

SEXUAL DIMORPHISM AND SPECIATION ON TWO ECOLOGICAL COINS: PATTERNS FROM NATURE AND THEORETICAL PREDICTIONS

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Adaptive divergence of phenotypes, such as sexual dimorphism or adaptive speciation, can result from disruptive selection via competition for limited resources. Theory indicates that speciation and sexual dimorphism can result from identical ecological conditions, but co-occurrence is unlikely because whichever evolves first should dissipate the disruptive selection necessary to drive evolution of the other. Here, we consider ecological conditions in which disruptive selection can act along multiple ecological axes. Speciation in lake populations of threespine sticklebacks (*Gasterosteus aculeatus*) has been attributed to disruptive selection due to competition for resources. Head shape in sticklebacks is thought to reflect adaptation to different resource acquisition strategies. We measure sexual dimorphism and species variation in head shape and body size in stickleback populations in two lakes in British Columbia, Canada. We find that sexual dimorphism in head shape is greater than interspecific differences. Using a numerical simulation model that contains two axes of ecological variation, we show that speciation and sexual dimorphism can readily co-occur when the effects of loci underlying sexually dimorphic traits are orthogonal to those underlying sexually selected traits.

KEY WORDS: Adaptive radiation, adaptive splitting, disruptive selection, ecological dimorphism, stickleback species pair.

Phenotypic differences between sexes or species can result from disruptive ecological selection, and can act to reduce competition in a population (Rueffler et al. 2006 and references therein). To understand more fully the process of differentiation in nature, either between or within species, it is necessary to investigate the forms adaptive splitting may take and the possible mechanisms that may explain the prevalence of sexual dimorphism and speciation. In this study, we employ two approaches to investigate the evolution of sexual dimorphism and speciation: an empirical approach to quantify the concurrent phenotypic variation between sexes and species in nature and a modeling approach to explore the conditions under which such variation can arise. Through these approaches we address the following questions in

threespine stickleback species pairs: (1) How do shape and size vary between sexes and species? (2) Is this phenotypic variation consistent with ecological disruptive selection? (3) Under what conditions might sexual dimorphism and speciation both evolve in a biologically realistic selective environment?

Ecological speciation is defined as the process by which barriers to gene flow evolve among populations as a result of ecologically based divergent selection (Rundle and Nosil 2005), and may also be adaptive speciation if the splitting process itself is an adaptive response to frequency-dependent disruptive selection (Dieckmann et al. 2004). Past models have explored adaptive speciation (Metz et al. 1996; Geritz et al. 1998; Doebeli and Dieckmann 2000, 2003) and the alternative outcomes of

sexual dimorphism and adaptive speciation in response to ecological disruptive selection (Bolnick and Doebeli 2003). Populations may split into separate lineages (Geritz et al. 1998; Kisdi and Geritz 1999; Doebeli and Dieckmann 2000, 2003) or evolve sexual dimorphism within a lineage (Slatkin 1984; Bolnick and Doebeli 2003), and either outcome can serve to dissipate disruptive selection. Whether speciation or sexual dimorphism occurs is influenced by the genetic independence of male and female traits and the strength of assortative mating (Bolnick and Doebeli 2003). Ecological sexual dimorphism and adaptive speciation can result from identical ecological conditions, but because either may eliminate the selective pressure driving the other, they have been called “two sides of the same ecological coin” (Bolnick and Doebeli 2003), and it has been assumed that they are unlikely to evolve in the same population.

Few empirical studies have addressed the potential for the simultaneous evolution of sexual dimorphism and speciation in response to resource competition, but some studies have investigated the relationship between species richness and sexual dimorphism in the context of ecological release. The presence of competitor species may constrain the evolution of sexual dimorphism, but following colonization of a new habitat without interspecific competition, sexual dimorphism can evolve to alleviate competition within the species (Schoener 1967, Shine 1989). For example, Poe and colleagues (2007) found that shifts from living in sympatry to solitary existence on an island were followed by increased sexual size dimorphism in *Anolis* lizards. In emydid turtles, however, Stephens and Wiens (2009) found only equivocal support for such a relationship. An empirical study on sticklebacks by Bolnick and Lau (2008) shows that sexual differentiation in trophic traits arises from intraspecific competition and mitigates disruptive selection. Certainly, ecological sexual dimorphism is prevalent in some species-rich groups, particularly in *Anolis* lizards (Butler and Losos 2002 and references therein) and some bird groups (woodpeckers: Selander 1966, hummingbirds: Temeles et al. 2000), which may have evolved under multiple ecological dimensions. The idea that multiple ecological dimensions can allow for coexistence of multiple groups is prevalent in niche partitioning and niche packing literature (Attrill and Power 2004, Friggens and Brown 2005, Gilbert et al. 2008). Sexual dimorphism allows for divergence in a population, but is limited to two groups, and subsequent ecological speciation could allow for the formation of additional groups that can more effectively fill niche space. Recently, Butler and colleagues (2007) showed that sexual differences contribute to ecomorphological diversity in *Anolis* lizards, with the sexes occupying nonoverlapping morphological space, similar to the space occupied by different species.

Theoretical work on the evolution of speciation and sexual dimorphism has focused on selective landscapes that include disruptive selection acting on a single ecologically important trait.

For example, individuals might compete for prey of differing sizes, where each individual is best able to consume a particular size. Competition will then be strongest between individuals that consume similar resources (Schluter 2003, Bolnick and Paull 2009). Those with extreme phenotypes may experience less competition, in which case selection on the trait will be disruptive. Competition in a single ecological dimension can be alleviated by adaptive splitting, either through sexual dimorphism or speciation (Bolnick and Doebeli 2003). In real systems however, disruptive selection may occur along any number of dimensions. For example, individuals might be differently adapted to forage on prey of different sizes and, at the same time, to forage on prey in different locations. To understand the evolution of adaptive divergence in complex selective landscapes, it is useful to consider multidimensional traits such as shape that may reflect adaptation to multiple selective pressures. Most previous studies of sexual dimorphism and species richness focused on sexual size dimorphism alone, an exception being a study by Butler and Losos (2002) which indicated that in *Anolis* lizards, interspecific ecological variation for each sex was related to shape more strongly than to size.

Threespine sticklebacks present an excellent opportunity to address patterns of sexual dimorphism and speciation empirically. Freshwater species pairs include limnetic and benthic species that evolved multiple, independent times from a marine ancestor (Schluter and McPhail 1992, 1993), and are prime examples of ecological speciation via divergent selection (Hatfield and Schluter 1999, Rundle et al. 2000, McKinnon and Rundle 2002, Boughman 2007). The process of speciation in the benthic/limnetic species pairs is thought to have both allopatric and sympatric phases (McPhail 1993, 1994, Rundle and Schluter 2004), likely with ecologically based divergent viability and sexual selection in both phases (McKinnon and Rundle 2002).

Speciation occurred rapidly but rarely as there are just seven lakes with species pairs in coastal British Columbia. Within stickleback species pairs, the small, narrow limnetic species feeds on zooplankton in open water and the large, deep-bodied benthic species feeds on invertebrates in the littoral zone. In marine and freshwater populations, head shape and overall body shape are under divergent selection through foraging efficiency, in which deeper bodies, larger heads, and stronger jaw mechanics enable better prey capture and consumption in a benthic environment whereas slender bodies and larger eyes enable specialization on small, limnetic prey (Webb 1984, Liem 1993, Caldecutt and Adams 1998, Blake 2004). Within benthic and limnetic species, the sexes may also differ in foraging niche as their life histories diverge, particularly during the breeding season. Males of both species build nests and care for young in shallow littoral areas, which is more benthic-like habitat; whereas female limnetics feed in pelagic habitat and come inshore primarily to mate and benthic females appear to use more limnetic-like habitat than males

(Bentzen and McPhail 1984). By examining interspecific and intersexual differences in traits under divergent selection, specifically head shape, which has been observed to affect foraging efficiency in species pairs (Bentzen and McPhail 1984, Schluter 1995), we can better understand the sources of selection causing adaptive divergence. Because trophic morphology is primarily in the head region, measures of head shape may best capture variation related to foraging niche divergence.

We used geometric morphometric analyses to quantify variation in head shape and body size for sticklebacks in two lakes in British Columbia. One of the lakes, Paxton Lake, currently contains a species pair. The second lake, Enos Lake, contained a species pair in the past (Larson 1976; McPhail 1984, 1992) but the species have recently undergone high levels of gene flow and have collapsed into a hybrid swarm (Taylor et al. 2006). Shape variation in Paxton Lake indicates how much interspecific and intersexual variation can be present concurrently. We compare this with Enos Lake to study a population in which the speciation process has collapsed.

We complement our empirical approach by using simulation models to test hypotheses about the environmental conditions that may lead to the patterns of variation we measure in nature. We expanded the Bolnick–Doebeli model (2003) to include resource competition in two ecological dimensions. Our primary objectives were to determine whether sexual dimorphism and speciation can evolve together when disruptive selection acts in more than one ecological dimension, and to identify the biological factors that make the co-occurrence of these two results more or less likely. The model is not meant to represent the evolutionary history of the species pairs precisely, as speciation in nature may occur in a variety of scenarios that are neither purely allopatric nor purely sympatric (McKinnon and Rundle 2002). Nor do we aim to directly test our model of adaptive splitting with empirical data from stickleback species pairs. Rather, our modeling approach explores the possibility of simultaneous speciation and sexual dimorphism and is combined with our empirical approach to offer insight into how environmental complexity may allow for multiple adaptive splits. In essence, we ask: What is the evolutionary outcome when there are two ecological coins?

Empirical Methods

FIELD COLLECTIONS AND SPECIES DESIGNATIONS

Fish were collected from Paxton and Enos Lakes over a two-week period in April 2008 using minnow traps and dip nets. The Enos Lake fish were chosen to represent the most extreme variation between individuals in the hybridizing population. Previous studies have documented a loss in species-distinctive shape (Taylor et al. 2006), so this sampling scheme was designed to give us the greatest ability to detect remaining differentiation. In both

lakes, individuals with even a small amount of nuptial coloration were identified as male and gravid individuals were identified as female.

Paxton Lake is inhabited by a benthic/limnetic stickleback species pair (McPhail 1992, Schluter and McPhail 1992). We identified individual fish to species using a two-step cluster analyses in SPSS (version 16) implemented in the first two principal components of shape and on centroid size, which are variables measured for each fish as described below. This method allowed us to determine the structure of clustering data without prior knowledge of species type (Baylac et al. 2003). We randomized the order of individuals and repeated the cluster analysis ten times. Models were compared with the assumption that none were favored a priori, and the optimal number of clusters was determined by comparing the Schwarz's Bayesian criterion (BIC) calculated for each potential number of clusters (Fraley and Raftery 1998). The most parsimonious model was identified by a low BIC value and large ratio of BIC change with the addition of more clusters, which indicates that dividing the data into additional clusters is less parsimonious. Our analysis identified two distinct morphometric clusters within each sex in the Paxton Lake population (Fig. S1 and Table S1). Individuals were assigned to the same cluster more than 98% of the time, which indicates that our categorization was robust. Size and shape of fish within clusters corresponded to the benthic and limnetic species described in earlier studies (e.g., Taylor et al. 2006), and thus we refer to these clusters as the benthic and limnetic species. We use these species assignments for species and species \times sex comparisons of shape and size. The optimal number of clusters is shown in bold in Table S1, determined by low BIC values, and large ratio of BIC changes and ratio of distance measures if more than one cluster. A lower cluster number is more parsimonious, and was chosen if BIC numbers were relatively low and there was not a substantial change in the number with additional clusters.

In Enos Lake, there is evidence of extensive hybridization and gene flow between what was historically two species (Taylor et al. 2006). We performed a clustering analysis, but found no evidence of separate morphometric clusters in the Enos Lake population (Table S1 and Fig. S1). Therefore, our designations for fish at the extremes are referred to as limnetic-like and benthic-like for Enos fish.

SHAPE AND SIZE ANALYSIS

We collected fish and measured shape differences between sexes, populations, and species of sticklebacks using a geometric morphometrics approach (Bookstein 1991, Rohlf 1999). From Paxton Lake, we analyzed 120 limnetics and 115 benthics and from Enos Lake we analyzed 110 fish, with similar sample sizes for males and females within each lake. We photographed the left side of live fish using a digital camera (Canon G7; Canon

U.S.A Lake Success, NY) against a constant neutral background to account for fluctuating natural light conditions. We used live, unanesthetized fish for the photographs because we were collecting other behavioral and genetic data on these individuals (reported elsewhere), which resulted in some variation in position. We selected 14 landmarks, 13 in the head region and one on the tail (Fig. 1). These landmarks were a subset of those used in previous studies of stickleback shape (Taylor et al. 2006; Albert et al. 2008). They mark solid homologous structures, except for pseudo-landmark 10 that captures the hump-shaped region in the dorsal part of the head. All landmarks were sufficiently reliable and reproducible in the photographs to be digitized using the program TPSDIG (Rohlf 1997). Only the 13 landmarks in the head were used in the shape analysis. The landmark in the tail was used in the overall size measure, as well as to align the photographs before shape measurement because we found that the photographs aligned better with this additional tail point.

We estimated size for each individual by calculating the geometric mean of the distance between each landmark and the centroid point of the polygon formed by all of the landmarks. This method standardized the measurement of size for fish with different shapes. We then aligned the x and y coordinates of the landmarks using a generalized procrustes alignment in the PAST program (Hammer et al. 2001, <http://folk.uio.no/ohammer/past/>), which removed nonshape variation such as isometric aspects of size, position, and orientation in the photograph. After alignment, we used the 13 landmarks on the head to obtain principal components that described the major variation in head shape. Principal components were calculated for Paxton Lake fish alone (the Paxton-only analysis) to look at the distribution of shapes within that lake and to differentiate both sexes and species, and components were calculated from both lakes together (the combined analysis) to compare the magnitude of sex differences and species differences within and between lakes. To determine whether there were effects of sex and species on shape within and between lakes, we used a multivariate analysis of covariance (MANCOVA) in SPSS (version 16) in which the dependent variables were the 26 principal components, and the independent variables were lake, species, sex, and their interactions, and the covariate was size. To compare statistically the magnitude of sex and species differences between lakes, we assigned species to two clusters in Enos Lake to identify the most benthic-like and most limnetic-like fish, even though the cluster analysis above indicates that there is only one cluster in Enos Lake. We also compared centroid size using univariate ANOVA with a posthoc Tukey test.

We compared the magnitude of sex and species differences in both lakes by measuring the distance between groups. To do this, we compared vectors comprised of the mean values for each shape variable. We calculated the length of the distance vector between groups, which we used as a measure of overall differences between

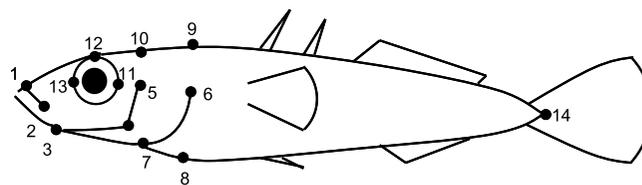


Figure 1. Location of the 14 landmarks used in the morphometric analysis of three-spined stickleback shape, based on the consensus configuration: (1) anterior tip of upper lip; (2) posterior extent of maxilla; (3) anterior edge of angular (or preopercular); (4) posterior edge of angular; (5) dorsal point of angular; (6) posteriodorsal extent of operculum; (7) posteroventral extent of preopercular; (8) posterior tip of ectocoracoid; (9) point of intersection between the dorsal midline and the line posterior to the top of the eye; (10) the midpoint of the line between the top of the eye and the intersection with the dorsal midline, immediately lateral to the supraoccipital notch; (11) posterior point of the left eye; (12) dorsal point of the left eye; (13) anterior point of the left eye; (14) caudal border of hypural plate at the lateral midline.

sexes or species for each lake. Vector length gives a single value for divergence, which we compared using t -tests. We also indicate the relative contribution of each trait axis to the differences between the groups as the absolute value of the difference between the groups for each trait divided by the length of the distance vector.

To determine whether some components of shape are allometric, we calculated Pearson correlations between size and the first five PC values for each lake. We also calculated partial correlations to partition out the effects of species or sex differences.

Empirical Results

In the combined analysis using fish from both Paxton and Enos Lakes, the first six principal components have eigenvalues greater than one in the correlation matrix, and together explain more than 83% of the variation in the data according to the variance-covariance matrix (Table S2). Although the correlation matrix gives a guideline of how many eigenvalues are informative, we used the variance-covariance matrix in all statistical analyses. Sexual dimorphism made up a substantial amount of the variation in head shape. In both Paxton and Enos Lakes, sexes separated along the first principal component (PC1) axis (Fig. 2). The amount of variation along PC1 was similar between the lakes. Within sexes in Paxton Lake, the shape data clustered into two groups along the second principal component (PC2) axis. This pattern of clustering was similar in the combined (Fig. 2) and in the Paxton Lake only (Fig. S1) analyses. The loadings of each landmark onto the PC axes for the combined data and for Paxton Lake only data are shown in Tables S3 and S4, respectively. The variances explained by the axes in Paxton Lake

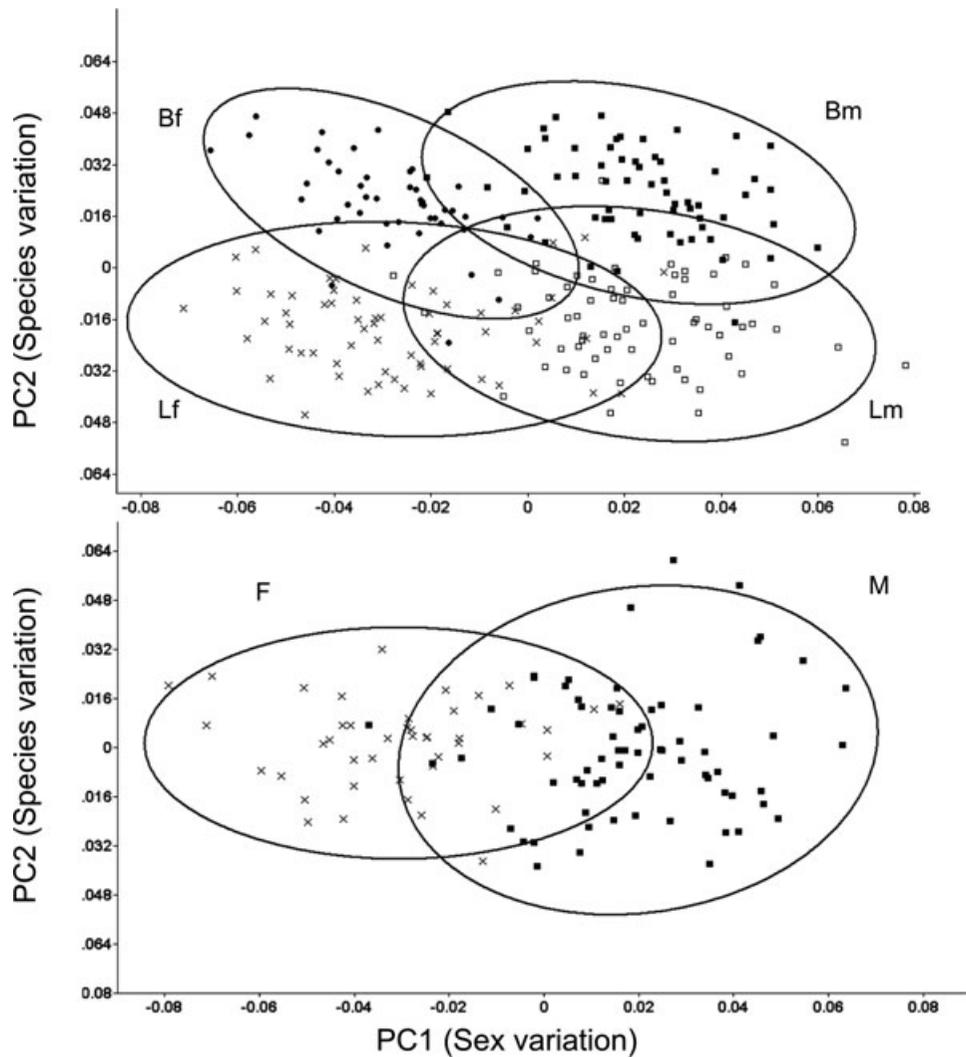


Figure 2. Scatterplot of fish from Paxton Lake (A) and Enos Lake (B) on PC1 and PC2 computed from combined data. In both lakes, sexes differ along PC1 with females (F) having smaller PC1 scores than males (M). In Paxton, species differ along PC2 with limnetic fish (L) having smaller PC2 scores than benthic fish (B), and there are not separate species groups for limnetic and benthics in Enos.

are shown in Table S5 and are similar to that explained by the lakes combined.

A visual representation of how shape differed between sexes and species is shown in Fig. 3. Thin-plate spline plots indicate the shape differences of the most extreme male, female, limnetic and benthic individuals compared to the consensus shape. Each shape is plotted on a grid that shows deformation from the square, consensus grid. Sexual dimorphism was determined mostly by anterior–posterior differences in the position of landmarks indicating head length, mouth size, and mouth orientation (see PC1 loadings in Table S4). Compared to females, males had lengthened heads and mouths, larger eyes, and greater spaces between their angulars and opercula. Shape differences between species were due mostly to dorsal–ventral variation in landmarks that corresponded to head depth, mouth orientation, and location of

the posterior edge of the angular (see PC2 loadings in Table S4). Compared to benthic fish, limnetic fish had shorter heads, larger eyes, mouths pointed more forward, angulars at greater angles; they are also more slender (Fig. 3).

In the combined data, sex differences were explained by PC1 (Table 1; $F_{1,341} = 511.2$, $P > 0.001$), which accounted for 37% of the variance in head shape. The Eta square value indicating effect size is highest for this comparison, at a value of 0.603. PCs 2, 4, and 5 also differed significantly between the sexes but had much smaller effect sizes. The species differences were explained primarily by PC2 ($F_{1,341} = 80.9$, $P < 0.001$, $\eta^2 = 0.194$) and to a lesser extent by PC1 ($\eta^2 = 0.068$). Lake differences are seen mostly in PC3 ($F_{1,341} = 75.5$, $P < 0.001$, $\eta^2 = 0.183$). The results that PC1 explains sex variation and PC2 explains species variation are seen more clearly in the analysis of Paxton Lake only

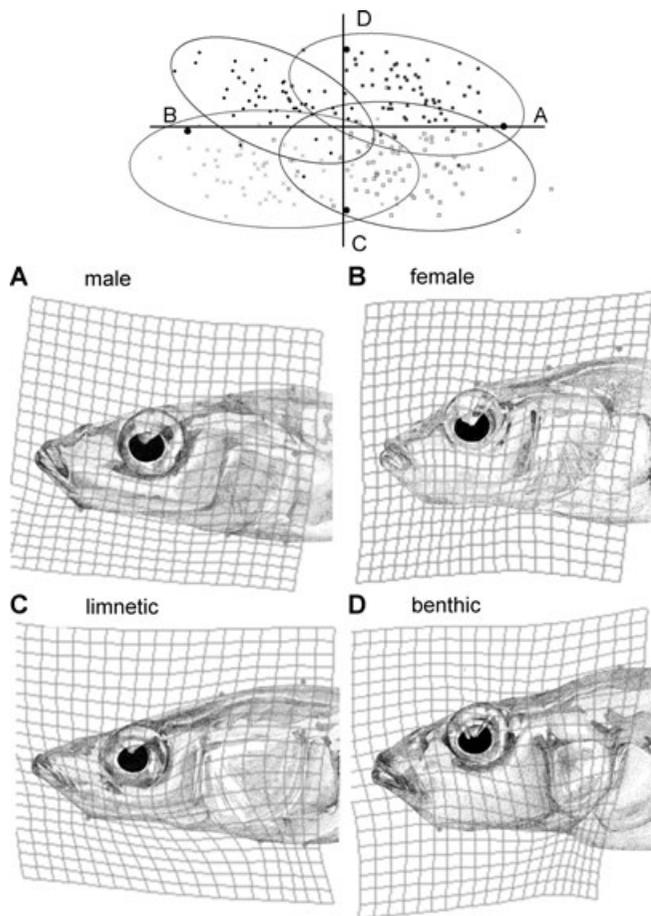


Figure 3. Thin-plate spline plots indicating shape deviation from the consensus shape. Male (A), female (B), limnetic (C), and benthic (D) shapes are indicated in their respective plots as well as on the scatterplot of shape in Paxton Lake (Figure 2A). To help visualize the shape differences, pen drawings were made on top of each thin-plate spline plot, showing the head traits based on the location of the landmarks in the spline plots (drawings by I. Cooper).

(Table S6) because the analysis then includes only the patterns within a species-pair lake.

We compared the magnitude of sex and species differences in both lakes by measuring the length of the vectors between the means of size and shape values between the groups. Table 2 shows the total divergence between species and sexes for each lake, measured as the length of the difference vectors between groups, which was calculated using shape and size data together as well as shape only. Also shown is the contribution of the first five PC axes to the total amount of divergence. Two-tailed *t*-tests indicate that sex differences in size and shape are significantly greater than species differences in Enos ($t = 28.5299$, $df = 218$, $P < 0.005$) but not in Paxton ($t = 1.5087$, $df = 268$, $P = 0.1326$). For shape only data, sex differences are greater than species differences in

both Enos ($t = 47.4273$, $df = 218$, $P < 0.005$) and Paxton ($t = 110.4936$, $df = 268$, $P < 0.005$).

In Paxton Lake, benthic fish were larger than limnetic fish (ANOVA; $F_{1,408} = 339.38$, $P < 0.001$), and both species showed sexual size dimorphism (Fig. 4A; (species \times sex effect: $F_{1,408} = 18.425$, $P < 0.0001$). Benthic females were larger than benthic males (Tukey; $P < 0.002$) whereas limnetic females were smaller than limnetic males ($P < 0.005$). In Enos Lake, limnetic-like females are smaller than limnetic-like males ($P < 0.008$) similar to Paxton; however, there is no size difference between the benthic sexes (Fig. 4B; $P = 0.998$). The difference between the lakes is mainly due to benthic-like females being smaller in Enos than in Paxton ($P < 0.001$).

To determine whether any aspects of shape are allometric, we calculated correlations between size and the first five principle components (Table 3). The lakes differ in which PCs are allometric. The largest positive correlation suggesting allometry is for PC2 in Paxton Lake. This correlation disappears when species is controlled for, but remains when sex is controlled for, which indicates that the shape difference between species may be partly caused by size differences. Size is not the entire explanation however, because the MANCOVA shows a significant effect of PC2 on species differences when size is included as a covariate ($F = 4.652$, $P = 0.032$). We found moderate correlations suggesting weaker allometric relationships for all the other PCs in Paxton Lake when either species or sex are controlled, indicating that part of the differences in shape between species and/or sex are due to size for these PCs. Weaker allometric relationships are seen for Enos Lake fish than for Paxton fish. PC5 is significantly correlated with size and remains so when sex is controlled for but not when species is controlled. PC1 is only significantly correlated with size when sex is controlled and PC3 is correlated when species is controlled.

Modeling Approach

We modified the model of Bolnick and Doebeli (2003) to investigate the conditions under which sexual dimorphism and adaptive divergence might evolve in the same population. Our model tracks the explicitly modeled genotypes of individuals in a diploid population with discrete generations that inhabits a single spatial patch. In each model generation, individuals undergo competition for resources followed by mating under a regime of female choice. We present the mathematical apparatus of the model in detail in Appendix S1.

Each model generation begins with a population of juveniles. The probability that a juvenile survives to reproductive age is governed by competition for resources. Resources are characterized by two ecological dimensions that we call simply *y* and *z* (Fig. 5A). For example, in the stickleback system dimension *y* might

Table 1. MANCOVA comparing head shape between sex, species, and lakes. PC values were calculated using fish from both lakes. All PC values were included in the comparison (and are reported in full in Table S6) but only the first 6 are shown here. Significant effects with the largest η^2 values are shown in bold for each independent variable (numerator df = 1 for all).

Source	Dependent Variable	Mean Square	<i>F</i>	<i>P</i>	η^2
Sex	PC1	1406.291	511.238	0.000	0.603
	PC2	54.701	20.942	0.000	0.059
	PC3	4.283	1.564	0.212	0.005
	PC4	18.133	10.510	0.001	0.030
	PC5	9.848	7.626	0.006	0.022
	PC6	0.879	0.958	0.328	0.003
Species	PC1	66.945	24.337	0.000	0.068
	PC2	211.239	80.872	0.000	0.194
	PC3	4.165	1.521	0.218	0.005
	PC4	0.225	0.130	0.718	0.000
	PC5	0.242	0.187	0.666	0.001
	PC6	0.686	0.748	0.388	0.002
Lake	PC1	0.775	0.282	0.596	0.001
	PC2	127.336	48.750	0.000	0.127
	PC3	206.723	75.464	0.000	0.183
	PC4	14.676	8.507	0.004	0.025
	PC5	16.262	12.593	0.000	0.036
	PC6	13.528	14.738	0.000	0.042
Size	PC1	74.074	26.929	0.000	0.074
	PC2	2.099	0.804	0.371	0.002
	PC3	24.562	8.966	0.003	0.026
	PC4	8.817	5.110	0.024	0.015
	PC5	15.479	11.987	0.001	0.034
	PC6	7.619	8.301	0.004	0.024
Sex × species	PC1	0.240	0.087	0.768	0.000
	PC2	7.847	3.004	0.084	0.009
	PC3	8.255	3.014	0.083	0.009
	PC4	47.018	27.252	0.000	0.075
	PC5	0.675	0.523	0.470	0.002
	PC6	0.894	0.974	0.324	0.003
Sex × lake	PC1	7.452	2.709	0.101	0.008
	PC2	28.603	10.951	0.001	0.032
	PC3	1.348	0.492	0.483	0.001
	PC4	17.579	10.189	0.002	0.029
	PC5	3.452	2.673	0.103	0.008
	PC6	10.662	11.616	0.001	0.033
Lake × species	PC1	57.137	20.771	0.000	0.058
	PC2	155.178	59.409	0.000	0.150
	PC3	2.491	0.909	0.341	0.003
	PC4	0.831	0.482	0.488	0.001
	PC5	0.013	0.010	0.921	0.000
	PC6	4.102	4.469	0.035	0.013
Sex × size	PC1	0.240	0.087	0.768	0.000
	PC2	7.847	3.004	0.084	0.009
	PC3	8.255	3.014	0.083	0.009
	PC4	47.018	27.252	0.000	0.075
	PC5	0.675	0.523	0.470	0.002
	PC6	0.894	0.974	0.324	0.003

Continued.

Table 1. Continued.

Source	Dependent Variable	Mean Square	<i>F</i>	<i>P</i>	η^2
Lake × sex × species	PC1	4.814	1.750	0.187	0.005
	PC2	0.806	0.309	0.579	0.001
	PC3	1.724	0.629	0.428	0.002
	PC4	17.178	9.957	0.002	0.029
	PC5	4.635	3.589	0.059	0.011
	PC6	3.549	3.866	0.050	0.011

represent food particle size and dimension *z* might represent particle location in the lake (e.g., benthic or limnetic). In nature, different phenotypes may control an individual’s ability to exploit resources characterized by different ecological axes. For example, in sticklebacks the size of prey items in an individual’s diet depends on body size whereas the taxonomic composition of the diet depends on gill raker number (Ingram et al., unpubl. data), and the taxonomic composition of an individual’s diet may be an indicator of that individual’s preferred foraging location. We allow an individual’s ability to exploit resources with character (*y,z*) to depend on two ecological phenotypes that we call *p_y* and *p_z*. In sticklebacks, *p_y* and *p_z* might represent body size, gill raker number, or elements of head or body shape. For consistency with our empirical results, we will let *p_y* and *p_z* be elements of head shape in examples used to illustrate the model. Individuals compete most intensely with other individuals that use similar resources, and thus that have similar ecological phenotypes (Schluter 2003, Bolnick and Paull 2009). Therefore, individual fitness is frequency dependent in both ecological dimensions. Complex in-

teractions in the effect of two or more ecological dimensions on resource density, or complex interactions in the effect of two or more phenotypes on competition between individuals, can result in selection for increased phenotypic variance in a population (Doebeli and Ispolatov 2010). For simplicity, we assume here that the effects of ecological dimensions on resource density, and the effects of ecological phenotypes on competition, are independent.

An individual’s ecological phenotypes are governed by two sets of explicitly modeled diallelic loci that we call the SD and SS locus sets. Phenotypes influenced by the SD locus set may have the potential to be sexually dimorphic, and phenotypes influenced by the SS locus set have the potential to be sexually selected, according to the mechanisms described below. The SD locus set is subdivided into SDM loci expressed only in males, SDF loci expressed only in females, and SDE loci expressed in either sex. An individual’s *p_y* phenotype is determined by the value of the alleles at the SD loci expressed in that individual, and by a factor *v_y* that determines the strength of effect of those alleles on the *p_y* phenotype. An individual’s *p_z* phenotype is determined by the

Table 2. Divergence of shape and size between species and sexes for Paxton and Enos Lake sticklebacks. Values shown are from an analysis of the magnitude of difference between sexes and species in each lake, and include estimates of total divergence and the contribution of the first four principal components to total divergence.

Lake	Factor	Total divergence ΔZ (vector length)	Contribution of each axis to total divergence					
		Shape and size	SE	Size	PC1	PC2	PC3	PC4
Paxton	Species	0.04990	0.00075	0.5846	0.0231	0.7924	0.1100	0.0929
	Sex	0.05165	0.00107	0.0527	0.9850	0.1029	0.0452	0.0857
		Shape only						
	Species	0.18389	0.00064	-	0.0063	0.2150	0.0299	0.0252
	Sex	0.26822	0.00043	-	0.1897	0.0198	0.0087	0.0165
		Shape and size						
Enos	Species	0.02314	0.00118	0.8226	0.2042	0.1893	0.2022	0.0509
	Sex	0.05167	0.00163	0.1252	0.9777	0.0446	0.1130	0.0652
		Shape only						
	Species	0.01316	0.00117	-	0.0194	0.0180	0.0192	0.0048
	Sex	0.05127	0.00101	-	0.9855	0.0450	0.1139	0.0657

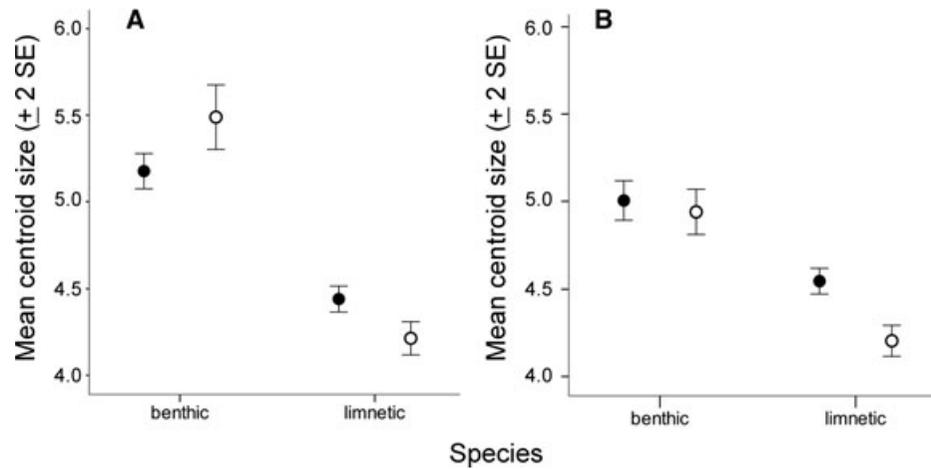


Figure 4. Mean centroid sizes (± 2 SE) of males (solid dot on left) and females (open circles on right) in Paxton (A) and Enos lakes (B). Although benthic stickleback are larger overall, the sexual size dimorphism varies according to species and lake. Limnetic females are smaller in both lakes, but benthic females are larger in Paxton lake. There is a reversal of sexual size dimorphism in Paxton Lake that does not appear in Enos Lake, which is due primarily to a change in benthic female size.

value of the alleles at that individual's SS loci and by an additive effect of the alleles at the SD loci expressed in that individual. A factor v_Z determines the strength of effect of alleles at SD loci on the p_Z phenotype (Fig. 5B). For example, this architecture might represent a case in which SS loci govern head depth or length and therefore affect benthic and limnetic swimming and foraging ability, whereas SD loci govern mouth shape and so may affect both gape width and benthic or limnetic foraging ability.

The genetic architecture of our model allows us to control two attributes of the population that we wish to investigate. First, we can constrain ecological phenotypes in the population to be sexually monomorphic (when the number of SDM and SDF loci is zero), or we can allow the ecological phenotypes to have the potential for sexual dimorphism (when the number of SDM and SDF loci is greater than zero). Second, we can allow the ecological effects of the SD and SS loci to range from orthogonal (when $v_Y > 0$ and $v_Z = 0$) to parallel (when $v_Y = 0$ and $v_Z > 0$). For example,

the orthogonal case might represent a population in which the only effect of SD loci is on mouth shape components that confer gape width. In this case, SD loci would affect the size of the particles an individual could consume, but would not affect foraging ability independent of particle size in the benthic or limnetic habitats. The parallel case might represent a population in which the only effect of SD loci is on mouth shape components that confer benthic or limnetic foraging ability, and in which SD loci have no effect on the size of particles an individual can consume.

Individuals in our model population reproduce sexually, and mate selection is by female choice. Females express an assortative mating phenotype p_{AM} , which can range from 0 to 1 and is governed by alleles at a set of loci we call the AM locus set. Females with positive p_{AM} mate assortatively, while females with $p_{AM} = 0$ mate randomly. Our model follows a number of recent models of adaptive speciation by not including the potential for disassortative mating (e.g., van Doorn et al. 2004; Bürger and

Table 3. Correlations between size and PC values for each lake. Pearson correlations are given when no other variables are controlled, and partial correlations are given when species or sex are controlled. Significant correlations are shown in bold, and suggest allometry.

Control variables	PC1		PC2		PC3		PC4		PC5		PC6	
	corr.	<i>P</i>	corr.	<i>P</i>	corr.	<i>P</i>	corr.	<i>P</i>	corr.	<i>P</i>	corr.	<i>P</i>
Paxton Lake												
None (df=233)	-0.059	0.371	0.615	0.000	-0.031	0.639	0.361	0.000	-0.193	0.003	-0.010	0.877
Species (df=232)	-0.108	0.099	-0.022	0.740	0.141	0.031	0.351	0.000	-0.134	0.040	0.013	0.838
Sex (df=232)	-0.192	0.003	0.613	0.000	-0.036	0.596	0.355	0.000	-0.202	0.002	-0.018	0.788
Enos Lake												
None (df=108)	0.006	0.953	-0.085	0.378	0.036	0.709	0.065	0.498	0.316	0.001	-0.021	0.827
Species (df=107)	0.077	0.426	-0.008	0.935	0.192	0.046	0.047	0.626	0.062	0.524	-0.059	0.545
Sex (df=107)	-0.276	0.004	-0.073	0.448	-0.006	0.950	0.038	0.697	0.288	0.002	-0.029	0.766

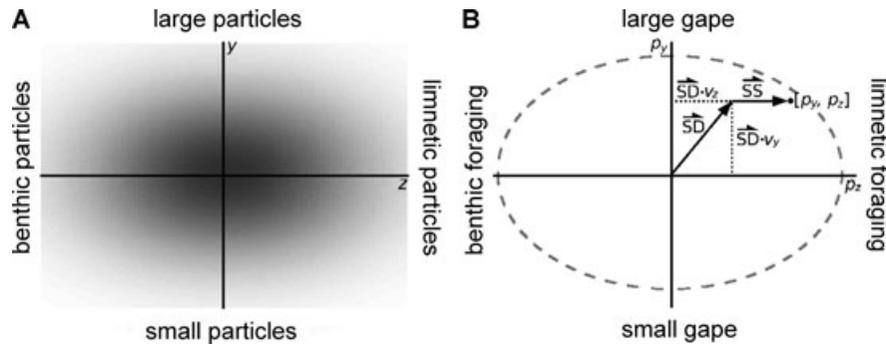


Figure 5. A graphic representation of ecological space and phenotype space in the model. (A) Axes represent ecological dimensions y and z . Dark areas represent resource types present at high density in the model system. (B) Axes represent ecological phenotypes p_y and p_z . Vectors represent the effects of alleles at the SS and SD loci in a representative individual. The magnitude of each vector represents the sum of all alleles at the corresponding loci. Alleles at SS loci affect only phenotype p_z , whereas alleles at SD loci affect phenotypes p_y and p_z . The relative effect of alleles at SD loci on each phenotype, and thus their direction of effect in phenotype space, is determined by the scalars v_y and v_z . Vector SD is parallel to vector SS when $v_y = 0$ and $v_z > 0$, and orthogonal to SS when $v_y > 0$ and $v_z = 0$. The ecological phenotype of the individual, $[p_y, p_z]$, is the sum of vectors SS and SD. The dotted ellipse in (B) represents an arbitrary isocline of resource density, and is included to illustrate the correspondence of the ecological and phenotype spaces. Text labels (e.g., large particles, large gape, limnetic foraging) correspond to the examples given in the text, but other examples that meet the assumptions of the model will be equally valid.

Schneider 2006; Bürger et al. 2006; Otto et al. 2008; Pennings et al. 2008). Assortatively mating females choose males that express sexually selected phenotypes, p_{SS} , similar to their own. Each individual's sexually selected phenotype is determined entirely by the alleles at that individual's SS loci. This might be the case if, for example, females choose mates based on head shape components associated with benthic or limnetic foraging ability, but not based on mouth shape. Each female that survives to adulthood is approached at random by surviving males, and either mates with or rejects those males according to her assortative mating phenotype and the difference between their sexually selected phenotypes. We impose a cost of choosiness on females by allowing each female that rejects a potential mate to die with some probability d before she is approached by the next male. Each female mates only once with the first male she selects, and then produces offspring and is removed from the population. Males are not removed from the population after mating, and thus may mate once, more than once, or not at all. Each offspring of each mating is assigned to be either male or female with equal probability, and inherits one allele from each parent at each SD, SS, and AM locus under free recombination. Each allele at each diallelic locus mutates to the opposite allele with probability μ . The offspring of each generation form the pool of juveniles in the next generation.

We simulated evolution in populations with or without the potential for sexual dimorphism, and when the ecological effects of the SS and SD locus sets ranged from orthogonal to parallel. Each simulation began with the invasion of a parental population into a novel habitat to which it was poorly adapted. Thus, we fixed all SS and SD loci in the initial population in a maladapted state. Because we were interested in the evolution of sexual dimorphism,

we set the initial population to be sexually monomorphic (i.e., we fixed identical alleles at corresponding SDF and SDM loci). To study the evolution of reproductive isolation, it was necessary to begin with a randomly mating population, and thus we fixed all AM loci at zero. Because alleles were fixed at every locus in the initial population, evolution was initially mutation limited. This initial population state was not intended to accurately represent ancestral stickleback populations, but rather to provide a common baseline to allow comparison between simulations. We set the resource distribution in the patch to create disruptive selection in ecological dimensions y and z on the randomly mating and sexually monomorphic population. This condition promotes the evolution of sexual dimorphism or speciation (de Cara et al. 2008; Otto et al. 2008), and so facilitates the study of phenotypic divergence. Our model allows us to compare the relative probabilities of speciation under different sets of parameter values, but because we deliberately chose initial conditions that favor divergence, our model should not be interpreted to offer absolute probabilities of speciation for any real system. We ran each simulation for 10^4 generations. We recorded the generation in which reproductively isolated populations emerged, and in populations capable of generating sexual dimorphism we recorded the generation in which sexual dimorphism emerged.

Ecological Model Findings

SEXUALLY MONOMORPHIC POPULATIONS

When populations were constrained to be sexually monomorphic, reproductive isolation often evolved in our simulations (Figs. 6 and 7). In the randomly mating population there is disruptive

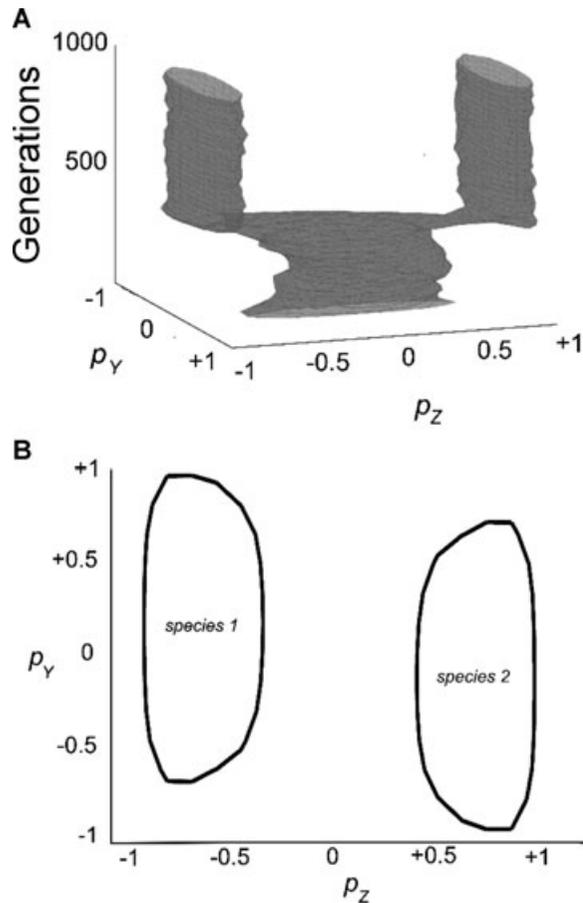


Figure 6. A representative simulation of the evolution of reproductive isolation in a sexually monomorphic population showing phenotype distributions over time (A) and at the end of the simulation (B). The y axes represent ecological phenotype p_Y and the x axes represent ecological phenotype p_Z . In both panels, the enclosed phenotype space contains 99% of individuals in each generation. (Simulation parameters: $N_{SD} = 6$, $N_{SDM} = 0$, $N_{SDF} = 0$, $N_{SDE} = 6$, $N_{SS} = 6$, $N_{AM} = 3$, $v_Y = 0.87$, $v_Z = 0.50$, $\sigma_Y = 1$, $\sigma_{cY} = 0.5$, $\sigma_Z = 5$, $\sigma_{cZ} = 0.85$, $K_m = 2200$, $r = 3$, $d = 0.01$, $\mu = 0.001$).

selection on ecological phenotypes p_Y and p_Z . A female with a mutation conferring assortative mating will be more likely than a randomly mating female to have offspring with the extreme p_Z phenotypes favored by selection, and those offspring are likely to carry assortative mating alleles. As a result, selection on the p_Z phenotype leads to an increase of assortative mating alleles in the population, and the density of assortative mating alleles becomes highest among individuals with extreme p_Z phenotypes. Because extreme p_Z phenotypes are relatively rare, assortatively mating females may fail to encounter their preferred mates. These females may accept less-preferred mates, or they may fail to mate entirely. Thus, selection against rare phenotypes due to mate choice partially offsets the positive effect of ecological viability selection on the invasion of assortative mating. When assortative mating becomes sufficiently strong, extreme p_Z phenotypes become com-

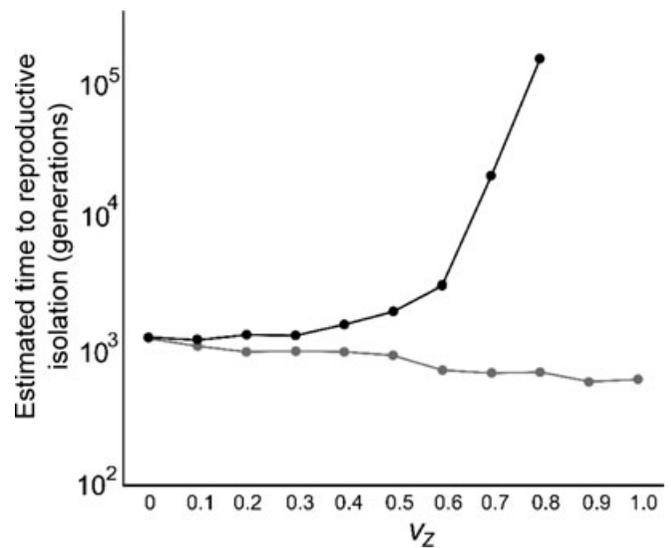


Figure 7. Expected times to reproductive isolation under disruptive selection for sexually monomorphic (gray line) and sexually dimorphic (black line) populations. Parameter v_Z measures the effect of SD loci on ecological phenotype p_Z . When v_Z is close to 1 (i.e., the ecological effects of the SD and SS loci are close to parallel), sexual dimorphism inhibits the evolution of reproductive isolation. When v_Z is close to 0 (i.e., the ecological effects of the SD and SS loci are close to orthogonal), reproductive isolation evolves readily even in the presence of sexual dimorphism. (Simulation parameters, sexually dimorphic populations: $N_{SD} = 12$, $N_{SDM} = 6$, $N_{SDF} = 6$. Sexually monomorphic populations: $N_{SD} = 6$, $N_{SDM} = 0$, $N_{SDF} = 0$. All populations: $N_{SS} = 6$, $N_{AM} = 3$, $\sigma_Y = 1$, $\sigma_{cY} = 0.5$, $\sigma_Z = 5$, $\sigma_{cZ} = 0.85$, $K_m = 2200$, $r = 3$, $d = 0.01$, $\mu = 0.001$.)

mon and intermediate p_Z phenotypes become rare. Frequency-dependent viability selection then favors intermediate p_Z phenotypes. However, because assortative mating is now strong, sexual selection against rare intermediate males overwhelms viability selection and drives the continued invasion of assortative mating alleles. This forces the evolution of reproductive isolation to completion (Fig. 8, see also Gilman and Behm, in press).

As the ecological effects of the SD and SS loci move from orthogonal to parallel, the SD loci contribute an increasing amount of variability to the p_Z phenotype. This allows the randomly mating population to achieve a wider range of p_Z phenotypes, and thereby reduces resource competition and the strength of disruptive selection on the p_Z phenotype. Interestingly, if selection on the p_Z phenotype remains disruptive when variation at the SD loci is maximized, reproductive isolation can sometimes evolve faster when the SD loci contribute to the p_Z phenotype than when they do not, even though disruptive selection has been weakened (Fig. 7). As assortative mating begins to invade the population, weak reproductive isolation between extreme p_{SS} phenotypes helps to maintain linkage disequilibria between alleles at the SD and SS loci. As the ecological effects of the SD and SS loci move

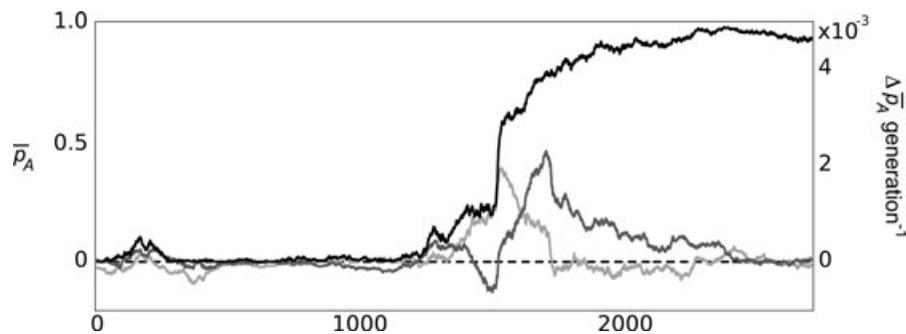


Figure 8. Invasion of assortative mating alleles (black line, scaled on left) into an initially randomly mating population. \bar{p}_A is the density of assortative mating alleles (i.e., the proportion of alleles at assortative mating loci that code for assortative mating behavior) in the population in each generation. Gray lines show the change in the density of assortative mating alleles in each generation, and are scaled on the right axis. The light gray line shows the change that occurs during the ecological selection phase, and the dark gray line shows the change that occurs during the mate selection phase of each generation. Early in the invasion of assortative mating, the process is driven by ecological selection. As extreme phenotypes become common, competition for extreme resources increases and ecological selection opposes the continued invasion of assortment. However, sexual selection by assortative females now favors extreme males, who tend to carry assortative mating alleles more than intermediate males. Because the increase in assortative mating due to sexual selection is greater than the decrease due to ecological selection, reproductive isolation proceeds to completion. (Simulation parameters: $N_{SD} = 6$, $N_{SDM} = 0$, $N_{SDF} = 0$, $N_{SDE} = 6$, $N_{SS} = 6$, $N_{AM} = 3$, $v_Y = 1$, $v_Z = 0$, $\sigma_y = 1$, $\sigma_{cy} = 0.5$, $\sigma_z = 5$, $\sigma_{cz} = 0.85$, $K_m = 2200$, $r = 3$, $d = 0$, $\mu = 10^{-4}$).

closer to parallel, these linkage disequilibria allow for an increasing amount of divergence of p_Z phenotypes between emerging p_{SS} groups. Each assortative allele then has a greater disruptive effect on the p_Z phenotype, and the relative fitness of assortative alleles early in the invasion process increases. Because the p_{SS} phenotype under sexual selection is determined by the SS but not the SD loci, extreme p_Z phenotypes created by the effect of alleles at SD loci do not suffer reduced fitness in the mate selection phase. When assortative mating becomes strong and SS loci in each emerging subpopulation near fixation, the linkage disequilibria between the SS and SD loci begin to decay. Increasing variability at the SD loci maintains variability in the p_Z phenotype in each subpopulation. Thus, resource competition within the reproductively isolated subpopulations is weaker when the ecological effects of the SD and SS loci are parallel, and ecological viability selection less strongly inhibits the completion of reproductive isolation in the parallel case. For these reasons, reproductive isolation in sexually monomorphic populations in our model sometimes evolved more quickly when the ecological effects of the SD and SS loci were parallel than when they were orthogonal.

A number of studies have shown that reproductive isolation can evolve in a population when a “magic trait” (sensu Gavrilets 2004) under frequency-dependent disruptive selection also serves as the basis for assortative mating (Dieckmann and Doebeli 1999; Otto et al. 2008; Pennings et al. 2008), and several potential magic traits have now been identified in nature (Grant 1986; Hawthorne and Via 2001; Jiggins et al. 2001; Podos 2001). Because the p_{SS} phenotype under sexual selection in our model is correlated by epistasis with the p_Z phenotype under frequency-dependent

disruptive selection, the p_{SS} phenotype is a magic trait. Our results suggest that the presence of loci that are not themselves subject to assortative mating (here, the SD loci) but which contribute to the ecological phenotype associated with a magic trait (here, the p_Z phenotype) may sometimes increase the rate at which reproductive isolation evolves. To our knowledge, this is the first time this mechanism has been described.

SEXUALLY DIMORPHIC POPULATIONS

When there was disruptive selection on ecological traits with the potential for sexual dimorphism in our model, sexual dimorphism usually evolved within 200 generations. This is because sexual dimorphism is strongly favored as soon as mutation has created variability at the SD loci. When the ecological effects of the SD and SS loci are parallel or nearly parallel (i.e., $v_Z > 0.6$ in our simulations), the emergence of sexual dimorphism greatly reduces the strength of disruptive selection on the p_Z phenotype. This was sufficient to slow or prevent the invasion of assortative mating and the evolution of reproductive isolation, even under parameter sets that had allowed reproductive isolation to evolve quickly in sexually monomorphic populations (Fig. 7). This supports the result of Bolnick and Doebeli (2003). When the ecological effects of the SD and SS loci are orthogonal or nearly orthogonal (i.e., $v_Z < 0.5$ in our simulations), the effect of the SS loci on the p_Z phenotype is weak and disruptive selection on the p_Z phenotype remains strong even in the presence of sexual dimorphism. In such cases, sexual dimorphism and reproductive isolation often evolved sequentially (e.g., Fig. 9). Assortative mating is favored by ecological viability

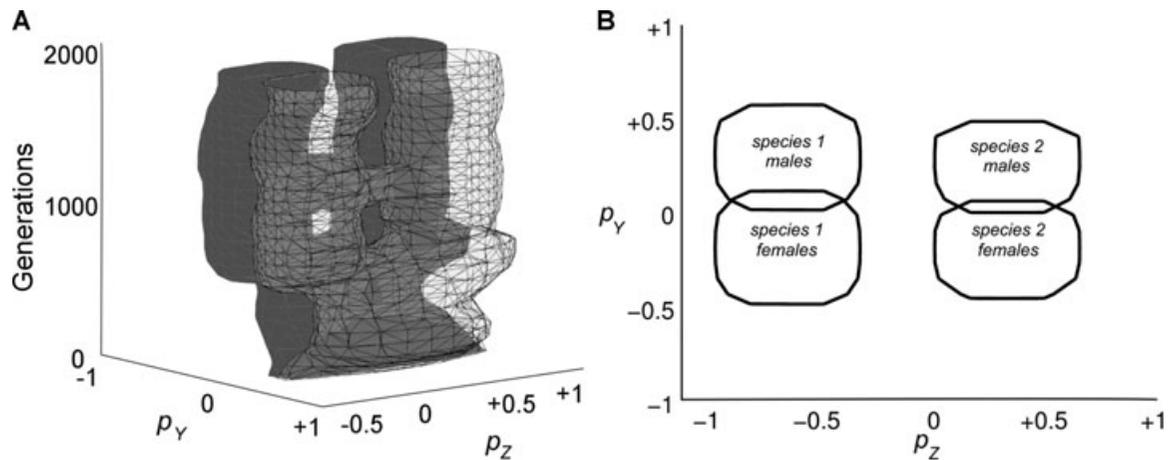


Figure 9. A representative simulation of the evolution of sexual dimorphism and reproductive isolation in the same population over time (A) and at the end of the simulation (B). The y axes represent ecological phenotype p_Y and the x axes represent ecological phenotype p_Z . (A) Male phenotypes are shown in dark gray and female phenotypes are shown in translucent gray. In both panels, the enclosed phenotype space contains 99% of individuals in each generation. Sexual dimorphism evolves quickly, whereas reproductive isolation evolves more slowly. There is a short bout of introgression near generation 1000. Sexual selection against hybrids during the introgression strengthens assortative mating, which stabilizes the incipient species pair. (Simulation parameters: $N_{SD} = 12$, $N_{SDM} = 6$, $N_{SDF} = 6$, $N_{SDE} = 0$, $N_{SS} = 6$, $N_{AM} = 3$, $v_Y = 1$, $v_Z = 0$, $\sigma_Y = 0.5$, $\sigma_{cY} = 0.25$, $\sigma_Z = 1$, $\sigma_{cZ} = 0.5$, $K_m = 200$, $r = 3$, $d = 0.01$, $\mu = 0.001$).

selection when there is disruptive selection on the p_Z phenotype, but viability selection acts directly on ecological phenotype and only indirectly on assortative mating phenotype. Moreover, the cost of assortative mating is potentially large early in the process of adaptive divergence, when male phenotypes favored by assortative females are rare. For these reasons, the evolution of adaptive divergence was generally slow relative to that of sexual dimorphism. Our qualitative results are unchanged in the biologically plausible case in which ancestral populations are allowed to be sexually dimorphic before the invasion of the novel habitat (results not presented).

When the ecological effects of the SD and SS loci were orthogonal or nearly orthogonal and populations were large, there was little difference in the rate at which reproductive isolation evolved in sexually monomorphic and sexually dimorphic populations (Fig. 7). When population size was very small ($N_e \sim 120$), we found that sexually dimorphic populations were sometimes more likely to evolve reproductive isolation than sexually monomorphic populations (Fig. 10). Reproductive isolation requires strong assortative mating. However, as the strength of assortative mating increases, mate selection by female choice tends to reduce genetic variability at sexually selected loci (Otto et al. 2008, Gilman and Behm, in review). In small populations, this can result in the fixation of all or nearly all sexually selected loci. This fixed state is ecologically maladapted, but is locally stabilized by sexual selection under female choice (Otto et al. 2008, Gilman and Behm, in press). Under biologically reasonable mutation rates (i.e., $< 10^{-4}$) our model populations often remained in the fixed state 10^4 generations or more, suggesting that detours through fixation may

be sufficient to prevent the evolution of reproductive isolation in some populations in nature. In our model, sexual dimorphism in resource use reduces competition between males and females, and so allows sexually dimorphic populations to maintain higher densities than sexually monomorphic populations given the same

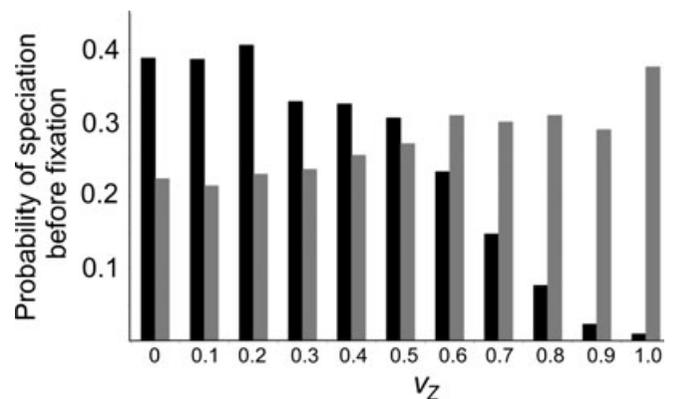


Figure 10. Probability that small sexually dimorphic (black bars) and sexually monomorphic (gray bars) populations speciate before alleles at all SS loci become fixed. Parameter v_Z measures the effect of SD loci on ecological phenotype p_Z . We ran 300 simulations for sexually dimorphic and sexually monomorphic populations at each value of v_Z . Effective population size in the randomly mating parental population before the evolution of sexual dimorphism was approximately 120 in all trials. (Simulation parameters, sexually dimorphic populations: $N_{SD} = 12$, $N_{SDM} = 6$, $N_{SDF} = 6$. Sexually monomorphic populations: $N_{SD} = 6$, $N_{SDM} = 0$, $N_{SDF} = 0$. All populations: $N_{SS} = 6$, $N_{AM} = 3$, $\sigma_Y = 1$, $\sigma_{cY} = 0.5$, $\sigma_Z = 5$, $\sigma_{cZ} = 0.85$, $K_m = 440$, $r = 3$, $d = 0.01$, $\mu = 0.001$).

resource distribution. Because sexually dimorphic populations were larger, they were less likely to fix at all sexually selected loci and were more likely to evolve reproductive isolation within the 10^4 generations we modeled. This effect appears to be restricted to very small populations. Stickleback populations ancestral to the species pairs in nature are believed to have had effective population sizes of at least 10^3 (Caldera and Bolnick 2008), and we were unable to detect a positive effect of sexual dimorphism on the rate of evolution of reproductive isolation in model populations of this size. However, some fresh water fish and amphibian populations have effective population sizes of 10^2 or less (Davis and Verrell 2005; Brede and Beebee 2006; Schmeller and Merila 2007; Lucentini et al. 2009), which is similar to the size of the small populations we modeled. Recent work suggests that speciation with gene flow may have occurred in some small amphibian populations (Niemiller et al. 2008), and in such populations sexual dimorphism in ecological traits might have the potential to increase the rate of speciation.

Discussion

We show empirically that there are substantial differences in head shape between the sexes and species in stickleback species pairs, and that these differences are greater between sexes than between species. Aspects of shape that differ between the sexes are not the same as those that differ between the species, although there is some overlap. This pattern suggests that the disruptive selection driving sexual dimorphism may be different from the disruptive selection driving interspecific divergence—this is the condition that underlies the orthogonal case of our model. Our empirical results show that selection also acts differently on size than on shape, as sexual shape dimorphism is similar for limnetic and benthic sticklebacks but the pattern of sexual size dimorphism is reversed. There is evidence that head shape is important for resource use in sticklebacks (Lavin and McPhail 1986), and variation in head shape within species (McPhail 1984; Schluter 1993, 1995) and sexes (Bentzen and McPhail 1984; Reimchen and Nosil 2004) has been shown to influence foraging. The fact that head shape varies in different ways between species and sexes may indicate that sexes and species are partitioning resources differently.

The extensive sexual dimorphism we observed is surprising in a system that has become a classic example of adaptive speciation (Schluter 1994, 1996, McKinnon and Rundle 2002). Bolnick and Doebeli (2003) concluded that sexual dimorphism and adaptive lineage splitting on the same ecological axis were unlikely to occur in the same population, but their model and its results may not be applicable to systems in which interspecific variation and sexual dimorphism allow resource partitioning in more than one ecological dimension. Extending resource partitioning to more than one ecological dimension may be applicable to more

biological systems. Multiple ecological dimensions appear to be found currently in Paxton Lake and may have been the case in the past for Enos Lake, and we conjecture that it may be common in nature. Our model allowed interspecific and sexual divergence to occur in the same or in different ecological dimensions, and we found that sexual dimorphism and adaptive species divergence can co-occur when disruptive selection acts on more than one axis of ecological niche variation. Our theoretical results showing divergence on multiple ecological axes are related to the multifarious selection hypothesis discussed by Nosil and colleagues (2009) and proposed by Rice and Hostert (1993). Speciation may be more likely if there are ecological dimensions in which sexual dimorphism does not occur, such as when there are multiple ecological axes. As in the Bolnick and Doebeli model, in our model sexual dimorphism arises quickly and precludes species divergence when the axes of ecological niche variation are parallel and therefore are effectively a single axis. However, sexual dimorphism does not eliminate disruptive selection on the population when the axes of ecological niche variation are orthogonal or close to orthogonal, and in such cases speciation may follow the evolution of sexual dimorphism. In contrast, the evolution of sexual dimorphism rarely followed speciation, simply because the evolution of sexual dimorphism was much more rapid than that of assortative mating.

Resource partitioning occurs along a benthic/limnetic ecological axis in threespine sticklebacks, and this is also the case in other freshwater fish, including cichlids, sunfish, whitefish, minnows, sticklebacks, perches, and salmonids (Robinson and Wilson 1994 and references therein). Competition for food results in the partitioning of foraging niches and specialization on limnetic or benthic prey items. In these fish taxa, limnetic fish have slender bodies with relatively small heads and large eyes and feed on zooplankton (Larson 1976; McPhail 1984, 1992, 1993; Skulason and Smith 1995; Robinson and Schluter 2000). Relatively larger eyes enable detection of small planktonic prey (Meer and Anker 1984, Hart and Gill 1994, Walton et al. 1994), and the small slender mouths facilitate their capture (Malmquist 1992). Benthic fish have deep bodies that maneuver to capture macroinvertebrates (Webb 1984; Blake 2004). Their large heads and jaws, higher supraoccipital crests, and a larger area between the angular and eye facilitate the insertion of large muscles necessary for jaw movement to consume macroinvertebrates (Liem 1993, Caldecutt and Adams 1998). Our shape measurements of limnetic and benthic stickleback species pairs follow these general shape patterns associated with foraging niche.

We found that sexual dimorphism in foraging morphology was distinct from species differences. Previous studies have found sexual dimorphism in dentition, in which males from solitary lakes have more teeth than females (Caldecutt et al. 2001.). The specific foraging niches that the sexes occupy are not fully known,

but in solitary lake populations, male sticklebacks tend to be more prevalent in littoral habitat where they have nests, and females are relatively more limnetic (Reimchen 1980). Consistent with this pattern, an analysis of stomach contents in a solitary population revealed that females have a greater frequency of limnetic prey items and males have greater frequency of benthic items (Reimchen and Nosil 2001), suggesting that the sexes specialize on different foraging niches. There is some indication that the sexes in sticklebacks are under similar patterns of disruptive selection in solitary populations (Bolnick 2004; Bolnick and Lau 2008), but we do not yet know how much disruptive selection on the sexes may be acting on axes orthogonal to those under disruptive selection between species. Differences between the sexes remain to be explored in depth and linked to functional differences in head shape as well as overall body shape. This is a promising area for future work.

We found reversed sexual size dimorphism between benthic and limnetic species of Paxton Lake, confirming previous studies (McPhail 1992). Because sexual size dimorphism is in opposite directions but sexual dimorphism in shape is similar between species, we can conclude that the sexual shape dimorphism is not simply the result of allometric differences between the sexes. Instead of being a correlated response to selection on body size, sexual dimorphism in shape is likely to be directly selected in both lakes. Reversed size dimorphism was found previously in Enos Lake as well (McPhail 1984), but has been lost. This loss of size dimorphism may be another consequence of genetic mixing due to hybridization or because ecological disturbance has weakened disruptive selection on size (Behm et al. 2010).

An additional selection pressure on stickleback shape and size is predation. The suite of predators differs in limnetic and benthic habitats (Reimchen 1980; Vamosi 2002) and may exert divergent selection on shape and size. Cutthroat trout and diving birds are important predators in limnetic habitat and may select for larger stickleback size (Reimchen 1991), more spines and plates (Reimchen and Nosil 2004), and shape that allows faster acceleration (Law and Blake 1996). Insect predators are more important in benthic habitat and may exert different selection on shape, favoring reduced spines (Reimchen and Nosil 2002), a larger lateral profile (Liem 1993), and deeper body (Walker 1997).

Sexual dimorphism in stickleback head shape is likely related to feeding differences and possibly to predation, but there may be an additional role for sexual selection. In many systems, sexual dimorphism is found to arise as a result of sexual rather than ecological selection, and sexual selection through both male–male competition and female choice may also contribute to head shape variation in sticklebacks. The longer head, larger cheek area, and longer throat of males may enhance the display of courtship coloration to females (McLennan and McPhail 1989; Bakker and Milinski 1993; Kraack et al. 1999) and also signal

territoriality to other males (McKinnon and McPhail 1996). The perception of color in this context may be influenced by shape because larger heads make for a larger signal. Additionally, males use their mouths in nest building, courtship, and fighting over territories (Bakker 1994, Foster 1994) and larger mouths may be advantageous for these purposes. Nest construction and defense ability may be important in both male–male competition and female choice. The addition of sexual selection to ecological selection on head traits may make simultaneous dimorphism and speciation even more feasible, as selection is more likely to be occurring along multiple axes. Even though restricted to ecological selection, our model frequently results in speciation, and in some cases both speciation and sexual dimorphism. In contrast, some models suggest that sexual selection acting alone is unlikely to result in speciation. For example, Van Doorn et al. (2004) indicate that adaptive speciation solely by sexual selection is unlikely because it requires some specific conditions such as frequency-dependent selection in both sexes. Whether this is generally the case is unknown; however.

Our model and the Bolnick and Doebeli model (2003) predict that sexual dimorphism should evolve more rapidly than reproductive isolation between species. If sexual dimorphism evolves before interspecific differences (Parent and Crespi 2009), and if sexual dimorphism can evolve freely in phenotype space, we would expect sexual dimorphism to orient along the ecological axis that allows it to eliminate the greatest amount of disruptive selection. Sexual dimorphism may also have been present in the ancestral oceanic stickleback (Kitano and Peichel 2007; Aguirre et al. 2008; Spoljaric and Reimchen 2008; Aguirre and Akinpelu 2010; Kume et al. 2010). These studies show varying sexual dimorphism in size and shape, however. Only some oceanic populations are dimorphic in either trait, and for those, which sex is larger in size and the nature of shape dimorphism vary.

Given these varying patterns, our ongoing research will evaluate sexual dimorphism in the direct oceanic ancestors of the benthic and limnetic species, which can reveal whether dimorphism results from evolutionary change primarily in males, females, or both sexes. We can also directly test the Bolnick and Doebeli (2003) model predictions that speciation and dimorphism trade-off, which would result in speciation being accompanied by a reduction in dimorphism compared to marine ancestors. We can also study solitary populations (that have not speciated), which should show greater dimorphism than species pairs if such a trade-off exists. Albert and colleagues (2007) found that the QTL with the largest effect on shape differences between benthic and oceanic sticklebacks mapped to the sex-determining region; our findings of sexual dimorphism in shape are in line with these earlier results. In addition to a genetic basis for shape differences between the sexes or species, habitat difference could also lead to plastic difference in shape (Day et al. 1994, Day and McPhail 1996,

Wund et al. 2008). The implications for ecological speciation are unclear, but recent reviews indicate that a role for plasticity is underappreciated (Crispo 2008, Pfennig et al. 2010), so this would be another interesting area to explore in future work.

The model indicates the feasibility of simultaneous ecological speciation and ecological sexual dimorphism, which we observe in the stickleback species pairs. Our model shows that two dimorphic species can arise along two ecological axes. This adaptive splitting within and between species is similar to what might be expected in ecological species packing under disruptive selection (Nosil and Reimchen 2005, Greenberg and Olsen 2010, Martin and Pfennig 2010). Although our model is not intended to imitate the conditions that gave rise to the benthic/limnetic stickleback species pairs, it would be interesting to investigate theoretically how varying starting conditions would affect the evolutionary outcome of disruptive selection (Bürger and Schneider 2006, Bürger et al. 2006). One aspect that would be especially interesting to explore is how a period of allopatric divergence followed by secondary contact compares to wholly sympatric conditions. It may be that the outcomes will be largely similar, and whether this is the case would have broad implications for much of the work currently being done on ecological speciation (Schluter 2000).

By considering disruptive ecological selection on two axes of phenotypic variation, we conclude that the simultaneous or sequential evolution of sexual dimorphism and speciation are more likely than when one axis of variation is involved, which may help to explain the profusion of sexual dimorphism in species-rich groups. Generally, there has been relatively little research on the ecological pressures resulting in sexual dimorphism, and such pressures are thought to be less common than sexual selection. Disruptive sexual selection is likely to make the coexistence of sexual dimorphism and interspecific differences even more common, but we show here that it is not a necessary condition. By recognizing this possibility, patterns of speciation and sexual dimorphism can be re-evaluated with an eye toward ecological disruptive selection.

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Supporting Information

The following supporting information is available for this article

Figure S1. PC1 vs. PC2 calculated separately for Paxton females (A), Paxton males (B), Enos females (C), and Enos males (D).

Table S1. Evaluation of number of morphological clusters in each lake.

Table S2. Eigenvalues and the percent variance explained by each PC in a variance–covariance matrix and a correlation matrix for data from Paxton and Enos Lakes.

Table S3. PC Loadings of the x and y coordinate of each landmark for the combined analysis of Paxton and Enos Lakes.

Table S4. Loadings of x and y coordinates for Paxton Lake fish along all 26 PC axes.

Table S5. Eigenvalues and the percent variance explained by each PC in a variance–covariance matrix and a correlation matrix for data from Paxton Lakes.

Table S6. MANCOVA for Paxton Lake only, comparing head shape between sex and species with size as a covariate.

Table S7. MANCOVA for both lakes comparing head shape between sex, species, and lake.

Table S8. Parameter values used in simulations.

Appendix S1. Details of the model

Supporting Information may be found in the online version of this article.

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