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Report

Maintenance of a Genetic Polymorphism with Disruptive Natural Selection in Stickleback

Kerry B. Marchinko,[1] Blake Matthews,[2] Matthew E. Arnegard,[3] Sean M. Rogers,[4] and Dolph Schluter[1,*]

[1]Biodiversity Research Centre and Department of Zoology, University of British Columbia, Vancouver, BC V6T 1Z4, Canada

[2]Department of Aquatic Ecology, EAWAG, 79 Seestraße, 6047 Kastanienbaum, Switzerland

[3]Human Biology and Basic Sciences Divisions, Fred Hutchinson Cancer Research Center, Seattle, WA 98109, USA

[4]Department of Biological Sciences, University of Calgary, Calgary, AB T2N 1N4, Canada

Summary

The role of natural selection in the maintenance of genetic variation in wild populations remains a major problem in evolution. The influence of disruptive natural selection on genetic variation is especially interesting because it might lead to the evolution of assortative mating or dominance [1, 2]. In theory, variation can persist at a gene under disruptive natural selection, but the process is little studied and there are few examples [3, 4]. We report a stable polymorphism in the bony armor of threespine stickleback maintained with a deficit of heterozygotes at the major underlying gene, *Ectodysplasin* (*Eda*) [5]. The deficit vanishes at the embryo life stage only to re-emerge in adults, indicating that disruptive natural selection, rather than nonrandom mating, is the cause. The mechanism enabling long-term persistence of the polymorphism is unknown, but disruptive selection is predicted to be frequency dependent, favoring homozygous genotypes when they become rare. Further research on the ecological and evolutionary processes affecting individual genes will ultimately lead to a better understanding of the causes of genetic variation in populations.

Results and Discussion

Adaptive processes maintaining genetic polymorphisms include heterozygote advantage, gene flow between locally adapted populations, and frequency-dependent natural selection, but they are difficult to distinguish in genomic data and their relative importance in nature is unclear [3, 4]. We explored this problem in a threespine stickleback population highly variable in bony armor plates and polymorphic at the major underlying gene. Kennedy Lake is a large, highly oligotrophic, and young lake (12,000 years) on Vancouver Island, Canada, whose stickleback were sampled previously in 1965 [6]. Armor plate number is strongly bimodal (Figure 1A). Completely plated individuals ("complete morph") are more common than individuals with few plates ("low morph"), and intermediate individuals ("partial morph") are rare. The frequency of armor morphs has changed little between samples taken over 45 years in 1965, 2004, 2006, 2008, and 2010 (Figure 1B). The frequency of the partial morph has declined since the 1965 collection, and the frequency of the complete morph has risen slightly (Figure 1B; contingency test of frequency differences among years: $\chi^2 = 22.1$, $df = 8$, $p = 0.005$). Mean generation time is most likely between 1 and 3 years, based on other populations [7, 8]. Kennedy Lake is inaccessible to highly armored stickleback from the Pacific Ocean (four cascades on the outlet river prevent upstream passage), ruling out gene flow between divergent marine and lake populations as a cause of the polymorphism.

Natural selection most likely plays a role in the polymorphism. Lateral plates have a defensive role, increasing survival after attack by piscivorous fish [9, 10]. Plates are also costly, reducing the growth rate of individual fish raised in fresh water [11, 12]. Low-plated and completely plated morphs differ in head morphology and in stable isotopes of carbon, $\delta^{13}C$, in muscle (Table S1 available online), possibly indicating a diet and/or habitat difference. Plate polymorphisms with appreciable frequencies of both low and complete morphs are uncommon in lake-resident stickleback populations of the region [13–15] but may occur more frequently in Europe [16–18].

Ectodysplasin (*Eda*) is the major gene responsible for variation in lateral-plate number in threespine stickleback [5, 13, 18, 19]. We confirmed that the plate polymorphism is strongly associated with *Eda* in Kennedy Lake (Goodman-Kruskal correlation [20]: $\gamma = 0.86 \pm 0.03$ SE; see the Supplemental Experimental Procedures). The majority of heterozygotes, $Eda^{C/L}$, are completely plated, with the *C* (complete) allele dominant over the *L* (low) allele (dominance coefficient = 0.87 ± 0.04 SE). *Eda* is pleiotropic and is linked to other genes that may also affect phenotype [5]. Thus, the polymorphism may be affected by selection on other traits, including head morphology (Table S1), neuromast pattern along the lateral line [21], and schooling behavior [22].

Remarkably, we found a persistent deficit of *Eda* heterozygotes in adult fish in each year (Figure 1C), as indicated by large positive Wright's F_{IS} coefficients (2006: $F_{IS} = 0.44$ [95% CI: 0.19, 0.67]; 2008: $F_{IS} = 0.36$ [0.19, 0.52]; 2010: $F_{IS} = 0.30$ [0.15, 0.45]; combined: $F_{IS} = 0.35$ [0.25, 0.45]). Accordingly, adult genotype frequencies deviated from Hardy-Weinberg expectation (2006: $\chi^2 = 10.37$, $p = 0.001$; 2008: $\chi^2 = 16.31$, $p < 0.0001$; 2010: $\chi^2 = 14.57$, $p = 0.0001$; $df = 1$). The heterozygote deficit was not associated with a genome-wide signature of population structure [23]. None of seven unlinked, putatively neutral microsatellite loci deviated significantly from Hardy-Weinberg expectation or from linkage equilibrium after correction for false discovery rate (Supplemental Experimental Procedures). Individual-based population assignment [24] with these loci strongly supported a single genetic cluster ($Pr[K = 1] = 0.99$). Moreover, the differences in microsatellite allele frequencies between the low and complete lateral-plate morphs explained less than 1% of the total allelic variation ($F^{ST} = 0.008$), similar to estimates of F^{ST} between stickleback populations experiencing high levels of gene flow [25]. These findings indicate that armor phenotypes belong to a single, panmictic population.

Heterozygote deficiency vanished in embryos collected from nests, indicating that assortative mating by *Eda* genotype

*Correspondence: schluter@zoology.ubc.ca

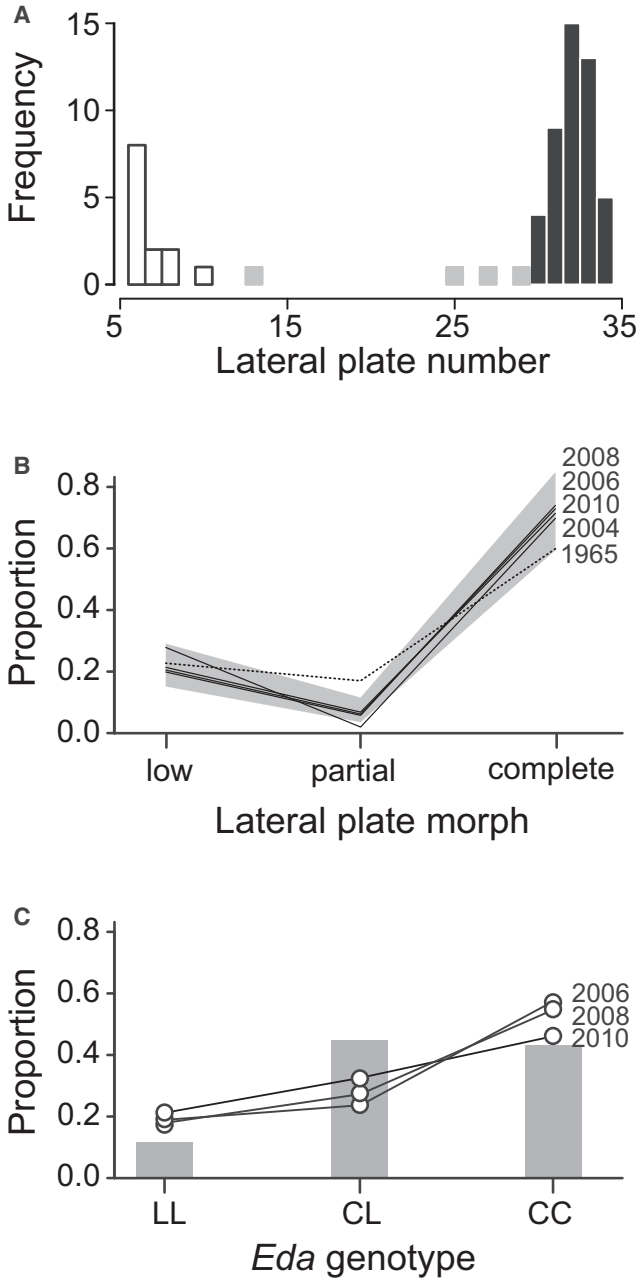

Figure 1. Lateral-Plate and *Eda* Genotype Frequencies

(A) The bimodal frequency distribution of the number of lateral plates in adult stickleback collected in 2006 (plate numbers were counted on the left side of the fish). Lateral-plate morph is indicated by shading: complete (black), partial (gray), and low (white).

(B) Morph frequencies in adult samples from 1965 (n = 35), 2004 (n = 329), 2006 (n = 63), 2008 (n = 135), and 2010 (n = 169). Shading indicates span of 95% confidence intervals (CIs) for proportions in 2010, to indicate levels of uncertainty.

(C) *Eda* genotype frequencies in adults collected in 2006, 2008, and 2010. Bars indicate Hardy-Weinberg expectations calculated from the average genotype frequencies over the three years. Sample sizes are as in (B).

See also Table S1.

is not the cause of the low frequency of adult heterozygotes. We genotyped the *Eda* locus of embryos in three to six eggs from each of 71 and 68 stickleback clutches collected during breeding seasons in June 2008 and June 2010. If the

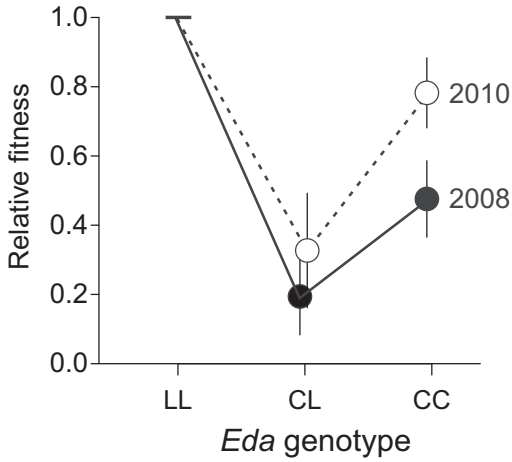

Figure 2. Estimated Fitness of *Eda* Genotypes Relative to $Eda^{L/L}$

Estimates are based on the genotype frequencies observed in 341 embryos and 135 adults sampled during the breeding season of June 2008, and 303 embryos and 169 adults sampled in June 2010. Points and error bars indicate the mean relative fitness ± SE estimated from 10,000 bootstrap resamples. See also Figure S1.

heterozygote deficiency in adults reflects assortative mating, then their embryos should show a similar deficit of *Eda* heterozygotes. Instead, embryos showed no deficiency of heterozygotes (2008: F_{IS} = 0.061 [95% CI: –0.07, 0.20]; 2010: F_{IS} = –0.05 [–0.19, 0.08]) (Figure S1) and no significant deviation from Hardy-Weinberg expectations at *Eda* (2008: χ^2 = 0.287, p = 0.592; 2010: χ^2 = 0.258, p = 0.612; *df* = 1). Adults exhibited significantly greater heterozygote deficiency than did embryos collected at the same time and place (F_{IS} 2008: difference of 0.30 [95% CI: 0.08, 0.51]; 2010, difference of 0.35 [0.15, 0.55]). Assortative mating between stickleback populations differing in lateral-plate number is well known [26], but assortative mating between armor morphs within populations has not been described.

Absence of heterozygote deficiency in embryos implies that the deficit in adults is regenerated each year by persistent disruptive natural selection. We estimated selection from the observed changes in genotype frequencies between life stages, from embryo to adult and from adult back to embryo, assuming random mating (Figures 2 and S1). Estimated selection coefficients are large, with the fitness of heterozygotes being only 20%–30% of the fitness of the most fit homozygote (product of selection and dominance coefficients 2008: *hs* = –0.81 [95% CI: –0.53, –0.96]; 2010: *hs* = –0.67 [–0.25, –0.86]; Figure 2). Selection against heterozygotes was indicated in both viability (from embryo to adult) and reproductive success (adult to embryo) (Figure S1), but the pattern is strongest when the two stages are combined (Figure 2). Estimated selection between adult and embryo stages is presumed to arise from differences in reproductive success via sexual, fecundity, or gametic selection. Intrinsic differences in survival of embryos prior to hatching is unlikely to contribute because lateral-plate morphs from Kennedy Lake differ little in hatching rate in the lab [12]. Embryo *Eda* frequencies varied significantly between sampling dates, but selection against heterozygotes was consistently observed (Figure S1).

Relative fitness was highest for the low $Eda^{L/L}$ genotype (Figure 2), which appears to have a growth advantage: low-plated adults from this study had slightly higher mean

standard length than did completely plated individuals (Table S1), consistent with a lab study [12]. A higher net fitness of the $Eda^{L/L}$ genotype is expected at equilibrium (Figure 2), given that the *L* allele is at a lower frequency than the *C* allele in the population. This higher fitness balances the disadvantage experienced by the *L* allele via random mating, which puts a higher fraction of all *L* allele copies into low-fitness heterozygotes than *C* allele copies simply because it is more rare.

There are few other examples from nature of stable genetic polymorphisms with heterozygote disadvantage. In stickleback, *Ectodysplasin* polymorphisms in other populations result from gene flow or represent transient states [13, 18, 27], although disruptive selection on *Eda* has been detected in at least one other study [17]. Such cases of heterozygote disadvantage are interesting for three reasons. First, despite the paucity of examples, disruptive selection on polymorphic loci is predicted to arise under many ecological conditions, such as when genotypes compete for food or enemy free space [28].

Second, population genetic theory indicates that it is difficult to maintain a stable polymorphism with heterozygote disadvantage unless selection is frequency dependent, favoring each homozygote when it becomes rare; otherwise, the rarer allele is driven rapidly to extinction [29]. Niche differentiation between genotypes may give rise to frequency-dependent selection, and morphological and dietary differences between morphs suggest the presence of ecological differences (Table S1), though this is not by itself sufficient evidence. Interactions with predators might give rise to frequency dependent selection, and other mechanisms are also possible [30].

Third, important evolutionary consequences can arise from such a polymorphism [1, 2, 29]. One possibility is the evolutionary modification of dominance, yielding heterozygotes whose fitness resembles that of one of the homozygote genotypes. Another possible consequence is the evolution of assortative mating, reducing the number of maladaptive heterozygotes produced. There are theoretical obstacles to both outcomes [31], and it is not simple to predict which is most likely. No assortative mating by *Eda* genotype was discovered here. It is tempting to consider whether the reduction in the frequency of the partial morph in Kennedy Lake from 1965 to the present, and an increase in the complete morph (Figure 1A), reflects an evolved change of dominance on lateral plates, but other explanations are possible. It is also conceivable that intermediate stages toward the evolution of assortative mating or dominance might incidentally give an unconditional advantage to one allele over the other and produce a third outcome, the elimination of the polymorphism. This might help to explain why most stickleback populations in the region are virtually fixed for either the low or the complete *Eda* genotype [14].

Eda thus provides a new opportunity to investigate polymorphism maintained in the face of low heterozygote fitness and its evolutionary outcomes. Our findings advance growing evidence for the role of selection in the maintenance of ecologically important genetic polymorphisms [3, 4, 32, 33] in wild populations and in particular point to the role of frequency-dependent selection maintaining variation within a single population. Our increasing ability to identify processes affecting evolutionary dynamics at individual genes will ultimately lead to a better understanding of the maintenance of genetic variation, still regarded as an unsolved problem [4, 34].

Experimental Procedures

Collections

We collected adult threespine stickleback in minnow traps and dip nets near the southern tip of the Clayoquot arm of Kennedy Lake on May 11, July 9, and Aug 17, 2004; June 11, 2006; June 12, 2008; and June 11, 2010. Fish were given a lethal dose of tricaine methanesulfonate (MS-222) before preservation. Formalin-preserved specimens from a sample collected on Aug 2, 1965, by G. Haythorne and D. Hagen were obtained from the Beaty Museum at the University of British Columbia (UBC 65-0506). Isotopic analysis and morphological measurements were conducted on the 2004 collection. Embryos were collected by snorkeling in 2008 and 2010. Detailed descriptions of sampling, storage and morphological measurements are found in the Supplemental Experimental Procedures. All procedures and experimental protocols were approved by the University of British Columbia Animal Care Committee and were in accordance with the Canadian Council on Animal Care.

Genotyping

A diagnostic indel locus (isolated from the locus Stn381 within intron six of the *Eda* gene [5]) was used to identify the genotype of lateral-plate morphs. Seven microsatellite loci, isolated and characterized by the Stanford Genome Research Center, were selected for genetic analyses: Stn301 (GenBank accession number BV678111, unpublished), Stn65 (G72254 [35]), Stn216 (BV102494 [36]), Stn250 (BV678075, unpublished), Stn387 (BV678140 [37]), Stn388 (BV678141 [37]), and Stn51 (G72248 [35]). A detailed description of genotyping methods is found in the Supplemental Experimental Procedures.

Relative Fitness and Selection Coefficients

Relative fitness for each *Eda* genotype during viability selection was calculated as the change in the observed frequency of a genotype from embryo to adult (frequency in adult divided by frequency in embryo), relative to the homozygous low $Eda^{L/L}$ genotype, which was set to 1. Relative fitness during the reproductive phase of the life cycle was calculated by setting the relative reproductive success of $Eda^{L/L}$ genotypes in adults to 1 and then solving for the fitness values of adult $Eda^{C/L}$ and $Eda^{C/C}$ genotypes generating observed embryo allele frequencies in nests sampled from the wild. Calculations of relative reproductive fitness used *Eda* genotype frequencies observed in randomly sampled adults and assume no assortative mating. Reproductive selection may result from differences in mate preference, viability, or fecundity among adults differing in *Eda* genotypes.

Relative fitness of each genotype across one generation (net selection) was calculated as the product of the relative fitness from viability and reproductive phases, again relative to the homozygous low $Eda^{L/L}$ genotype (set to 1). Selection coefficients *s* were calculated as 1 minus the relative fitness of $Eda^{C/C}$ homozygote, whereas 1 minus the relative fitness of the $Eda^{C/L}$ heterozygote is *hs* [38].

Statistical Analyses

Statistical analyses were conducted using R 3.0.1 (R Foundation for Statistical Computing; http://www.r-project.org). Approximate likelihood-based 95% CIs for morph proportions were calculated using the glm function. Heterozygote deficiency was quantified using Wright's coefficient of inbreeding (F_{IS}),

$$F_{IS} = 1 - \frac{H}{2pq},$$

where *H* is the frequency of the heterozygotes, and *p* and *q* represent allele frequencies [38]. CIs for F_{IS} in adult samples from 2006, 2008, and 2010 were calculated using 10,000 bootstrap resamples. CIs for embryo genotype frequencies and F_{IS} were estimated from a bootstrap distribution generated from 10,000 resamples of whole clutches, rather than individual embryos. The 95% CIs for the selection coefficients associated with the homozygote $Eda^{C/C}$ and heterozygote $Eda^{C/L}$ genotypes (*s*, and *hs* respectively) for viability, reproductive selection, and net selection were estimated from bootstrap resamples of genotypes of embryo whole clutches and adults.

Tests of genetic diversity and Hardy-Weinberg equilibrium were carried out with GENEPOP v.4.0 [39]. Genetic differentiation in allele frequencies between low and complete morphs was tested in ARLEQUIN (v3.5). The most likely number of populations (from K = 1 to K = 3) was assessed with STRUCTURE [24].

Supplemental Information

Supplemental Information includes one figure, one table, and Supplemental Experimental Procedures and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2014.04.026.

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References

1. Durinx, M., and Van Dooren, T.J.M. (2009). Assortative mate choice and dominance modification: alternative ways of removing heterozygote disadvantage. Evolution *63*, 334–352.
2. Rueffler, C., Van Dooren, T.J.M., Leimar, O., and Abrams, P.A. (2006). Disruptive selection and then what? Trends Ecol. Evol. *21*, 238–245.
3. Hedrick, P.W. (2006). Genetic polymorphism in heterogeneous environments: the age of genomics. Annu. Rev. Ecol. Evol. Syst. *37*, 67–93.
4. Mitchell-Olds, T., Willis, J.H., and Goldstein, D.B. (2007). Which evolutionary processes influence natural genetic variation for phenotypic traits? Nat. Rev. Genet. *8*, 845–856.
5. Colosimo, P.F., Hosemann, K.E., Balabhadra, S., Villarreal, G., Jr., Dickson, M., Grimwood, J., Schmutz, J., Myers, R.M., Schluter, D., and Kingsley, D.M. (2005). Widespread parallel evolution in sticklebacks by repeated fixation of *Ectodysplasin* alleles. Science *307*, 1928–1933.
6. Hagen, D.W., and McPhail, J.D. (1970). The species problem within *Gasterosteus aculeatus* on the Pacific coast of North America. J. Fish. Res. Board Can. *27*, 147–155.
7. Baker, J.A. (1994). Life history variation in female threespine stickleback. In The Evolutionary Biology of the Threespine Stickleback, M.A. Bell and S.A. Foster, eds. (Oxford: Oxford University Press), pp. 144–187.
8. DeFaveri, J., and Merilä, J. (2013). Variation in age and size in Fennoscandian three-spined sticklebacks (*Gasterosteus aculeatus*). PLoS ONE *8*, e80866.
9. Reimchen, T.E. (1994). Predators and morphological evolution in threespine stickleback. In The Evolutionary Biology of the Threespine Stickleback, M.A. Bell and S.A. Foster, eds. (Oxford: Oxford University Press), pp. 240–276.
10. Reimchen, T.E. (2000). Predator handling failures of lateral plate morphs in *Gasterosteus aculeatus*: functional implications for the ancestral plate condition. Behaviour *137*, 1081–1096.
11. Barrett, R.D.H., Rogers, S.M., and Schluter, D. (2008). Natural selection on a major armor gene in threespine stickleback. Science *322*, 255–257.
12. Marchinko, K.B., and Schluter, D. (2007). Parallel evolution by correlated response: lateral plate reduction in threespine stickleback. Evolution *61*, 1084–1090.
13. Kitano, J., Bolnick, D.I., Beauchamp, D.A., Mazur, M.M., Mori, S., Nakano, T., and Peichel, C.L. (2008). Reverse evolution of armor plates in the threespine stickleback. Curr. Biol. *18*, 769–774.
14. Hagen, D.W., and Gilbertson, L.G. (1972). Geographic variation and environmental selection in *Gasterosteus aculeatus* L in Pacific Northwest, America. Evolution *26*, 301–312.
15. Moodie, G.E.E., and Reimchen, T.E. (1976). Phenetic variation and habitat differences in *Gasterosteus* populations of the Queen Charlotte Islands. Syst. Biol. *25*, 49–61.
16. Münzing, J. (1963). The evolution of variation and distributional patterns in European populations of the three-spined stickleback, *Gasterosteus aculeatus*. Evolution *17*, 320–332.
17. Zeller, M., Lucek, K., Haesler, M.P., Seehausen, O., and Sivasundar, A. (2012). Signals of predation-induced directional and disruptive selection in the threespine stickleback. Evol. Ecol. Res. *14*, 193–205.
18. Raeymaekers, J.A.M., Konijnendijk, N., Larmuseau, M.H.D., Hellemans, B., De Meester, L., and Volckaert, F.A.M. (2014). A gene with major phenotypic effects as a target for selection vs. homogenizing gene flow. Mol. Ecol. *23*, 162–181.
19. Cano, J.M., Matsuba, C., Mäkinen, H., and Merilä, J. (2006). The utility of QTL-Linked markers to detect selective sweeps in natural populations—a case study of the *EDA* gene and a linked marker in threespine stickleback. Mol. Ecol. *15*, 4613–4621.
20. Agresti, A. (2010). Analysis of Ordinal Categorical Data (Hoboken: Wiley).
21. Wark, A.R., Mills, M.G., Dang, L.-H., Chan, Y.F., Jones, F.C., Brady, S.D., Absher, D.M., Grimwood, J., Schmutz, J., and Myers, R.M. (2012). Genetic architecture of variation in the lateral line sensory system of threespine sticklebacks. G3 (Bethesda) *2*, 1047–1056.
22. Greenwood, A.K., Wark, A.R., Yoshida, K., and Peichel, C.L. (2013). Genetic and neural modularity underlie the evolution of schooling behavior in threespine sticklebacks. Curr. Biol. *23*, 1884–1888.
23. Castric, V., Bernatchez, L., Belkhir, K., and Bonhomme, F. (2002). Heterozygote deficiencies in small lacustrine populations of brook charr *Salvelinus Fontinalis* Mitchill (Pisces, Salmonidae): a test of alternative hypotheses. Heredity (Edinb) *89*, 27–35.
24. Pritchard, J.K., Stephens, M., and Donnelly, P. (2000). Inference of population structure using multilocus genotype data. Genetics *155*, 945–959.
25. Bolnick, D.I., Caldera, E.J., and Matthews, B. (2008). Evidence for asymmetric migration load in a pair of ecologically divergent stickleback populations. Biol. J. Linn. Soc. Lond. *94*, 273–287.
26. McKinnon, J.S., and Rundle, H.D. (2002). Speciation in nature: the threespine stickleback model systems. Trends Ecol. Evol. *17*, 480–488.
27. Bell, M.A., Aguirre, W.E., and Buck, N.J. (2004). Twelve years of contemporary armor evolution in a threespine stickleback population. Evolution *58*, 814–824.
28. Doebeli, M. (2011). Adaptive Diversification (Princeton: Princeton University Press).
29. Wilson, D.S., and Turelli, M. (1986). Stable underdominance and the evolutionary invasion of empty niches. Am. Nat. *127*, 835–850.
30. Ayala, F.J., and Campbell, C.A. (1974). Frequency-dependent selection. Annu. Rev. Ecol. Syst. *5*, 115–138.
31. Gavrilets, S. (2003). Perspective: models of speciation: what have we learned in 40 years? Evolution *57*, 2197–2215.
32. Fitzpatrick, M.J., Feder, E., Rowe, L., and Sokolowski, M.B. (2007). Maintaining a behaviour polymorphism by frequency-dependent selection on a single gene. Nature *447*, 210–212.
33. Subramaniam, B., and Rausher, M.D. (2000). Balancing selection on a floral polymorphism. Evolution *54*, 691–695.
34. Barton, N.H., and Keightley, P.D. (2002). Understanding quantitative genetic variation. Nat. Rev. Genet. *3*, 11–21.
35. Peichel, C.L., Nereng, K.S., Ohgi, K.A., Cole, B.L.E., Colosimo, P.F., Buerkle, C.A., Schluter, D., and Kingsley, D.M. (2001). The genetic architecture of divergence between threespine stickleback species. Nature *414*, 901–905.
36. Colosimo, P.F., Peichel, C.L., Nereng, K., Blackman, B.K., Shapiro, M.D., Schluter, D., and Kingsley, D.M. (2004). The genetic architecture of parallel armor plate reduction in threespine sticklebacks. PLoS Biol. *2*, E109.
37. Gow, J.L., Peichel, C.L., and Taylor, E.B. (2006). Contrasting hybridization rates between sympatric three-spined sticklebacks highlight the fragility of reproductive barriers between evolutionarily young species. Mol. Ecol. *15*, 739–752.
38. Hedrick, P. (2000). Genetics of Populations (Sudbury: Jones and Bartlett).
39. Rousset, F. (2008). genepop'007: a complete re-implementation of the genepop software for Windows and Linux. Mol. Ecol. Resour. *8*, 103–106.