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Ecological Impacts of Reverse Speciation in Threespine Stickleback

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http://dx.doi.org/10.1016/j.cub.2016.01.004

SUMMARY

Young species are highly prone to extinction via increased gene flow after human-caused environmental changes [1–6]. This mechanism of biodiversity loss, often termed reverse speciation or introgressive extinction, is of exceptional interest because the parent species are typically highly differentiated ecologically. Reverse speciation events are potentially powerful case studies for the role of evolution in driving ecological changes, as the phenotypic shifts associated with introgressive extinction can be large and they occur over particularly short timescales. Furthermore, reverse speciation can lead to novel phenotypes [1, 2], which may in turn produce novel ecological effects. Here we investigate the ecological shift associated with reverse speciation in threespine stickleback fish using a field study and a replicated experiment. We find that an instance of introgressive extinction had cascading ecological consequences that altered the abundance of both aquatic prey and the pupating aquatic insects that emerged into the terrestrial ecosystem. The community and ecosystem impacts of reverse speciation were novel, and yet they were also predictable based on ecological and morphological considerations. The study suggests that knowledge about the community ecology and changes in functional morphology of a dominant species may lead to some predictive power for the ecological effects of evolutionary change. Moreover, the rapid nature and resultant ecological impacts associated with reverse speciation demonstrates the interplay between biodiversity, evolutionary change, and ecosystem function.

RESULTS

Stickleback species pairs consist of a "benthic" ecotype that consumes primarily benthic invertebrates (e.g., amphipods and chironomid larvae) and a "limnetic" ecotype whose diet is dominated by zooplankton (e.g., copepods) (Figure 1) [7]. Species pairs of stickleback have evolved independently five times in coastal southwestern British Columbia, Canada [8, 9]. Reverse speciation, marked by the dissolution of the genetic differences between ecotypes, occurred between 1994 and 2002 in one species pair inhabiting Enos Lake, leaving a single hybrid population with a mix of benthic and limnetic alleles (Figure 1) [2, 10, 11]. The vast majority of lakes in coastal southwestern British Columbia contain a single generalist population of stickleback with an intermediate phenotype, often halfway between the two ecotypes in morphology (Figure S1), and diet [7]. Generalist populations are thought to be similar to the early freshwater-adapted populations that were the first stage in the origin of species pairs [7]. We use them in this experiment as a proxy for a "pre-speciation" freshwater state. The availability of extant species pairs, generalist populations, and a benthic-limnetic pair that has undergone reverse speciation allows for a unique investigation of the ecological effects of reverse speciation. Here we focus on two contrasts: (1) species pairs compared to the introgressive extinction population of Enos Lake, to determine the ecological consequences of reverse speciation, and (2) generalist populations compared to the introgressive extinction population, to determine whether reverse speciation has led to a novel morphology and ecological impact compared to the "pre-speciation" state.

In addition to differences in diet, sympatric species of stickleback show divergence in morphology [8], particularly in functional traits related to prey capture [7, 8, 12, 13]. Previous analyses indicate that the genes underlying many divergent phenotypic traits of benthic and limnetic ecotypes have largely (though not entirely) additive effects in hybrids, which are intermediate in most traits [13, 14]. We examined the morphology of the reverse speciation population in Enos Lake using a morphological axis designed to discriminate between benthic and limnetic ecotypes. We found that reverse speciation (mean of −3.62) has yielded a morphology that is five times closer to that of the benthic (mean of −5.44) ecotype than the limnetic (mean of 5.97) (Figure S1). Thus, reverse speciation in Enos Lake did not lead to a full return to the generalist intermediate morphology (mean of 0.29). Instead, the reverse speciation population is significantly more benthic like in morphology than generalist populations (Figure S1) (t = 8.34, degrees of freedom [df] = 139, p < 0.001; Cohen's d = 1.43). The benthic-like shift of the Enos Lake form is most likely partly a genetic consequence of the decline in the frequency of the limnetic ecotype in the early stages of collapse [2, 15]. Given the established links between morphology and diet in stickleback, we can make predictions about how the loss of the species pairs and the shift of the hybrid toward a more benthic morphology will alter prey abundances and ecosystem function. As such, we expected

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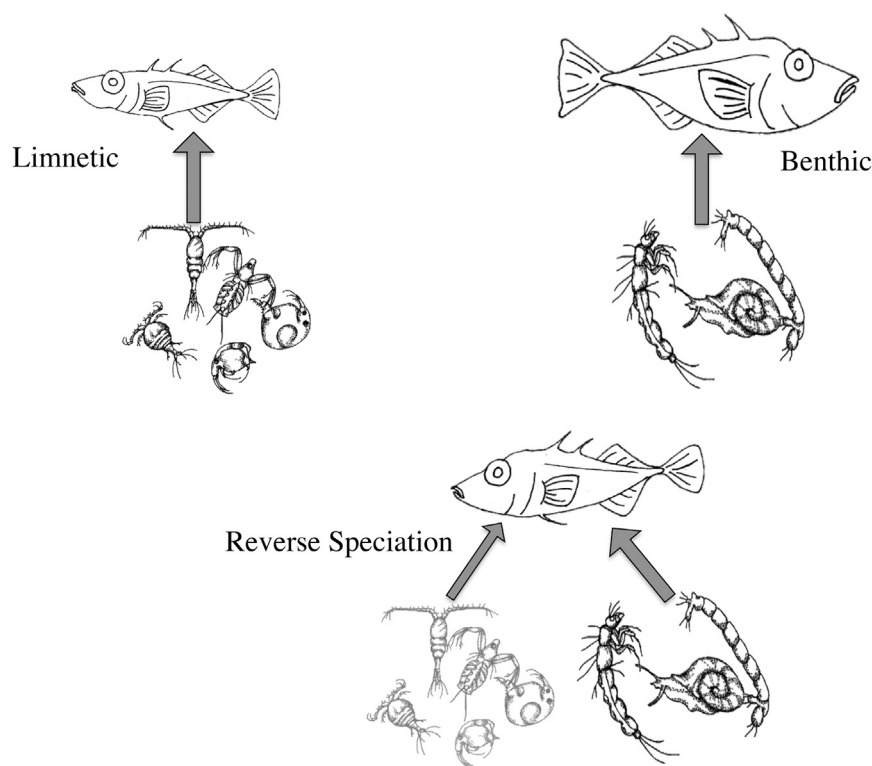
Figure 1. A Simplified Cartoon Illustrating the Trophic Relationships of the Benthic and Limnetic Stickleback Ecotypes and the Population Resulting from Reverse Speciation

The thickness of the arrows indicates the relative consumption of each prey group. A morphological and ecological comparison revealed that after reverse speciation, stickleback were more similar to benthic ecotypes than to limnetic ecotypes. The placement of the reverse speciation fish on the horizontal, intermediate between the benthic and limnetic ecotypes but closer to the benthic reflects the morphology after introgressive hybridization. See also Figures S1 and S2.

the ecological consequences of reverse speciation to include stronger trophic impacts on the benthic community, reduced trophic impacts on the plankton community, and cascading effects on ecosystem function relative to both extant species pairs [16, 17] and to generalist intermediate populations. Thus, we expected reverse speciation to yield novel ecological consequences relative to other populations of stickleback in the study.

**Ecological Consequences of Reverse Speciation**

To test the ecological effects of reverse speciation, we first compared the zooplankton communities between lakes, which we found to differ dramatically (Figure 2; Table S1). Enos Lake is dominated by the small cladoceran *Bosmina sp.*, which is rare in all the other lakes (Table S1). The zooplankton community of Enos Lake was composed of 54% *Bosmina sp.*, compared to 1.7% (±2.4%) and 0.6% (±0.4%) for species pair and generalist lakes, respectively. This difference in community composition matches results from previous studies that documented an increase in *Bosmina* abundance in experimental mesocosms that housed only benthic stickleback [18, 19]. Prior to reverse speciation, *Bosmina* was heavily consumed by the limnetic ecotype and was rarely, if ever, consumed by benthic stickleback in Enos Lake [10] (Table S2). Relaxation of predation by stickleback on *Bosmina* after the shift in phenotype during introgressive extinction could thus explain their high abundance in Enos Lake. Previous whole-lake manipulation studies have shown that reducing numbers of zooplanktivorous fish often leads to increased abundances of cladocerans [20]. We also carried out a replicated mesocosm experiment and found a trend toward greater total zooplankton biomass (Figure S3) and a corresponding decrease

in phytoplankton biomass (t = 2.92, df = 31, p = 0.006; Cohen's *d* = 0.95) (Figure S4) in the reverse speciation treatment compared to the species pair treatment. However, the effect of reverse speciation on the zooplankton community was not as large in the mesocosm as we observed in the native lake (Table S1).

Our mesocosm experiment included treatments containing stickleback from species pair, reverse speciation, and pre-speciation generalist lakes, allowing us to further investigate the effects of reverse speciation on littoral prey, insect emergence, and ecosystem properties. As predicted from a shift to a more benthic morphology, the abundance of non-chironomid benthic invertebrates was reduced by ∼40% in introgressive extinction replicates relative to mesocosms containing extant species pairs of stickleback (Figure 4) (t = −2.33, df = 29, p = 0.027; Cohen's *d* = 1.16). In contrast to the decrease of non-chironomid invertebrates, we observed an approximately 2-fold increase in insect emergence in the introgressive extinction treatment compared to the species pair treatment (t = −3.28, df = 29, p = 0.003; Cohen's *d* = 1.06). This increase was produced almost entirely by chironomids, which made up ∼99% of the abundance of aquatic insects emerging from the mesocosms into the terrestrial traps. These consequences agree with previous studies showing that benthic ecotypes deplete the abundance of many invertebrate groups but can facilitate chironomid abundance compared with the effects of limnetic ecotypes [18, 19].

The impacts of reverse speciation were also observable at the ecosystem level, where we found a 9% decrease in decomposition compared to the species pair treatment over the course of the mesocosm experiment (t = 2.43, df = 28, p = 0.022; Cohen's *d* = 1.02) (Figure 3). This finding is consistent with elevated predation by reverse speciation fish on benthic invertebrates, many of which contribute to leaf litter decomposition. We also observed a significant difference in dissolved organic carbon (DOC), with mesocosms containing Enos Lake hybrids having ∼17% greater DOC than species pair replicates (t = −3.12, df = 30, p = 0.004; Cohen's *d* = 1.19). Speciation in stickleback has previously been shown to alter carbon composition in an
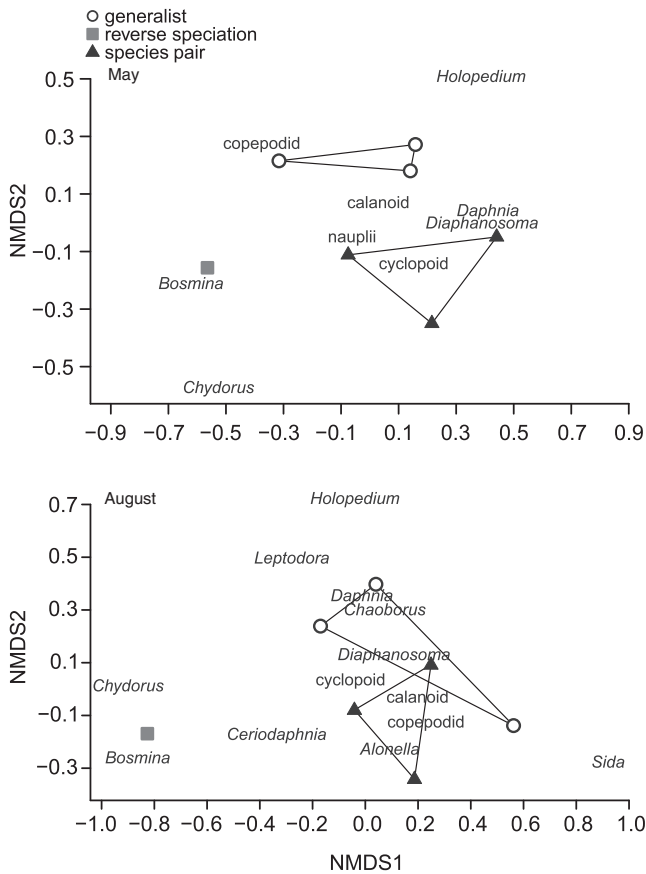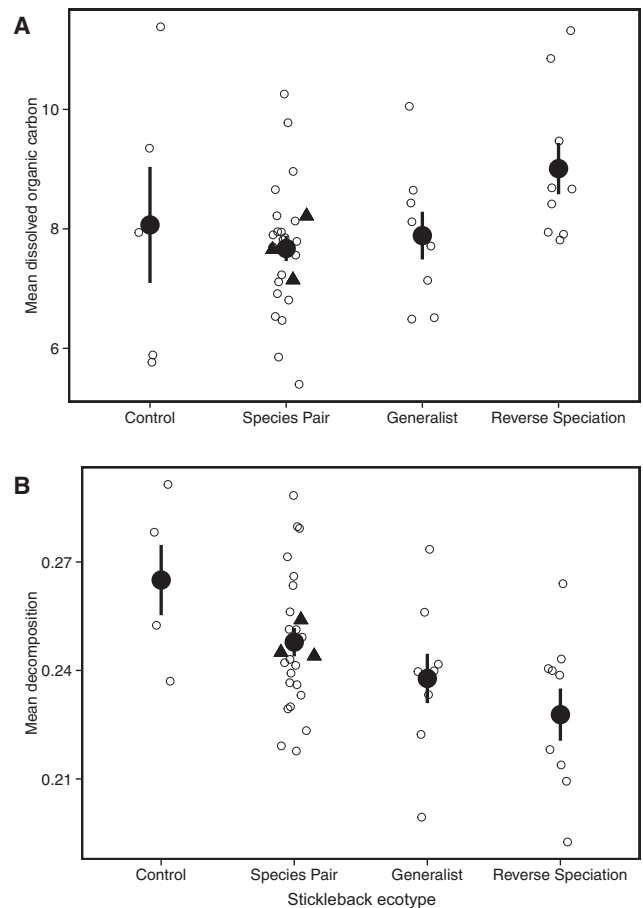
Figure 2. Ordinations of the Zooplankton Community Found in Natural Lakes Containing Different Forms of Stickleback in May and August

Each point represents the position of a single lake on the first two dimensions of variation. Prey species names are placed at their respective positions along the two axes. Calanoid and copepodid are two distinct life stages of calanoid copepods. Nauplii are an early life stage of both calanoid and cyclopoid copepods. See also Tables S1 and S2.

Figure 3. The Effects of Stickleback Ecotype on Ecosystem Functioning in the Mesocosm Experiment

Dissolved organic carbon (μg/l) at the end of the study (A) and decomposition, measured as mass lost in grams (B). Open circles indicate measurements of individual tanks, and filled circles indicate means of each treatment (shown with ±1 SE). Triangles indicate the mean for each extant species pair.

experimental system relative to the ancestral generalist phenotype [18].

Novel ecological Consequences

Given the strong evidence that reverse speciation has both morphological and ecological consequences, we sought to understand whether introgressive extinction wholly reverses the ecological effects of speciation or creates a novel ecological impact. Data from the mesocosm experiment suggest that, in many respects, introgressive extinction caused a reversion back to the ecological impacts of the pre-speciation generalist state, supporting the idea of introgressive hybridization simply undoing the ecological effects of speciation. However, the abundance of emerging insects showed a significant difference between reverse speciation and the generalist treatments (t = −3.62, df = 14, p = 0.003; Cohen's d = 1.77) (Figure 4), with introgressive extinction replicates showing increased emergence, as did the field zooplankton collections (Figure 2). These data demonstrate that reverse speciation in Enos Lake, which has produced a novel phenotype, has correspondingly novel

ecological impacts that were nevertheless predictable based on known associations between morphology and diet.

DISCUSSION

The relationship between species loss and ecosystem function has been an area of intense focus in ecology, with many studies highlighting the impact of extinction on community and ecosystem processes [21, 22]. Yet studies to date have focused exclusively on the effects of extinction by demographic decline, and little is known about how the alternative extinction process, reverse speciation through introgressive hybridization, impacts ecosystems [1, 2]. Species communities that have assembled by recent adaptive radiation in otherwise species-poor environments seem especially prone to introgressive extinction, in part because reproductive barriers may be highly sensitive to environmental context [1–6, 23]. These species are often young yet highly differentiated ecologically, showing strong divergence in both resource use and functional traits, such as gill raker number in zooplanktivorous fishes [2, 4] and beak size in seed-eating
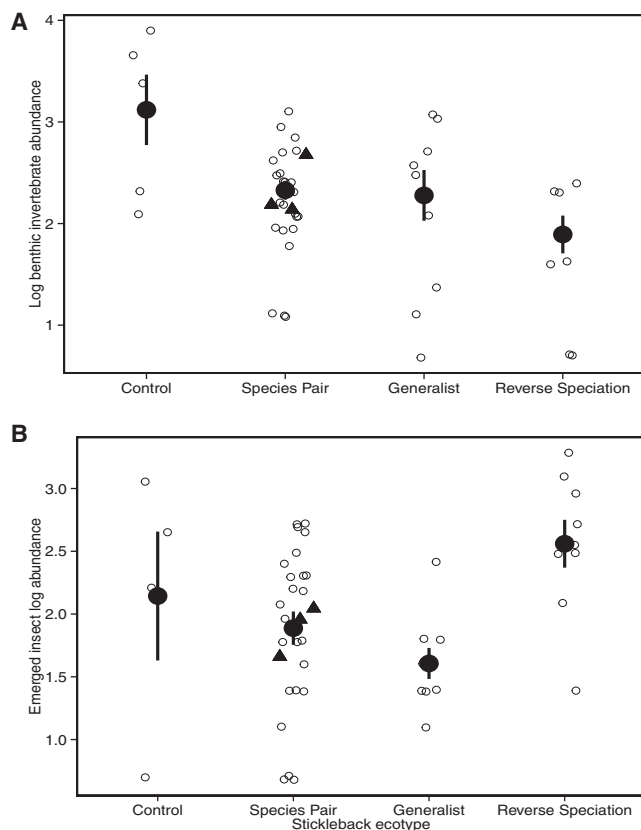
Figure 4. The Effects of Stickleback Ecotype on the Invertebrate Community in the Mesocosm Experiment

Log abundance of non-chironomid benthic invertebrates (A) and log abundance of emerging insects from the mesocosm experiment (B). Open circles indicate measurements of individual tanks. Filled circles indicate means of each treatment (shown with ±1 SE). Triangles indicate the mean for each of the three extant species pairs. See also Figures S3 and S4.

finches [5, 24]. Here we show that reverse speciation can have wide-ranging ecological effects (Figures 2, 3, and 4). These impacts were largely on prey species and were found to extend out from modifications of the abundance of aquatic insects to aquatic ecosystem function and even to the aerial environment through modifications of the abundance of emerging insects.

Studies to date that have documented reverse speciation find that demographic decline may occur simultaneously, leading to genetic idiosyncrasies that shape the morphology of the resulting population [2, 4, 5]. Hybridization during introgressive extinction most likely also produces a wide range of novel phenotype combinations, which could be influenced by mate choice asymmetries and might be subject to strong natural selection [13, 25]. Our study suggests that these case-specific idiosyncrasies may be important in ultimately determining the ecological impacts of introgressive extinction. Nevertheless, our data demonstrate that the phenotypic changes associated with reverse speciation can provide some predictability to the ecological effects of this rapid phenotypic shift. Utilizing this relationship between morphology and ecological impacts could lend some predictive power when projecting the impacts of evolutionary changes on ecosystems.

The number of cases of reverse speciation is growing, and with most examples to date invoking anthropogenic habitat alterations as a driving factor [1, 2, 4–6], they seem likely to continue to climb. Our field and experimental evidence illustrates that this form of extinction can alter community structure and ecosystem function. Given the rapid nature of reverse speciation in Enos Lake [2], our results also underscore the role that rapid phenotypic evolution can play in shaping ecology. The rapid nature and the resultant ecological impacts of species collapse highlight the importance of explicitly protecting the phenotypic diversity among populations that haven't attained complete reproductive isolation, as these populations face the unique threat of introgressive extinction [3, 4, 6, 26–28]. Studying cases of species collapse offers an opportunity to develop a more thorough understanding of the relationship between ecology and evolution that could ultimately assist in conserving young interspecific diversity.

EXPERIMENTAL PROCEDURES

Phenotypic Analysis and Field Collection

We used minnow traps to collect stickleback from seven lakes containing four types of stickleback in early April 2011: benthic and limnetic specialists from the three lakes with independently derived pairs, generalists from three lakes containing a single species of stickleback, and reverse speciation fish from Enos Lake. Generalist populations were chosen based on three criteria: (1) the fish community had to match that of species pair lakes and Enos Lake, which contain only stickleback and cutthroat trout and (2) they had to be roughly similar in size (surface area and max depth), and (3) the lake had to be within a 30 km radius of the species pair lakes and Enos lake. Fish for morphological analysis were collected separately and were stained, and the left side of each fish was photographed from overhead. Images were used to obtain 24 landmarks based on bony structures for each fish for comparative morphometric analysis [28]. We placed phenotypes of all fish along this benthic to limnetic continuum by using the discriminant function axis that best differentiated benthic and limnetic individuals taken from extant species pairs [29]. We generated this function from landmark data on a randomly chosen half of a sample of 91 limnetic and 101 benthic fish collected from each of the species pairs. Scores were then calculated for the remaining half of the species pair fish to ensure the axis correctly differentiated between ecotypes. Assignment to the correct group was 100% and variation between ecotypes accounted for 84% of the total variation observed along this axis, as measured by R^2 of a single-factor ANOVA of the two groups, using only measurements in the second half of the fish. We then used this function to obtain shape scores for individuals from generalist populations and Enos Lake.

We collected zooplankton samples from the same seven lakes on May 17–23 and again August 12–18, 2012. In each lake we collected two 5 m and one 10 m vertical zooplankton samples using a 30 cm diameter zooplankton tow constructed from 80 μm mesh with a cod end. All zooplankton samples were sub-sampled to one-sixteenth volume, stained with Rose bengal, and then counted with at least the first 20 individuals of each taxon measured to obtain average sizes for each taxon. Lakes containing generalist and species pairs of stickleback broadly overlap in their physical and chemical features, with no significant difference in their means [30].

Mesocosm Experiment

We set up an array of 50 1,136 l mesocosms, which were filled; seeded with sterilized play sand, zooplankton (community dominated by copepods with some cladocerans [e.g., *Bosmina, Daphnia*] present), and benthic mud from nearby expereran ponds; and allowed to settle for ∼4 weeks. Mesocosms were then randomly assigned to one of four treatments: species pair, generalist, reverse speciation (Enos Lake), and a fish-free control. Generalist and reverse speciation treatments were replicated nine times each. Each of the three species pairs was replicated nine times, for a total of 27 replicates. Five tanks were set up as fish-free controls. Stickleback for the experiment

were held in the lab for at least 1 week before being placed into mesocosms. A previous study has shown that plasticity in other stickleback populations can have ecological effects [31], but the morphological differences between benthic and limnetic ecotypes are largely genetic, even when they are reared on different diets [32]. Here we used wild-caught individuals to ensure that the phenotypes present in the experiment reflect those present in the natural lakes. Handling of stickleback in this experiment adhered to an approved University of British Columbia animal care protocol (no. 11-0402). Fish were collected with permission from Fisheries and Oceans Canada (SARA permit no. 197) and Ministry of Forests, Lands, and Natural Resource Operations of British Columbia (permit no. NA/SU10-68002). After fish introduction, mesocosms were surveyed at least once per day for dead or dying stickleback, which were replaced within 12 hr until the conclusion of the experiment on June 30, 2011. To match biomass approximately between treatments containing fish, we stocked different numbers of stickleback (generalist = 4, species pair = 2 benthic and 3 limnetic, and reverse speciation = 4). Species pair treatments received more limnetics than benthics to simulate relative abundances in the natural lakes (D.S., unpublished data) and as a way match biomass (4–5 g/mesocosm) between treatments. Variation in the number of individuals between treatments could lead to differences in metabolic demands between treatments (as smaller individuals tend to have higher mass-specific metabolic rates [33]). We sampled the communities of phytoplankton, zooplankton, benthic invertebrates, and insects that emerged from the mesocosms at the end of the study. We examined decomposition, photosynthetically active radiation, DOC, and gross primary productivity to monitor ecosystem function.

We treated lake as a fixed effect in our statistical analyses, recognizing the small number of stickleback species pairs and the unique nature of reverse speciation in Enos Lake. Comparisons used planned contrasts between the means of extant species pairs and the Enos Lake population. We also calculated standard effect sizes (Cohen's d), and we report them for each significant result. We focus on descriptive statistics with field zooplankton data because reverse speciation has occurred in stickleback only once.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, four figures, and two tables and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2016.01.004.

AUTHOR CONTRIBUTIONS

S.M.R. and D.S. conceived of the study. S.M.R. carried out the experiment and field sampling. S.M.R. and D.S. carried out the analysis, wrote the manuscript, and made revisions.

ACKNOWLEDGMENTS

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