

Hendry Vineyard stickleback: testing for contemporary lake–stream divergence

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ABSTRACT

Background: Threespine stickleback (*Gasterosteus aculeatus*) show patterns of trait divergence between habitats that are repeated across many watersheds. For example, stream stickleback generally have deeper bodies than do lake stickleback. Parallel divergence of this sort is often used to infer the power and predictability of natural selection, but how rapidly such lake–stream differences can evolve is unknown. An opportunity to address this uncertainty was provided when two reservoirs on the Hendry Vineyard in Napa, California, were constructed in the early 1970s and filled with water (and thus stickleback) from the adjacent creek.

Question: Do reservoir and creek stickleback on the Hendry Vineyard show morphological divergence typical of lake and stream stickleback?

Samples: Stickleback from the reservoirs and creek in each of two years, combined with stickleback from six lake–stream pairs on Vancouver Island, British Columbia.

Methods: Multivariate analyses of univariate traits and geometric morphometric variables.

Results: Reservoir and creek stickleback differed only marginally in morphology, especially compared with lake and stream stickleback from Vancouver Island. In contrast to low divergence between habitats on the Hendry Vineyard, morphological variation was very high within habitats.

Conclusions: Reservoir–creek divergence in Hendry Vineyard stickleback is seemingly constrained by a combination of weak divergent selection, high gene flow, and limited time for divergence. On the other hand, the high variation within these populations might be used to study the origins and fate of phenotypic variation.

Keywords: adaptive radiation, constraint, contemporary evolution, diversification, local adaptation, natural selection, rapid evolution, stickleback.

INTRODUCTION

Natural selection is the primary force shaping the diversity of life. This conclusion first emerged from evidence that the traits of species are well suited for their environments and ways of life (Darwin, 1859; Simpson, 1944; Mayr, 1963; Cain, 1964; Endler, 1986; Schluter, 2000; Bell, 2008). **More**

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formal support came from two general observations. First, the repeated and independent evolution of similar traits in similar environments ('parallel' or 'convergent' evolution) implies that natural selection predictably matches organismal traits to environmental requirements (Schluter, 2000; Arendt and Reznick, 2008; Losos, 2011; Wake *et al.*, 2011). Second, the rapidity of adaptive evolution when environments change ('rapid' or 'contemporary' evolution) implies that natural selection can accomplish its work in very short order (Hendry and Kinnison, 1999; Reznick and Ghalambor, 2001; Hendry *et al.*, 2007, 2008). These and other lines of evidence have led to the common perception that natural selection is powerful and predictable (Cain, 1964; Endler, 1986; Bell, 2008).

The above observations and conclusion appear strongly supported by research on threespine stickleback (*Gasterosteus aculeatus*). Regarding parallel evolution, many studies have documented similar phenotypic changes in independent stickleback lineages colonizing similar environments. Examples include adaptation to marine versus freshwater environments (McKinnon *et al.*, 2004; Colosimo *et al.*, 2005; Bell and Aguirre, 2013; Ravinet *et al.*, 2013b), benthic versus limnetic environments (Schluter and McPhail, 1992; Willacker *et al.*, 2010), different predation environments (Reimchen *et al.*, 2013), and – the focus of our study – lake versus stream environments (Reimchen *et al.*, 1985; Lavin and McPhail, 1993; Berner *et al.*, 2009, 2010; Kaeuffer *et al.*, 2012; Lucek *et al.*, 2012a; Ravinet *et al.*, 2013a). Moreover, this parallel phenotypic evolution is often accompanied by parallel genetic changes (Shapiro *et al.*, 2004; Colosimo *et al.*, 2005; Chan *et al.*, 2010; Hohenlohe *et al.*, 2010; Jones *et al.*, 2012a, 2012b), and it can generate substantial reproductive isolation (McKinnon and Rundle, 2002; Hendry *et al.*, 2009). Regarding contemporary evolution, studies of stickleback populations colonizing new environments have revealed that adaptive changes can occur in only years to decades (Klepaker, 1993; Bell *et al.*, 2004; Adachi *et al.*, 2012; Leaver and Reimchen, 2012; Aguirre and Bell, 2012; Bell and Aguirre, 2013). Moreover, this contemporary evolution of traits can initiate the onset of reproductive isolation (Furin *et al.*, 2012). On the surface, then, research on stickleback strongly reinforces the perception that natural selection is powerful and predictable.

Considered more deeply, however, the above assertions are seen to be caricatures of a more complex and nuanced reality. First, phenotypic differences are often present among independent populations in similar environments (Hendry and Taylor, 2004; Kaeuffer *et al.*, 2012; Leinonen *et al.*, 2012), and adaptation to similar environments does not always involve similar genetic changes (Roesti *et al.*, 2012; Lucek *et al.*, 2012b). These results show that adaptive evolution often involves substantial unpredictable (non-parallel) components. Second, the colonization of new environments does not always lead to contemporary evolution (Hunt *et al.*, 2008; Berner *et al.*, 2010), nor does it always cause reproductive isolation (Berner *et al.*, 2009; Hendry, 2009). These results show that an abrupt shift in natural selection does not always lead to rapid adaptive changes. In reality, then, research on stickleback also indicates that natural selection has limits, constraints, and unpredictabilities, as has also been argued more generally (Barton and Partridge, 2000; Lenormand, 2002). The key task is to delineate when these complications arise and how strong they are in natural populations.

Lake and stream stickleback provide excellent opportunities for studying the power and predictability of natural selection, as well as its limits and constraints (Reimchen *et al.*, 1985; Lavin and McPhail, 1993; Reusch *et al.*, 2001; Aguirre, 2009; Berner *et al.*, 2009; Kaeuffer *et al.*, 2012; Ravinet *et al.*, 2013a). Particularly useful in these endeavours are lake–stream pairs where one habitat was recently colonized by stickleback from the other habitat. For example, several studies have examined lake–stream divergence in stickleback that colonized Switzerland in the middle to late nineteenth century, revealing the presence of modest divergence that only partly parallels that seen in the native range (Berner *et al.*, 2010; Lucek *et al.*, 2012a). In the present paper, we

introduce a system where colonization has been much more recent and the ancestral environment is known.

When A.P. Hendry was on sabbatical at his family's vineyard in Napa, California, his children (A.S. Hendry and C.A. Hendry) found stickleback in the creek and two reservoirs on the property. We here use these populations to explore the power and predictability of natural selection by considering two questions: (1) How much phenotypic divergence has occurred since the reservoir populations were founded by pumping water (and thus stickleback) from the creek in the early 1970s? (2) To what extent is phenotypic divergence between Hendry reservoir and creek stickleback parallel to that between lake and stream stickleback in older (post-glacial) stickleback systems (Hendry and Taylor, 2004; Berner *et al.*, 2008; Kaeuffer *et al.*, 2012)? Evidence that divergence has been substantial and parallel would reinforce assertions of the power and predictability of natural selection. Evidence to the contrary would point towards important limits, constraints, and unpredictabilities.

We sampled Hendry Vineyard stickleback from the creek and both reservoirs in each of two years. We then used univariate measurements and geometric morphometrics to quantify a suite of ecologically relevant traits that show genetically based divergence in other lake–stream pairs (Lavin and McPhail, 1993; Hendry *et al.*, 2002, 2011; Sharpe *et al.*, 2008; Berner *et al.*, 2011). We analysed these data in multivariate analyses that partitioned the variance among effects of year, sex, and sampling site. In addition, we used the geometric morphometric data to make quantitative comparisons to divergence between lake and stream stickleback from post-glacial populations on Vancouver Island, British Columbia. We did not find noteworthy divergence between reservoir and creek stickleback, and so we discuss the likely limits and constraints on natural selection. In addition, we introduce an interesting and perhaps unique property of these populations: they show exceptionally high levels of within-habitat variation. We suggest that Hendry Vineyard stickleback might therefore provide an excellent opportunity to study the origins and fate of morphological variation.

METHODS

Unbaited minnow traps were used to sample stickleback in May 2009 (37 from Reservoir 1, 51 from Reservoir 2, and 47 from the creek) and July 2010 (60 from Reservoir 1, 54 from Reservoir 2, and 85 from the creek). The traps were spaced haphazardly across at least half of the shoreline of each reservoir and at several locations in the creek (Fig. 1); the latter being places where ground water inputs maintained pools throughout the year. Within 2 h of capture, the fish were euthanized with clove oil and immediately placed on a standard background with a ruler. Following Kaeuffer *et al.* (2012), several pins were inserted along the margin of the fish so as to indicate important landmarks not otherwise distinguishable on photographs (Fig. 2). The left side of the fish was then photographed with a tripod-mounted Nikon D700 fitted with a 100 mm Sigma macro lens. Afterwards, each fish was labelled and preserved in 95% ethanol.

After at least a year of preservation, the fish were dissected to determine sex and digital calipers (nearest 0.01 mm) were used to measure a series of ecologically relevant traits. The measured traits included the number of lateral plates (both sides of the body), lengths of the first and second dorsal spines (from the anterior insertion), lengths of the left and right pelvic spines (from the posterior insertion), and the width of the pelvic girdle (at its widest). All measurements were made in triplicate, transformed to \log_{10} values, and found to be highly repeatable according to the proportion of total variance due to differences among

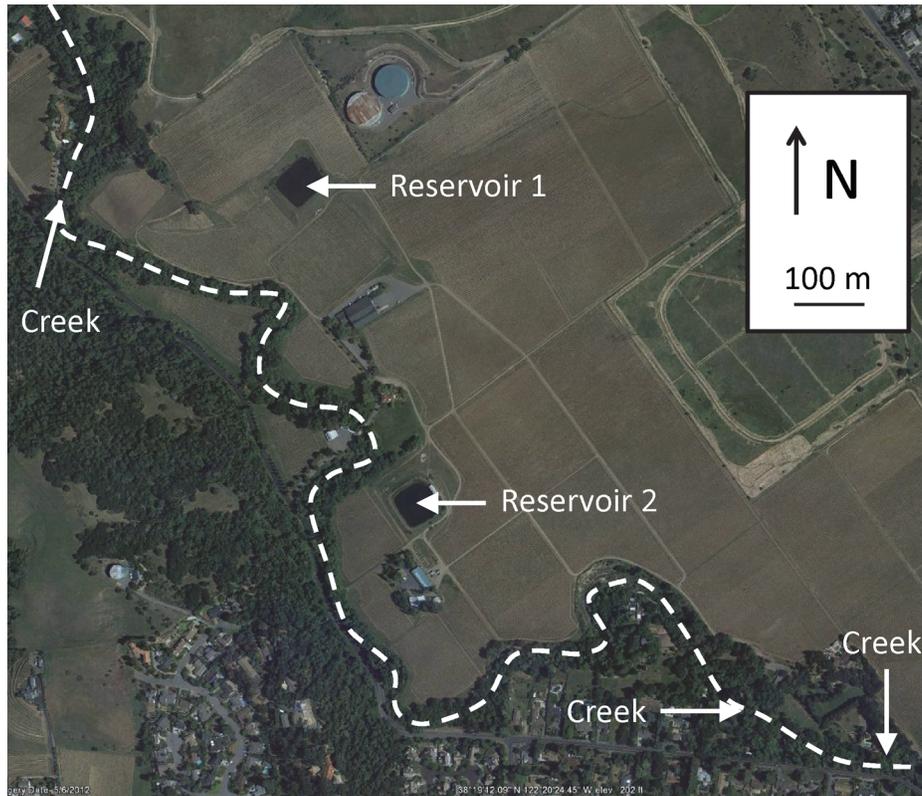


Fig. 1. Google Earth image of the Hendry Vineyard, showing the two reservoirs (Reservoir 1 and Reservoir 2), the creek (dashed line), and the sampling sites (arrows).

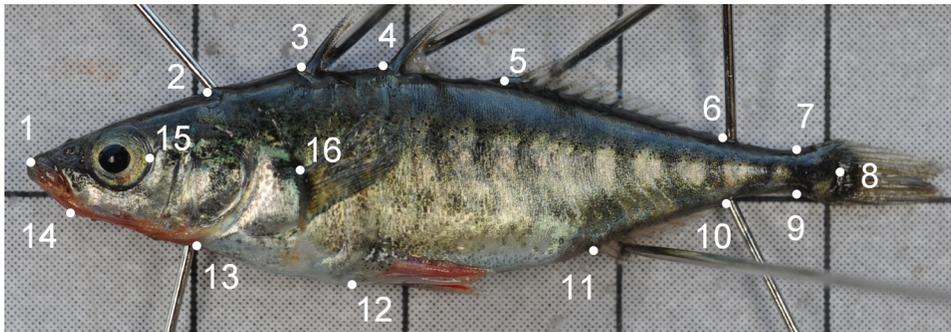


Fig. 2. A stickleback image showing the geometric morphometric landmarks as white circles, as well as the pins used to denote some landmarks and to extend the spines and fins. These landmarks are the same as those described in detail by Kaeuffer *et al.* (2012).

individuals (Arnqvist, 1998): first dorsal spine = 0.87, second dorsal spine = 0.79, left pelvic spine = 0.98, right pelvic spine = 0.94, and pelvic girdle = 0.96. All subsequent analyses used the median value of the three measurements so as to further increase precision and accuracy. These data were analysed using multivariate analysis of covariance

(MANCOVA): the covariate was centroid size (from geometric morphometrics – see below); the predictor variables were year (2009 and 2010), site (Reservoir 1, Reservoir 2, and creek), and sex; and the response variables were the \log_{10} trait measurements. Statistical inference was based on Wilks' lambda and effect sizes were assessed with partial eta-squared (Langerhans and DeWitt, 2004). In addition, a discriminant analysis was used to determine how well individuals from the three sites (years and sexes pooled) could be separated from each other.

Body shape was analysed from the photographs. To facilitate comparison of Hendry Vineyard stickleback with stickleback from other lake–stream systems, we obtained the photographs taken in a previous study (Kaeuffer *et al.*, 2012). That study examined lake and stream stickleback in each of six independent watersheds on Vancouver Island: Misty Lake and its inlet stream, Beaver Lake and its outlet stream, Pye Lake and its outlet stream, Robert's Lake and its outlet stream, Village Bay Lake and its inlet stream, and Boot Lake and its outlet stream. Sample sizes were 36–40 fish for each of the 12 sites. Although some univariate traits also were measured on these Vancouver Island fish, we do not analyse them here owing to concerns about comparability (the measurements were taken by different people). By contrast, similar photographs ensured complete comparability between studies for analyses of body shape.

Following Kaeuffer *et al.* (2012), tpsDig2 (Rohlf, 2010a) was used to place 16 landmarks on each fish in the same locations as for that previous study (Fig. 2). In addition to this *de novo* placement of landmarks on Hendry Vineyard stickleback, all previous landmark placements for the Vancouver Island stickleback were checked and corrected as necessary. We then used tpsRelw (Rohlf, 2010b) to rotate, translate, and scale landmark coordinates with generalized least-squares superimposition. tpsRelw was also used to calculate centroid sizes (sum of squared deviations from the geometric position that minimizes that sum), uniform components (shared variation across landmarks in the x–y planes), partial warps (non-shared variation of individual landmarks in the x–y planes), and relative warps (principal components based on the partial warps and uniform components).

MANCOVA was used to analyse total shape variation and to extract canonical variates that optimized variation between samples. The canonical variates were then visualized in tpsRegr (Rohlf, 2011) following the suggestions of Langerhans and DeWitt (2004). For these analyses, centroid size was the covariate, all partial warps and uniform components were the response variables, statistical inferences were based on Wilks' lambda, and effect sizes were based on partial eta-squared. Three different data sets were analysed. The first MANCOVA included only Hendry Vineyard stickleback and was used to examine variation between years (2009 and 2010), sites (Reservoir 1, Reservoir 2, and creek), and sexes. These data were also analysed in a discriminant analysis to determine how well individuals from the three sites (years and sexes pooled) could be separated from each other. The second MANCOVA combined Hendry Vineyard stickleback (years, sexes, and reservoirs combined) and all Vancouver Island stickleback. This analysis was intended to inform the magnitude and direction of divergence between the Hendry reservoirs and creek in relation to that between the Vancouver Island lakes and streams. The third MANCOVA included only the Vancouver Island stickleback. Compared with the combined data set (the second MANCOVA), this analysis informed the effect of Hendry Vineyard stickleback on inferences regarding parallelism across lakes and streams.

Table 1. MANCOVA results for analyses of Hendry Vineyard stickleback

Term	<i>F</i>	d.f. (num, den)	<i>P</i>	Partial η^2
Univariate measurements				
Centroid size	146.40	7, 287	<0.001	0.781
Site	8.91	14, 574	<0.001	0.178
Year	14.72	7, 287	<0.001	0.264
Sex	6.22	7, 287	<0.001	0.132
Site \times Year	3.11	14, 574	<0.001	0.070
Site \times Sex	1.19	14, 574	0.281	0.028
Year \times Sex	0.98	7, 287	0.446	0.023
Site \times Year \times Sex	1.12	14, 574	0.335	0.027
Geometric morphometrics				
Centroid size	12.04	28, 294	<0.001	0.534
Site	9.40	56, 588	<0.001	0.472
Year	13.48	28, 294	<0.001	0.562
Sex	33.04	28, 294	<0.001	0.759
Site \times Year	0.41	56, 588	<0.001	0.362
Site \times Sex	1.70	56, 588	0.002	0.140
Year \times Sex	1.81	28, 294	0.009	0.147
Site \times Year \times Sex	1.30	56, 588	0.077	0.110

Note: Separate analyses were performed for univariate measurements and for geometric morphometrics. Sample sizes (see denominator degrees of freedom) are a bit lower for the univariate traits because some preserved fish were provided to other investigators (for geometric morphometrics, the sex of these fish was determined from photographs).

RESULTS

For univariate traits, all main effects (site, year, and sex) and the site \times year interaction were highly significant, whereas the other two-way interactions and the three-way interaction were not (Table 1). The greatest amount of variation was explained by centroid size, followed by year, site, and sex. The first canonical axis separated the creek from the two reservoirs, whereas the second differentiated the two reservoirs. Overall, however, the univariate traits did not do a good job of discriminating among stickleback from the different sites: overall success in classifying individuals back to their collection site was only 53.9%. Considering the different habitats, 53.8% of creek fish were correctly classified back to the creek and 82% of reservoir fish were correctly classified back to the reservoirs.

For geometric morphometric analysis of Hendry Vineyard stickleback, all main effects (site, year, and sex) were highly significant, as were all two-way interactions (Table 1). The greatest amount of variation was explained by sex, followed by year, centroid size, and then site. The first canonical axis separated the creek from the reservoirs, whereas the second separated the two reservoirs. Overall classification success in discriminant analysis was 81.7% among the three sites, with 89.4% of creek fish correctly classified back to the creek and 95.0% of reservoir fish correctly classified back to the reservoirs. Although stickleback from the different habitats thus could be discriminated from each other, and although the effect of site was highly significant, even the most extreme means along the

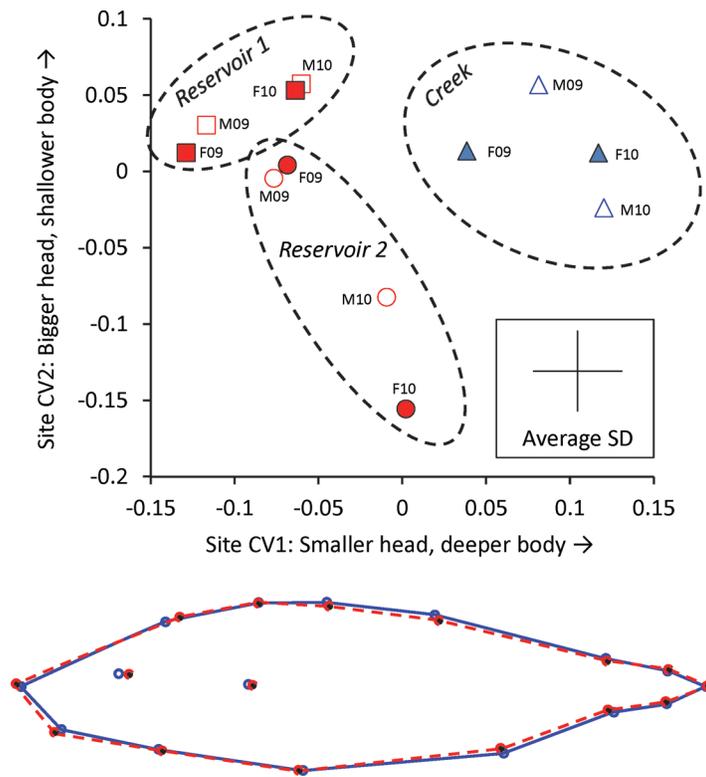


Fig. 3. Results of MANCOVA depicted as site/year/sex-specific means along the two canonical variates discriminating the three sites on the Hendry Vineyard (for statistics, see Table 1). Points are labelled by sex (M or F) and year (09 or 10), and average standard deviations are shown in the inset. Deformations from tpsRegr that correspond to the two most extreme means along CV1 are shown at the bottom: Reservoir 1 2009 females are the dashed outline and creek 2010 males are the solid outline.

first canonical axis were barely distinguishable when visualized in tpsRegr (Fig. 3). The small difference that was present reflected slightly shallower bodies and larger heads for reservoir stickleback than for creek stickleback.

For geometric morphometric analysis combining the Hendry Vineyard and Vancouver Island watersheds (Table 2), the greatest amount of variation was explained by centroid size, followed by watershed and habitat (lake/reservoir vs. stream/creek). The canonical axis optimized to discriminate between habitats showed that stream stickleback were deeper bodied and had longer caudal peduncles than lake stickleback, although the most extreme site means along this axis were not very different when visualized in tpsRegr (Fig. 4). The magnitude of divergence between populations along this habitat axis varied dramatically among watersheds, and was by far the smallest for the Hendry Vineyard stickleback (Fig. 4).

For geometric morphometric analysis of the Vancouver Island watersheds only (i.e. excluding Hendry Vineyard stickleback), all terms were again highly significant (Table 2). This time, however, the habitat (lake vs. stream) term explained by far the most variation. In

Table 2. MANCOVA results for analyses of geometric morphometric data

Term	<i>F</i>	d.f. (num, den)	<i>P</i>	Partial η^2
With Hendry Vineyard				
Centroid size	34.13	28, 762	<0.001	0.556
Watershed	16.53	168, 4492	<0.001	0.377
Habitat	9.05	28, 762	<0.001	0.250
Watershed \times Habitat	6.35	168, 4492	<0.001	0.189
Without Hendry Vineyard				
Centroid size	25.37	28, 430	<0.001	0.623
Watershed	11.92	140, 2128	<0.001	0.435
Habitat	63.92	28, 430	<0.001	0.806
Watershed \times Habitat	7.01	140, 2128	<0.001	0.312

Note: Separate analyses were performed with and without Hendry Vineyard stickleback (years, sexes, and reservoirs pooled). ‘Watershed’ represents the six Vancouver Island watersheds (and Hendry Vineyard in the first analysis) and ‘habitat’ represents lake versus stream (and reservoirs versus creek in the first analysis).

addition, the lake–stream canonical axis showed a large and consistent difference in body depth between lake and stream fish within each watershed (Fig. 5). Comparison of these results with those from the above combined analysis, shows that divergence between the Hendry reservoirs and creek is much lower than, and not parallel with, divergence between the Vancouver Island lakes and creeks.

While processing Hendry Vineyard stickleback, we noted that the morphological variation among individuals was very high. To quantify this observation, we performed an exploratory analysis of total geometric morphometric variation within the Hendry sites and within the Vancouver Island sites. We first calculated the scores for each fish on each relative warp axis when all populations were in the analysis. The variance in these scores was then summed across all of the relative warps within each population. This analysis revealed that shape variation within the Hendry reservoirs and creek was higher than that within any of the Vancouver Island lakes and streams (Fig. 6). (Variation was also high for particular combinations of sex and year within the Hendry Vineyard sites.)

DISCUSSION

Despite many previous studies documenting morphological divergence between lake and stream stickleback (Reimchen *et al.*, 1985; Lavin and McPhail, 1993; Hendry *et al.*, 2002; Hendry and Taylor, 2004; Berner *et al.*, 2008, 2009, 2010; Aguirre, 2009; Kaeuffer *et al.*, 2012; Ravinet *et al.*, 2013a), we did not find evidence of noteworthy divergence between reservoir and creek stickleback on the Hendry Vineyard. This conclusion is supported by three observations. First, although phenotypic divergence between creek and reservoir stickleback was statistically significant, it was very weak and explained little of the overall variance. Second, when analysed together with six lake–stream pairs from Vancouver Island, Hendry Vineyard stickleback showed much lower divergence than did any of the other pairs. Third, lake–stream divergence for the Vancouver Island pairs was much greater and more consistent (i.e. more parallel) when Hendry Vineyard stickleback were excluded from the analysis. We can see four potential reasons why

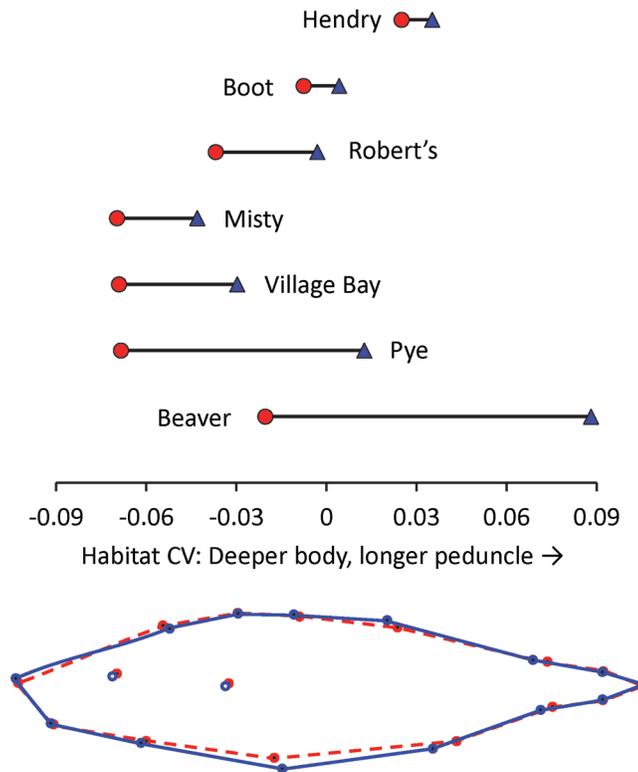


Fig. 4. Results of MANCOVA including all sites (Vancouver Island and Hendry Vineyard; years, sexes, and reservoirs pooled) depicted as means along the ‘habitat’ (creek/stream vs. reservoir/lake) canonical variate (for statistics, see Table 2). Lake/reservoir means are shown by circles and creek/stream means by triangles. Deformations from tpsRegr that correspond to the two most extreme means along the canonical variate are shown at the bottom: Misty Lake stickleback are the dashed outline and Beaver outlet stream stickleback are the solid outline.

divergence is low between creek and reservoir stickleback on the Hendry Vineyard: divergent selection might be weak, gene flow might be high, divergence time might be too short, and appropriate genetic variation might be lacking. We now consider each explanation in turn.

Divergent selection might be weaker between Hendry reservoir and creek stickleback than between Vancouver Island lake and stream stickleback. Although divergent selection has not been directly measured in any of the populations, two observations suggest that divergence in diets is a reliable surrogate. First, stream stickleback almost exclusively consume benthic prey whereas lake stickleback also often consume limnetic prey (Berner *et al.*, 2008, 2009; Kaeuffer *et al.*, 2012). Second, lake–stream diet divergence is generally a very good predictor of lake–stream phenotypic divergence (Berner *et al.*, 2008; Kaeuffer *et al.*, 2012). Although we have not yet quantified diets for Hendry Vineyard stickleback, the reservoirs are certainly more benthic environments (shallower and smaller) than are the Vancouver Island lakes. We would thus expect less diet divergence between Hendry reservoir and creek stickleback than between the Vancouver Island lake and stream stickleback – and the

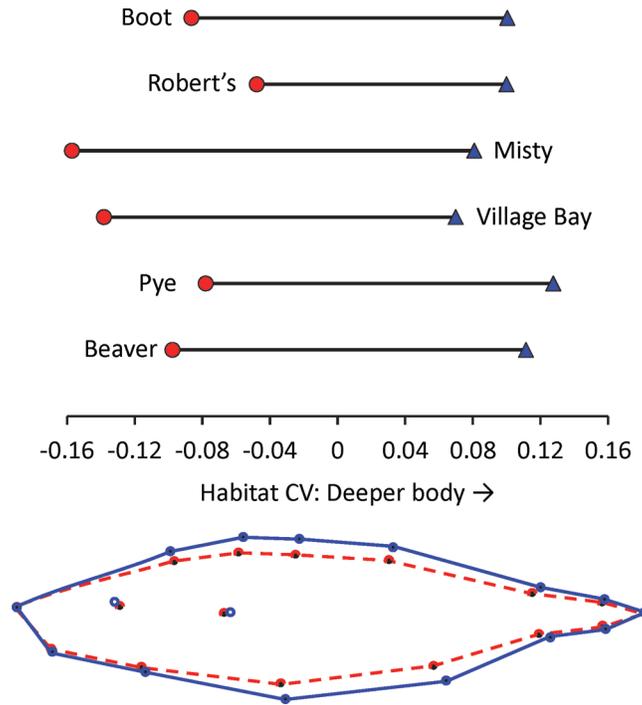


Fig. 5. Results of MANCOVA including only Vancouver Island sites (i.e. Hendry Vineyard fish excluded) depicted as means along the ‘habitat’ (stream vs. lake) canonical variate (for statistics, see Table 2). Lake means are shown by circles and stream means by triangles. Deformations from tpsRegr that correspond to the two most extreme means along the canonical variate are shown at the bottom: Misty Lake is the dashed outline and Pye outlet stream is the solid outline.

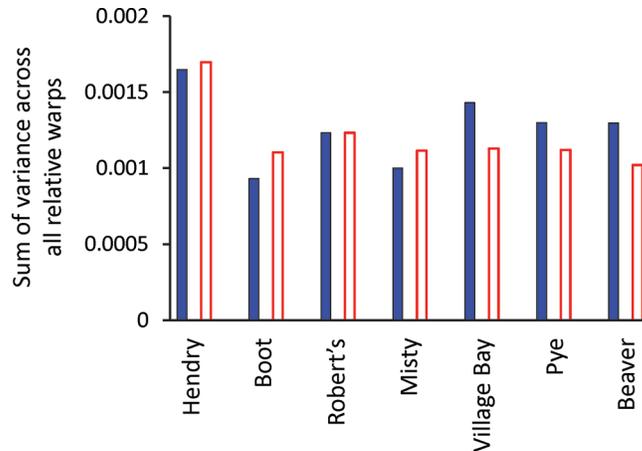


Fig. 6. The total amount of geometric morphometric variance in the Hendry Vineyard populations (sexes, years, and the two reservoirs pooled) and in the Vancouver Island populations (sexes pooled). Creek/stream sites are shown as solid bars and reservoir/lake sites as open bars.

consequence should be weaker divergent selection. However, weak divergent selection cannot be the full story because the reservoir and creek environments are certainly very different (A.P. Hendry, personal observation).

Gene flow might be high in Hendry Vineyard stickleback because water continues to be pumped from the creek into the reservoirs, as well as between the reservoirs. Overflow could also occur from the reservoirs into the creek. If gene flow is high, it could theoretically constrain evolution even in the presence of divergent selection (Hendry *et al.*, 2001; Lenormand, 2002; Garant *et al.*, 2007). In fact, gene flow does appear to constrain adaptive divergence in some Vancouver Island lake–stream pairs (Hendry *et al.*, 2002; Hendry and Taylor, 2004; Moore *et al.*, 2007; Roesti *et al.*, 2012), although not the pairs re-analysed here. To gain some initial insight into gene flow on the Hendry Vineyard, Ella Bowles and Sean Rogers at the University of Calgary screened 23 reservoir and 23 creek stickleback from our 2009 samples for allelic variation at four microsatellite loci. Their unpublished results indicate that reservoir–creek genetic divergence is very low, lower in fact than in any of the Vancouver Island lake–stream pairs. Gene flow thus might be an important constraint on divergence between Hendry Vineyard reservoir and creek stickleback.

The amount of time for divergence has been limited on the Hendry Vineyard because the reservoirs have only existed since the early 1970s. The *maximum* time for stickleback divergence is thus only about 35 generations. Even if divergent selection is present and gene flow is not overpowering, noteworthy divergence might simply take more time. On the one hand, this argument seems weak given the many examples of phenotypic changes in stickleback that have colonized new environments on similar or shorter time scales (Klepaker, 1993; Bell *et al.*, 2004; Adachi *et al.*, 2012; Aguirre and Bell, 2012; Leaver and Reimchen, 2012; Bell and Aguirre, 2013). On the other hand, stickleback colonizing Switzerland now show only modest lake–stream divergence after more than 100 years (Bernier *et al.*, 2010; Lucek *et al.*, 2012a). Limited time for divergence therefore might be a factor for Hendry Vineyard stickleback, at least in combination with other constraints.

Limited genetic variation for adaptation is another possible explanation for low trait divergence. Indeed, several stickleback studies have argued for such constraints in particular instances – and we here provide just two examples. First, pelvic phenotypes changed very slowly in a fossil lineage of stickleback, probably owing to the recessive and low-frequency nature of the necessary alleles (Hunt *et al.*, 2008; Bell and Aguirre, 2013). Second, reduced lateral plate number – a typical condition in freshwater stickleback – has apparently not occurred in some Scandinavian populations owing to limited genetic variation, with the populations instead evolving smaller plates (Leinonen *et al.*, 2012). Notwithstanding such examples, a constraint from limited variation seems unlikely for Hendry Vineyard stickleback because phenotypic variation, including in the traits normally showing lake–stream divergence (e.g. body depth), was very high in the ancestral creek population and in the derived reservoir populations (Fig. 6).

In summary, we do not have a smoking gun for why phenotypic divergence between Hendry reservoir and creek stickleback is so low. Instead, several factors all have the potential to contribute: divergent selection might be relatively weak, gene flow might be high, and more time might be necessary. Further work will be required to disentangle these different effects but the upshot is that selection – or at least its outcome – has here not been particularly rapid, powerful, or repeatable.

An opportunity in extreme variation?

Levels of geometric morphometric variation in Hendry Vineyard stickleback were very high – higher, in fact, than in any of the 12 Vancouver Island populations to which they could be directly compared (Fig. 6). Even more dramatic variation was evident in craniofacial shape (Fig. 7), although this has yet to be formally quantified. Some (but clearly not all) of this variation could be associated with differences between the sexes (Fig. 3), and some would seem to represent malformations. Yet even these malformed individuals were apparently doing fine in nature as they were large and in good condition. We suggest that this extreme variation might be useful for studying the origins and fate of morphological variation. We first summarize some of the key questions in this area and then outline some logical next steps for research.

Many questions surround the origin and fate of phenotypic variation (Stern, 2000). First, to what extent are novel morphological variants the result of genetic versus plastic (including epigenetic) differences (West Eberhard, 2003; Moczek *et al.*, 2011)? Second, to what extent do novel variants arise through protein coding versus regulatory changes (Hoekstra and Coyne, 2007; Stern and Orgogozo, 2008)? Third, what is the distribution of fitness effects of new mutations (Lynch *et al.*, 1999)? Fourth, when a new favourable mutation arises, what conditions favour its retention and spread – as opposed to its loss through drift (Orr, 1998, 2010)? One potential contributor is frequency dependence owing to natural or sexual selection (Olendorf *et al.*, 2006; Hosoi *et al.*, 2010). Fifth, how do multiple new variants interact – that is, do they show epistasis or ‘clonal interference’ (Gerrish and Lenski, 1998)? Sixth, are the effects of genes of major effect transitory during the course of adaptation (Agrawal *et al.*, 2001)? Seventh, to what extent does colonization of new environments lead to ecological release that inflates variance and promotes evolution (Yoder *et al.*, 2010)? Although many of these questions have been addressed in theory, laboratory studies, and comparisons among populations, opportunities to examine different variants currently segregating in natural populations have proven elusive.

Perhaps the extreme morphological variation in Hendry Vineyard stickleback (Figs. 6 and 7) could be used to address some of these questions. For starters, it would be good to ascertain whether high variation is a general property of stickleback in this geographical region, or whether it is specific to the Hendry Vineyard. A logical next step would be to use artificial crosses to assess the developmental, genetic, and environmental basis for the different morphological variants. The variation then could be explored through the many developmental and genetic analysis tools available for stickleback, including genome scans, gene expression assays, association mapping, and candidate genes (Kingsley and Peichel, 2007; Jones *et al.*, 2012b; Roesti *et al.*, 2012). In addition, the success of particular variants could be assessed in nature by photographing, tagging, and genotyping individuals with different phenotypes/genotypes, and then tracking their survival (mark–recapture) and reproductive success (parentage analysis). In combination, these various approaches could be used to test many of the general hypotheses outlined above.

Summary

Stickleback populations in the Hendry Vineyard reservoirs and creek have not diverged in a manner consistent with post-glacial stickleback populations in lakes and streams. The precise reason is not yet known but likely relates to a combination of relatively weak divergent selection, high gene flow, and limited time for divergence. These results provide



Fig. 7. A selection of cropped images showing extreme morphological variation in the Hendry Vineyard stickleback. These are a haphazard selection across sexes, years, and sites.

balance to the typical assertion in stickleback research that divergence between environments is a nearly ubiquitous outcome of divergent selection. Although stickleback divergence on the Hendry Vineyard was low between habitats, it was very high within habitats. This variation provides a promising substrate for studying the origins and fates of morphological variation.

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