	r_s	r_s^2	Coef	r_s	r_s^2	Coef	r_s	r_s^2
RW1	0.12	0.05	0	-0.21	-0.21	0.04	-0.43	-0.46	0.21	-0.53	-0.72	0.52
RW2	0.71	0.72	0.52	-0.04	0.14	0.02	0.65	0.59	0.34	-0.31	-0.34	0.12
RW3	0.69	0.66	0.43	-0.54	-0.29	0.08	-0.44	-0.56	0.31	0.49	0.30	0.09
RW4	-0.18	0.20	0.04	0.13	0.04	0	-0.13	-0.43	0.18	-0.60	-0.59	0.35
RW5	0.16	0.39	0.15	0.94	0.85	0.72	-0.35	-0.34	0.12	0.116	0.08	0.01
Net displacement	-0.28	-0.04	0	0.37	0.92	0.84	0.67	0.3	0.09	1.72	0.26	0.07
Cumulative distance upstream	1.36	0.49	0.24	-0.16	-0.83	0.7	-0.01	0	0	0.77	0.26	0.07
Upstream orientation	1.47	0.25	0.06	0.51	0.91	0.83	-0.43	0.22	0.05	-1.1	-0.24	0.06
Flow regime	-0.27	0.14	0.02	-0.24	-0.24	0.06	0.97	0.92	0.84	-0.29	-0.29	0.08

Table E5: Canonical solution for body shape predicting rheotactic behavior for the two canonical functions in non-breeding wild-caught lake and stream fish

Standardized canonical function coefficients ($\boldsymbol{coef}$), structure coefficients($\boldsymbol{r_s}$), squared structure coefficients ($\boldsymbol{r_s^2}$) are given for each of the canonical functions.

	Function 1			Function 2			Function 3			Function 4		
Variable	Coef	r_s	r_s^2	Coef	r_s	r_s^2	Coef	r_s	r_s^2	Coef	r_s	r_s^2
RW1	0.79	0.63	0.39	-0.46	-0.24	0.06	0.39	0.48	0.23	-0.37	-0.56	0.31
RW2	-0.51	-0.33	0.11	-0.55	-0.19	0.04	-0.38	-0.30	0.09	-0.60	-0.78	0.62
RW3	-0.57	-0.35	0.12	-0.22	-0.12	0.01	0.51	0.33	0.11	-0.23	-0.31	0.09
RW4	-0.51	-0.25	0.06	0.19	0.03	0.00	0.78	0.67	0.45	0.11	0.18	0.03
RW5	-0.06	-0.07	0.00	-1.09	-0.69	0.48	-0.09	0.00	0.00	0.33	0.72	0.52
Net displacement	0.37	0.11	0.01	0.76	0.94	0.89	0.35	0.30	0.09	-1.21	-0.11	0.01
Cumulative distance upstream	0.73	0.32	0.10	-0.33	-0.83	0.69	0.32	-0.07	0.01	-1.16	-0.45	0.20
Upstream orientation	0.68	0.57	0.33	-0.15	0.19	0.04	0.49	0.64	0.40	0.68	0.48	0.23
Flow regime	0.61	0.56	0.31	0.19	0.21	0.04	-0.76	-0.80	0.64	0.20	0.07	0.01

Table E6: Canonical solution for body shape predicting rheotactic behavior for each of the four canonical functions in lateral-line-ablated lake and stream fish prior to the breeding season

Standardized canonical function coefficients ($\boldsymbol{coef}$), structure coefficients($\boldsymbol{r_s}$), squared structure coefficients ($\boldsymbol{r_s^2}$) are given for each of the canonical functions.

	Function 1			Function 2			Function 3			Function 4		
Variable	Coef	r_s	r_s^2	Coef	r_s	r_s^2	Coef	r_s	r_s^2	Coef	r_s	r_s^2
Body mass	0.75	-0.33	0.11	1.46	0.12	0.01	3.75	0.54	0.29	-1.15	-0.21	0.04
Standard length	1.05	-0.44	0.19	-0.64	-0.11	0.01	-2.61	0.31	0.1	-1.90	-0.42	0.18
Pelvic width	0.73	0.02	0	-1.45	-0.22	0.05	0.53	0.50	0.25	0.58	0.23	0.05
Width at pectoral fin	-0.47	-0.26	0.07	1.31	0.26	0.07	-2.00	0.359	0.13	0.96	0.145	0.02
Width at preoperculum	-2.43	-0.65	0.42	-0.88	-0.13	0.02	0.65	0.38	0.14	1.52	-0.21	0.04
Net displacement	-0.59	0.06	0	0.55	0	0	0.39	-0.32	0.1	-1.23	-0.94	0.89
Cumulative distance upstream	-0.79	-0.39	0.15	1.06	0.54	0.3	0.53	0.47	0.22	-0.23	0.58	0.33
Upstream orientation	0.17	0.04	0	0.55	0.46	0.21	-0.87	-0.88	0.78	0.31	-0.09	0.01
Flow regime	0.88	0.83	0.68	0.43	0.4	0.16	0.26	0.38	0.15	-0.03	-0.09	0.01

Table E7: Canonical solution for body size predicting rheotactic behavior for each of the four canonical functions in lateral-line-ablated lake and stream fish

Standardized canonical function coefficients ($\boldsymbol{coef}$), structure coefficients($\boldsymbol{r_s}$), squared structure coefficients ($\boldsymbol{r_s^2}$) are given for each of the canonical functions.

	Function 1			Function 2		
Variable	Coef	r_s	r_s^2	Coef	r_s	r_s^2
Fin Length	1.75	0.68	0.47	-0.26	0.73	0.53
Fin Area	-1.29	0.15	0.02	1.21	0.99	0.98
Net displacement	-0.594	-0.656	0.43	1.388	0.461	0.21
Cumulative distance upstream	0.083	0.485	0.23	1.048	0.142	0.02
Upstream orientation	0.025	-0.133	0.02	-0.443	-0.117	0.01
Flow regime	-0.752	-0.762	0.58	-0.451	-0.352	0.12

Table E8: Canonical solution for pectoral fin size predicting rheotactic behavior for the two canonical functions in lateral-line-ablated lake and stream fish.

Standardized canonical function coefficients ($\boldsymbol{coef}$), structure coefficients($\boldsymbol{r_s}$), squared structure coefficients ($\boldsymbol{r_s^2}$) are given for each of the canonical functions

	Function 1			Function 2		
Variable	Coef	r_s	r_s^2	Coef	r_s	r_s^2
Fin Length	1.26	0.48	0.23	0.45	0.88	0.77
Fin Area	-1.17	-0.34	0.11	0.64	0.94	0.89
Net displacement	0.68	0.03	0	0.33	0.21	0.05
Cumulative distance upstream	1.07	0.73	0.54	-0.13	-0.46	0.21
Upstream orientation	-0.06	-0.37	0.14	0.47	0.63	0.4
Flow regime	-0.5	-0.35	0.12	-0.75	-0.77	0.59

CV1 (full model): $R_c = 0.72$, Wilks's $\lambda = 0.46$, $P = 0.044$; CV2: $R_c = 0.22$, Wilks's $\lambda = 0.92$, $P = 0.62$

Table E9: Canonical solution for pectoral fin size predicting rheotactic behavior for both canonical functions in non-breeding lab-reared lake and stream fish

Standardized canonical function coefficients ($\boldsymbol{coef}$), structure coefficients($\boldsymbol{r_s}$), squared structure coefficients ($\boldsymbol{r_s^2}$) are given for each of the canonical functions. Important variates (structural coefficient >0.3) are indicated in bold for significant CVs.

	Function 1			Function 2			Function 3			Function 4		
Variable	Coef	r_s	r_s^2	Coef	r_s	r_s^2	Coef	r_s	r_s^2	Coef	r_s	r_s^2
Body mass	2.10	0.12	0.01	2.6	0.41	0.17	-0.42	0.67	0.45	0.27	0.61	0.37
Standard length	-1.74	-0.32	0.1	-1.12	0.32	0.1	1.14	0.81	0.66	-0.27	0.37	0.14
Pelvic width	-0.52	0.15	0.02	0.75	0.53	0.28	-0.39	0.42	0.17	-0.13	0.40	0.16
Width at pectoral fin	0.73	0.454	0.21	-1.62	0.041	0	1.123	0.614	0.38	-0.38	0.545	0.3
Width at preoperculum	-0.85	0.06	0	-0.61	0.05	0	-0.66	0.26	0.07	1.26	0.96	0.91
Net displacement	-0.54	-0.19	0.04	-0.26	0.1	0.01	1.09	0.88	0.78	0.1	-0.42	0.18
Cumulative distance upstream	-0.89	-0.61	0.37	-0.44	-0.47	0.22	0.24	-0.45	0.21	0.81	0.45	0.2
Upstream orientation	-0.01	0.44	0.19	0.52	0.52	0.27	0.38	0.28	0.08	0.92	0.68	0.46
Flow regime	-0.55	-0.66	0.43	0.82	0.67	0.44	-0.29	-0.14	0.02	-0.16	-0.32	0.1

Table E10: Canonical solution for body size predicting rheotactic behavior for each of the four canonical functions in non-breeding lab-reared lake and stream fish

Standardized canonical function coefficients ($\boldsymbol{coef}$), structure coefficients($\boldsymbol{r_s}$), squared structure coefficients ($\boldsymbol{r_s^2}$) are given for each of the canonical functions.

	Function 1			Function 2			Function 3			Function 4		
Variable	Coef	r_s	r_s^2	Coef	r_s	r_s^2	Coef	r_s	r_s^2	Coef	r_s	r_s^2
RW1	0.84	0.38	0.14	0.68	0.57	0.32	0.11	0.40	0.16	0.00	-0.05	0
RW2	0.07	0.39	0.15	-0.47	-0.49	0.24	0.92	0.75	0.57	-0.14	-0.03	0
RW3	0.98	0.60	0.36	0.00	-0.62	0.37	-0.60	-0.42	0.18	-0.48	-0.30	0.09
RW4	-0.26	-0.34	0.12	0.14	0.36	0.13	0.16	0.10	0.01	-1.00	-0.86	0.74
RW5	0.48	-0.04	0	0.64	0.52	0.27	-0.05	0.11	0.01	-0.01	0.23	0.05
Net displacement	0.63	0.08	0.01	0.57	0.07	0.01	-0.22	-0.11	0.01	-0.9	-0.99	0.98
Cumulative distance upstream	0.94	0.72	0.52	0.87	0.22	0.05	0.19	0.35	0.12	0.12	0.56	0.31
Upstream orientation	-0.31	-0.59	0.35	1.07	0.7	0.5	-0.15	-0.38	0.14	0.07	0.12	0.02
Flow regime	-0.41	-0.22	0.05	0.18	0.08	0.01	0.93	0.92	0.84	-0.13	-0.32	0.1

Table E11: Canonical solution for body shape predicting rheotactic behavior for each of the four canonical functions in non-breeding lab-reared lake and stream fish

Standardized canonical function coefficients ($\boldsymbol{coef}$), structure coefficients($\boldsymbol{r_s}$), squared structure coefficients ($\boldsymbol{r_s^2}$) are given for each of the canonical functions.

	Function 1			Function 2		
Variable	Coef	r_s	r_s^2	Coef	r_s	r_s^2
Fin Length	1.93	0.78	0.61	-0.81	0.62	0.39
Fin Area	-1.31	0.39	0.15	1.64	0.92	0.85
Net displacement	-0.571	-0.302	0.09	-1.014	-0.944	0.89
Cumulative distance upstream	0.382	-0.052	0	-0.369	0.69	0.48
Upstream orientation	-0.051	-0.39	0.15	0.029	-0.8	0.64
Flow regime	-1.235	-0.67	0.45	0.468	0.683	0.47

Table E12: Canonical solution for pectoral fin size predicting rheotactic behavior for both canonical functions in wild-caught breeding lake and stream fish

Standardized canonical function coefficients ($\boldsymbol{coef}$), structure coefficients($\boldsymbol{r_s}$), squared structure coefficients ($\boldsymbol{r_s^2}$) are given for each of the canonical functions. Important variates (structural coefficient >0.3) are indicated in bold for significant CVs.

	Function 1			Function 2			Function 3			Function 4		
Variable	Coef	r_s	r_s^2	Coef	r_s	r_s^2	Coef	r_s	r_s^2	Coef	r_s	r_s^2
Body mass	-1.25	0.69	0.48	-3.70	0.07	0.01	-1.56	0.00	0.00	4.81	0.46	0.22
Standard length	1.57	0.74	0.55	2.97	0.26	0.07	-3.56	-0.05	0.00	-2.01	0.39	0.16
Pelvic width	-1.30	0.57	0.33	1.64	0.33	0.11	2.60	0.16	0.02	1.38	0.48	0.23
Width at pectoral fin	-0.10	0.65	0.42	-0.04	0.08	0.01	0.07	0.06	0.00	-3.01	0.27	0.07
Width at preoperculum	1.82	0.83	0.69	-0.66	0.07	0.00	2.61	0.16	0.02	-0.82	0.33	0.11
Net displacement	-0.93	-0.93	0.87	1.01	0.34	0.12	-1.69	0.10	0.01	1.33	-0.03	0.00
Cumulative distance upstream	0.43	0.69	0.48	1.07	-0.21	0.04	0.68	0.04	0.00	1.29	0.69	0.48
Upstream orientation	0.06	-0.86	0.75	-0.53	0.10	0.01	2.22	0.49	0.24	-0.35	-0.06	0.00
Flow regime	-0.52	0.21	0.04	-1.22	-0.76	0.58	-0.64	-0.09	0.01	0.20	0.61	0.37

Table E13: Canonical solution for body size predicting rheotactic behavior for each of the four canonical functions in wild-caught breeding lake and stream fish

Standardized canonical function coefficients ($\boldsymbol{coef}$), structure coefficients($\boldsymbol{r_s}$), squared structure coefficients ($\boldsymbol{r_s^2}$) are given for each of the canonical functions.

	Function 1			Function 2			Function 3			Function 4		
Variable	Coef	r_s	r_s^2	Coef	r_s	r_s^2	Coef	r_s	r_s^2	Coef	r_s	r_s^2
RW1	0.35	0.37	0.14	-0.69	-0.56	0.31	0.10	0.35	0.12	-0.63	-0.38	0.15
RW2	0.44	0.65	0.42	0.19	-0.03	0.00	0.63	0.61	0.37	0.73	0.46	0.21
RW3	0.65	0.61	0.37	0.02	0.06	0.00	-0.76	-0.73	0.54	0.10	0.25	0.06
RW4	-0.37	-0.34	0.12	0.19	0.05	0.00	0.06	-0.10	0.01	0.54	0.29	0.08
RW5	0.25	0.24	0.06	0.80	0.76	0.58	0.15	0.20	0.04	-0.48	-0.51	0.26
Net displacement	2.01	0.36	0.13	0.53	-0.62	0.39	0.22	0.62	0.38	1.47	0.32	0.10
Cumulative distance upstream	0.83	0.29	0.09	-0.82	0.35	0.12	-1.43	-0.79	0.63	0.23	-0.40	0.16
Upstream orientation	-0.90	0.20	0.04	-1.52	-0.80	0.64	-0.24	0.56	0.32	-1.47	-0.07	0.00
Flow regime	0.50	0.43	0.19	0.82	0.49	0.24	0.90	-0.15	0.02	-0.70	-0.74	0.55

Table E14: Canonical solution for body shape predicting rheotactic behavior for each of the four canonical functions in wild-caught breeding lake and stream fish

Standardized canonical function coefficients ($\boldsymbol{coef}$), structure coefficients($\boldsymbol{r_s}$), squared structure coefficients ($\boldsymbol{r_s^2}$) are given for each of the canonical functions.

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