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PARTIAL REPRODUCTIVE ISOLATION OF A RECENTLY DERIVED RESIDENT-FRESHWATER POPULATION OF THREESPINE STICKLEBACK (*GASTEROSTEUS ACULEATUS*) FROM ITS PUTATIVE ANADROMOUS ANCESTOR

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Abstract

We used no-choice mating trials to test for assortative mating between a newly derived resident-freshwater population (8 – 22 generations since founding) of threespine stickleback (*Gasterosteus aculeatus*) in Loberg Lake, Alaska and its putative anadromous ancestor as well as a morphologically convergent but distantly related resident-freshwater population. Partial reproductive isolation has evolved between the Loberg Lake population and its ancestor within a remarkably short time period. However, Loberg stickleback readily mate with morphologically similar, but distantly related resident-freshwater stickleback. Partial pre-mating isolation is asymmetrical; anadromous females and smaller, resident-freshwater males from Loberg Lake readily mate, but the anadromous males and smaller Loberg females do not. Our results indicate that pre-mating isolation can begin to evolve in allopatry within a few generations after isolation as a correlated effect of evolution of reduced body size.

Keywords

Assortative mating; asymmetric mate choice; rapid evolution; pre-mating isolation; speciation

Reproductive isolation is the hallmark of species under the biological species concept (Mayr 1963; Harrison 2010). The allopatric speciation model is generally believed to describe the most common geographic context of speciation, leading to reproductively isolated sister species by way of adaptation to specific environments or genetic drift. While describing a spatial pattern, this model does not stipulate the processes that lead to reproductive isolation (Coyne and Orr 2004; Dieckmann et al. 2004). Those processes must be studied directly in young species and populations undergoing speciation to understand how reproductive isolation evolves.

Investigation of contemporary evolution, as opposed to comparing sister species, allows the processes of speciation to be observed directly (McPhail 1994; Harrison 2010). Populations in the initial stages of divergence can be studied to analyze the critical points of divergence and the mechanisms driving them (McPhail 1994; Johannesson 2001; Hendry et al. 2000, 2007). Such studies have garnered considerable interest in recent years (e.g., Thompson

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1998; Hendry et al. 2000, 2007; Hendry 2004; Rundle and Nosil 2005; Kitano et al. 2008; Chamberlain et al. 2009).

Hagen (1967) and McPhail (1969) were the first to investigate reproductive isolating mechanisms between phenotypically contrasting, sympatric and parapatric populations of threespine stickleback (*Gasterosteus aculeatus* species complex), but numerous studies of isolation between sympatric biological species of threespine stickleback have followed (reviewed by McPhail 1994; McKinnon and Rundle 2002; Boughman 2007). The postglacial adaptive radiation of threespine stickleback provides an excellent model to study rapid evolution and speciation. As glaciers receded, marine and anadromous (collectively oceanic) populations repeatedly colonized Holarctic freshwater environments, where they adapted to local conditions and evolved numerous traits that differ from those of their oceanic ancestors (reviewed by Bell and Foster 1994, McPhail 1994).

Two recurrent types of sympatric or parapatric pairs of threespine stickleback ecotypes, benthic – limnetic, and resident-freshwater – anadromous, often exhibit positive assortative mating (reviewed by McPhail 1994; McKinnon and Rundle 2002; Boughman 2007). Positive assortative mating is one form of pre-mating isolation and results from the propensity of similar phenotypes to mate (Dobzhansky 1971; Coyne and Orr 2004). Stickleback ecotypes exhibit positive assortative mating in laboratory trials irrespective of phylogeny (Taylor 1999; Taylor and McPhail 2000; Vines and Schluter 2006), and discriminate against individuals from closely related populations with contrasting morphological traits (Nagel and Schluter 1998; Rundle et al. 2000; McKinnon et al. 2004). Thus, positive assortative mating is associated with phenotypic divergence that evolved in response to ecological differences (Schluter and McPhail 1992; Rundle and Schluter 1998; Schluter 2000a, 2001; McKinnon et al. 2004). This pattern provides strong evidence for parallel speciation within the threespine stickleback species complex (Rundle et al. 2000; Boughman et al. 2005).

The cues for assortative mating can include behavior, nuptial coloration, body size, pheromones, microhabitat selection, and other properties (Wootton 1976, 1984). Previous studies have shown that body size is an important cue for assortative mating in threespine sticklebacks (Nagel and Schluter 1998; Rundle et al. 2000; Ishikawa and Mori 2000; McKinnon et al. 2004; Albert 2005; Boughman et al. 2005; Kitano et al. 2009; but see Jones et al. 2008). Anadromous stickleback possess standing genetic variation for body size, are able to evolve in response to selection when colonizing fresh water (McGuigan et al. 2011), and are smaller than their parents when raised in fresh water (Mori 1990; Kristjansson 2005; Marchinko and Schluter 2007). Thus, phenotypic plasticity might facilitate assortative mating based on body size, leading to partial reproductive isolation after only one generation of fresh water residence (Adams and Huntingford 2004; von Hippel and Weigner 2004; Yukilevich and True 2006).

In this study we investigate pre-mating isolation via assortative mating in a recently derived population of lake-resident stickleback and a population representing its anadromous ancestor (Bell 2001; Bell et al. 2004; Arif et al. 2009; Aguirre and Bell 2012; Johnston et al. 2012). We show that partial pre-mating isolation has evolved within eight to 22 generations without secondary contact between the ancestral and derived populations. Numerous studies since Hagen's (1967) original work on this problem have demonstrated reproductive isolating mechanisms between phenotypically contrasting sympatric and allopatric threespine stickleback populations, but none has used populations that diverged in historical times. We also provide suggestive evidence that this partial isolation is an incidental byproduct of adaptation to lake conditions in the young freshwater isolate.

Materials and Methods

Study Populations

Sticklebacks were collected from Loberg Lake (61.560° N, 149.258° W), Corcoran Lake (61.573° N, 149.691° W), and Rabbit Slough (61.534° N, 149.266° W) in the Matanuska-Susitna Valley, Alaska (Supplementary Figure). The original resident stickleback population of Loberg Lake was exterminated with rotenone in 1982 by the Alaska Department of Fish and Game to promote the survival and growth of stocked trout and salmon. Annual inspections of Loberg Lake prior to 1990 did not detect stickleback (A.C. Havens, pers. comm.), but the lake must have been colonized sometime between 1983 and 1988. Extensive phenotypic divergence has occurred in this population since sampling began in 1990. The 1990 sample resembled anadromous specimens, contrasting sharply with most lake stickleback in the region for lateral plate morphology (nearly monomorphic for the complete morph; Bell 2001; Bell et al. 2004), operculum shape (Arif 2009), and body shape (Aguirre and Bell 2012). Today, it closely resembles other lake populations in the region for these and other traits.

Since Loberg Lake was colonized between 1983 and 1988, generation time in the original Loberg Lake population was two years (Havens et al. 1984), and there is evidence of two cohorts in the current population (Bell et al. 2004), the fish used in this study most likely represent the eighth to eleventh generation since colonization. Assuming conservatively a one year generation time, it may have been founded 22 generations before our study was performed. Regardless, it has diverged very recently from anadromous *G. aculeatus*.

The Rabbit Slough population was chosen for this study both to represent the anadromous ancestor from which the extant Loberg Lake population has diverged and the most likely anadromous population to come into sympatry with it. Rabbit Slough is 2.5 km from Loberg Lake, within the same drainage (Palmer Slough), and it is a potential source for anadromous stickleback that founded the extant Loberg Lake population. It is not known how Loberg Lake was colonized because it has no outlet, but genetic diversity of the Loberg Lake population is near the high end of variation for lake populations in the region (Aguirre 2007), suggesting that it was founded naturally by a large number of individuals. The possibility of gene flow from existent freshwater stickleback (e.g., adjacent populations) cannot be ruled out, but it is unlikely because Loberg is an isolated kettle lake, surrounded by steep slopes and has no surface tributaries or outflow (Bell et al. 2004; Aguirre and Bell 2012). Even if the Rabbit Slough anadromous population is not ancestral to the extant Loberg Lake population, anadromous *G. aculeatus* exhibit little genetic (Cresko 2000; Taylor and McPhail 2000; Aguirre 2007; Hohenlohe et al. 2010) or morphological (Walker and Bell 2000; Aguirre et al. 2008) divergence locally and regionally. The Rabbit Slough population also has a bimodal size-frequency distribution (see Results), which allowed us to use its two size classes to investigate the effect of size on mating behavior.

The Corcoran Lake population was chosen from 40 lake populations in the Matanuska-Susitna Valley studied by Walker (1997) and Aguirre (2009) for the similarity of its body shape and size to that of the Loberg Lake stickleback. An analysis of microsatellite variation indicated that the Loberg Lake population is more distantly related to the Corcoran Lake population than to the Rabbit Slough population (Aguirre 2007). Thus, comparison of reproductive isolation of the Loberg Lake population from the Rabbit Slough and Corcoran Lake populations allows us to infer whether reproductive isolation depends more strongly on morphological (and presumably ecological) divergence or genealogical relationship.

Sampling and Holding Procedures

Reproductive adult stickleback were collected from all three sites between 21 May and 30 June, 2004 and 01 May and 06 July, 2005. We used unbaited 0.64 cm wire mesh minnow traps that were set overnight on the bottom along the lake shorelines and in Rabbit Slough. Fish were transported live in coolers with aerated water to the lab in Anchorage. They were held outdoors in aerated 1600 L pools until they were moved indoors into 56 L aquaria. Males and females were separated by population in the outdoor pools. Both the outdoor pools (mean temperature 12.9 °C) and the indoor aquaria (mean temperature 15.6 °C) were supplied with aerated well water. The photoperiod in the lab conformed to ambient conditions, increasing from 18:6 (light:dark) to 20:4 h before decreasing to 18.5:5.5. Fish were fed frozen brine shrimp (*Artemia* sp.) *ad libitum* daily.

Mating Trials

One hundred and seventy-seven no-choice mating trials were conducted in 2004 and 2005 to evaluate spawning success (e.g., see Rundle and Schluter 1998). No-choice trials were used because males in the same aquarium interfere with each other's courtship behavior, and larger males generally prevail (Rowland 1989). Assessment of female mate preference using her orientation to isolated males in separate chambers can be unreliable (Hay and McPhail 1975; Hagen et al. 1980). However, female stickleback mate preference is similar in choice and no-choice experiments (Vines and Schluter 2006), and we opted for the latter.

Trials were conducted in 56 L aquaria (60cm × 31cm × 32 cm), each with a bio filter, a terra cotta pot (10.2 cm opening) for cover, and nesting material consisting of a Petri dish full of sand and plant fibers from Rabbit Slough. The aquaria were visually isolated from one another to prevent neighboring fish from interacting. Each aquarium was assigned an experimental group (i.e. Corcoran, Loberg, Rabbit Slough large or Rabbit Slough small males) sequentially to ensure that lighting and heating were random among groups and human traffic in the lab would not produce group effects. Thereafter, individual males from each population were randomly assigned to aquaria.

Males were placed into the aquaria individually and allowed to build nests. They were induced to build nests by placing a 1 L bottle containing a gravid female into their aquarium for 15 minutes a day if they did not immediately start nest construction. Males that failed to build a nest within seven days were replaced. Once a nest was complete and the male had "crept through" (bored through the nest to produce a tunnel, signifying readiness to court; Rowland 1994), females were introduced.

During each trial, individual females from a single, predetermined population were placed sequentially into a male's aquarium until spawning occurred. Each female was introduced directly into the male's tank after she had acclimated to indoor water temperature in isolation for at least 20 min. Females that failed to spawn within 12 min were removed and checked for ripeness by squeezing the abdomen and inspecting the cloaca for mature eggs; every female introduced was ripe. The male was allowed 10 min after failing to spawn to recover between female introductions.

In 2004, we introduced up to 15 females per trial, but we discovered that mating was unlikely after the first eight females. Ninety-six percent of 48 trials that produced spawning occurred with one of the first eight females. Therefore, in 2005, a maximum of eight females was offered to each male. Trials were run as nests and gravid females became available. The date on which trials were performed did not affect mating success of any of the trial types during either year with all trials combined and dates sequentially numbered (2004, t-test, $t = 0.35$, $df = 45$, $P = 0.728$; 2005, t-test, $t = 1.40$, $df = 125$, $P = 0.165$).

Females were used only once, and males were used for only one trial, though multiple females from the same population may have been introduced to his aquarium before spawning occurred. Following spawning, the standard length (SL, distance from the anterior tip of upper jaw to posterior end of hypural plate) of each male and female was measured using digital calipers. Males were killed with an overdose of MS-222 and frozen. Females were removed and used in unrelated experiments.

The four experimental groups from the three study populations are referred to as: L, Loberg; C, Corcoran; RS, Rabbit Slough large; and RSs, Rabbit Slough small (~16% of returning stickleback in 2005). A k-means cluster analysis revealed that the median SL of the two Rabbit Slough size classes differed by 2 SDs, which was significant for both males and females (t-test, males: $t = 16.15$, $df = 81$, $P < 0.001$, females: $t = 23.85$, $df = 232$, $P < 0.001$). The female's population is listed first and the male's is second (female-male) for all mating trial pairings. Trials pairing L with all other groups were used to assess assortative mating, and trials pairing males and females within each group served as controls.

Three measures of mating propensity were analyzed to estimate the degree of assortative mating. They are not all mutually exclusive, but each incorporates unique information. These measures were (1) number of females introduced until successful spawning, (2) frequency of spawning by the first female, and (3) frequency of spawning within eight or fewer presented females. All three of these measures give a single number for each male. Male courtship behaviors were recorded as well (biting, boring, creeping through, dorsal pricking, fanning, gluing, leading, and zigzagging). Of these, only biting and dorsal pricking, which were the only behaviors that differed significantly between resident-freshwater and anadromous fish, were used to determine if male aggression changed among sequential female introductions. Differences in SL of the male and female were used to test for size effects on the probability of mating.

Statistical Methods

Statistical analyses were carried out using SPSS (version 11.5). Non-parametric tests were used because samples were small and the data had non-normal distributions. The results of parametric tests were qualitatively indistinguishable from those of the non-parametric tests. The Kruskal-Wallis test was used to compare the ten individual trial types (L female with L male, RS female with L male, etc.) for differences. Multiple comparisons were carried out using the Dunnett T3 test or Bonferroni corrected multiple comparisons, where appropriate. A t-test was used to compare groups when there were sufficiently large sample sizes. Mann-Whitney U tests were used to determine if larger females or males were more likely to spawn than smaller ones; all females and males were included and sexes were analyzed separately, so female spawning success is on an individual basis, while male spawning success is on a trial (multiple females) basis. Standard length difference (ΔSL) between paired males and females was calculated as the absolute value of $\varphi SL - \sigma SL$.

To determine whether male courtship behaviors changed with successive female introductions (1–8), the slope of the male's frequency of biting and dorsal pricking per minute across all females introduced was calculated for males that did not mate with the first female. These slopes were then plotted for all successful males and a regression line of the slopes added to evaluate change across the number of females introduced. Neither the regression line for biting nor that for dorsal pricking differed significantly from zero. Therefore, male behavior across female introductions does not appear to be a confounding factor in the analysis of mating propensity.

Results

Female SL differed significantly among the four groups (Kruskal-Wallis H-test, $\chi^2 = 477.03$, $df = 3$, $P < 0.001$; Fig. 1A). In addition to significant size differences between RS or RSs and freshwater fish, C females were bigger than L females (Bonferroni corrected multiple comparison, groups = 4, mean difference = 1.76mm, $P = 0.001$). Male SL also differed among all groups (Kruskal-Wallis H-test, $\chi^2 = 142.09$, $df = 3$, $P < 0.001$) except between L and C (Bonferroni corrected multiple comparison, groups = 4, mean difference = 0.89mm, $P > 0.05$). Females were significantly larger than males in the anadromous groups (t-test: RS, $t = -10.76$, $df = 216$, $P < 0.001$; RSs, $t = -5.76$, $df = 93$, $P < 0.001$) but not in the freshwater groups (t-test: Loberg, $t = -0.69$, $df = 384$, $P = 0.493$; Corcoran, $t = -1.18$, $df = 144$, $P = 0.240$; Fig. 1A). C females were significantly larger than L males (t-test: $t = 3.11$, $df = 169$, $P < 0.01$).

One-hundred-seventy-seven trials were performed (Table 1). Seventy-nine percent of the males successfully mated with one of the first eight females offered to him, with no difference between the two years that trials were conducted (t-test: $t = 1.66$, $df = 173$, $P = 0.196$). Both RSs female with RSs male and RSs female with L male trials had 100% success within eight female introductions (Table 1, Fig. 2B). The lowest success rate, at only 21%, was in the L female with RS male trials. Trials within (C female with C male, L female with L male) and between (C female with L male, L female with C male) Loberg and Corcoran lake fish did not differ for any measure of success (Table 2). All three measures of assortative mating followed the same general pattern across trial types (Fig. 2). Asymmetric mate choice was observed in trials between the RS and L populations (Table 2). Matings rarely occurred between L females and RS males, but did usually occur between L females paired with RSs or C males. By contrast, L males had an equal probability of mating with RS females as with L, C or RSs females.

Courtships involving larger females were more likely to be successful than those with smaller females, but male size was not a significant influence on courtship success (Fig. 1B). The effect of female SL on success is not as clear when trial types are analyzed individually. The RSs female with RSs male trials were the only ones with a significant effect of female body size on mating success, with larger females being more likely to spawn (Mann-Whitney U-test, $U = 52.5$, $n = 31$, $P = 0.008$). C females are significantly larger than L females and males. Although not significant, the data trended toward L males mating with smaller C females (difference in mean SL = -2.8 mm; Mann-Whitney U-test, $n = 54$, $U = 174.0$, $P = 0.105$) and C males mating with larger L females (difference in mean SL = 2.4; Mann-Whitney U-test, $n = 54$, $U = 131.0$, $P = 0.097$).

We compared L female with RS male (mean $\Delta SL = -23.1$ mm) to L female with RSs male (mean $\Delta SL = -11.5$ mm) trials and RS female with L male (mean $\Delta SL = 28.7$ mm) to RSs female with L male (mean $\Delta SL = 14.2$ mm) to examine the effect of size on mating success. There was no significant difference in the L males' success with the large vs. small RS females for any of the assortative mating measures (Table 2). However, the larger RS males were significantly less successful than RSs males with L females for two of the three measures (Table 2).

Discussion

Morphological divergence comparable to that between reproductively isolated freshwater threespine stickleback populations evolved from the ancestral conditions of anadromous stickleback (Aguirre et al. 2008) in the Loberg Lake population in approximately 21 years (Bell et al. 2004; Aguirre 2007; Arif et al. 2009; Aguirre and Bell 2012). We found that

there is partial pre-mating isolation between the ancestral anadromous RS and the derived freshwater L populations because RS males and L females do not readily mate. Our study is the first to examine isolating mechanisms between a representative of an ancestral stickleback (i.e., Rabbit Slough) and a freshwater descendent that has evolved from it during a short period in allopatry.

The use of no-choice mating trials with multiple female introductions to each male must be interpreted with caution. Ecological cues for mate suitability (e.g., nesting male microhabitat selection; see Hagen 1967; Bentzen and McPhail 1984) are missing, and wild males and females may choose from several members of the opposite sex simultaneously or in quick succession (Foster 1994). Our experimental design forced individuals to reject a receptive mate based on its qualities in the absence of alternative mates or ecological cues. It is conservative because the choice is based only on qualities of a single individual and possibly the male's nest, and individuals should be less likely to reject an undesirable potential mate in isolation than in the presence of other potential mates or an interpretable environmental context. In addition, we examined only one potential isolating mechanism, assortative mating, which is one of many mechanisms possible.

Mate Choice and Size Effects

This study provides evidence that morphology is important in the mate choice of threespine stickleback. The morphologically similar but phylogenetically distant L and C resident lake stickleback were as likely to mate with each other as with members of their own populations (Table 2). The L population has evolved the reduced body size typical of resident-freshwater stickleback in Southcentral Alaska (Fig. 1A). Similar body size is likely a major contributor to the mating compatibility of the L and C populations.

Asymmetric mate choice was observed between anadromous and Loberg fish. The small L males mated readily with large RS females (consistent with the preference of males for larger females with more eggs and the “supernormal stimulus”; Rowland 1989), but the large RS males and small L females rarely mated with each other (Fig. 2). Additionally, there was a trend for RS females to mate more readily with RS males than with L males (Fig. 2A&B). This is consistent with other studies (Hay and McPhail 1975; Ishikawa and Mori 2000; Scott 2004; McKinnon et al. 2004) and probably would have been significant with larger sample sizes. Such an asymmetry in mate choice suggests that one or both of the sexes had a preference and that body size is an important criterion, though it does not rule out the importance of coloration or other cues that also differ between the L and RS populations.

Male aggression is an important component of stickleback courtship and was found to be plastic in the derived Loberg population. Compared to courtship with their own females, L males significantly increased aggressive behavior (biting and dorsal pricking) toward RS females, equaling male aggression in RS-RS pairs (Furin, unpublished data). Nagel and Schluter (1998) found that limnetic males increased aggression toward larger benthic females in sympatric freshwater species pairs. RS males performed typical courtship behaviors, except for a reduction in dorsal pricking (Furin, unpublished data), toward L females, but in most trials the females fled and hid (Furin 2006), suggesting that the L females were rejecting the RS males. L females may have viewed the anadromous male as a threat instead of a mate because he was large (Rowland 1994). Similarly, Kitano et al. (2009) found that Pacific Ocean threespine stickleback females terminated courtship in response to the aggressive courtship behavior of Japan Sea males. Mating occurred in a small number of trials using RS males and L females (Fig. 2B), so in the absence of other choices, some level of introgression by means of this pairing could occur.

Intersexual selection in *G. aculeatus* is complex because males provide all the parental care, so both males and females have cause to discriminate mates (Wootton 1976, 1984; McPhail 1994). In our study, RS females recognized L males as potential mates and did not significantly discriminate against them, despite the major morphological changes to the L population (Bell et al. 2004; Aguirre 2007; Arif et al. 2009; Aguirre and Bell 2012), while RS males and L females rarely mated. These results are consistent with the findings of Jones et al. (2006) in which hybrid progeny of anadromous females were more numerous than hybrid progeny of resident-freshwater females in a Scottish resident-freshwater and anadromous species pair. They suggested that this result was due to the presence of more anadromous females, but our study indicates that their result could be explained by asymmetrical assortative mating based on body size. Additionally, Kozak et al. (2009) found that female choice may be more important than male choice to pre-mating isolation in sympatric threespine stickleback species pairs. They determined that both sexes recognize mates, but only females prefer to mate with conspecifics, with body size being an important criterion. This study provides additional evidence of a difference between anadromous and resident-freshwater females in mate choice, with anadromous females tolerating morphologically divergent (smaller) males.

Results from trials with the two size classes of Rabbit Slough stickleback provide evidence for positive assortative mating. Mating was significantly more likely between the smaller RSs males and L females than between larger RS males and L females, indicating again the role of positive size-assortative mating in pre-mating isolation between L females and RS males. L female with RSs male mating trials were as successful as L female with L male trials, which contrasted sharply with the results for L female with RS male trials (Fig. 2B). Although not significant, there was a trend for L males with RSs females to mate more readily than L males with RS females (Fig. 2B). Female choice based on positive size assortative mating may be taking place here and has been observed in other populations (Rundle et al. 2000; McKinnon et al. 2004; Vines and Schluter 2006; Kozak et al. 2009). An alternative reproductive strategy of RSs males and females cannot be ruled out as a confounding factor. Both smaller males and females (RSs fish) might be less selective for mates because small males should have difficulty establishing territories and small females should be less attractive to courting males (Dufresne et al. 1990; Whoriskey and FitzGerald 1994).

The likelihood of matings between L males and RS females may be lost with more time in allopatry, but selection against hybridization based on production of unfit progeny in sympatry may be required to complete reproductive isolation. For example, allopatric “black” females in the Chehalis River preferred males with ancestral red breeding coloration, but sympatric “black” females showed no preference. These data suggest that ancestral preference for red persisted in allopatry, where it was impossible for females to select the wrong male, but was selected against in sympatry, where hybrids with reduced fitness could be produced (McPhail 1969; Bell 1976; Scott and Foster 2000). The limnetic-benthic species pairs in British Columbia evolved positive assortative mating via reinforcement (Rundle and Schluter 1998; Albert and Schluter 2004), which contributes strongly to maintenance of separate demes in sympatry (Gow et al. 2007). Limnetic males readily mate with limnetic females and not the larger sympatric benthic females, while allopatric limnetic males readily mate with the larger allopatric benthic females, indicating reproductive character displacement (Albert and Schluter 2004). The possibility of losing the preference for larger females in allopatry could be tested through mating trials between older resident-freshwater populations (such as C) and an allopatric anadromous population (RS). The possibility of reproductive character displacement leading to stronger size-based assortative mating in sympatry could be tested through trials from a habitat in which

reproductively isolated resident-freshwater and anadromous stickleback breed in sympatry, such as in nearby Mud Lake (Karve et al. 2008; Bell et al. 2010).

Phenotypic plasticity resulting in smaller body size when anadromous fish develop through sexual maturity in fresh water (Mori 1990; McKinnon et al. 2004; Kristjansson 2005; Marchinko and Schluter 2007) coupled with assortative mating based on body size could retard gene flow after only one generation of residence in fresh water. This would allow divergent selection to increase heritable ecological differences, including size differences, between individuals resident in fresh water and members of the same population that retain anadromy (von Hippel and Weigner 2004; McGuigan et al. 2011; see also West-Eberhard 1989, 2003). Gene flow may therefore be restricted in sympatry by mate choice even before two demes diverge genetically, facilitating genetic divergence in response to selection based on contrasting ecologies and leading to more complete reproductive isolation. The effectiveness of assortative mating initiated by phenotypic plasticity for body size should increase thereafter, as selection for smaller body size in fresh water increases heritable size difference between anadromous ancestors and the freshwater descendants.

Conclusion

The rate at which reproductive isolating mechanisms evolve is an important, unresolved issue (Coyne and Orr 2004). If species can form rapidly, ephemeral barriers to gene flow (e.g., beaver dams, habitat gaps produced by drought) may leave no evidence after they disappear but persist long enough for isolating mechanisms to form. This study provides a snapshot of the early stages of reproductive isolation between allopatric biological species of threespine stickleback. It looks beyond sympatric species pairs to the origin of resident-freshwater populations from oceanic ecotypes. Adaptation to the freshwater environment occurs rapidly (Klepaker 1993; Bell 2001; Bell et al. 2004; Arif et al. 2009; Gelmond et al. 2009; Aguirre and Bell 2012), and there is strong evidence that ecological traits (Schluter and McPhail 1992; Schluter 2000a,b), particularly body size (Nagel and Schluter 1998; McKinnon et al. 2004; Boughman et al. 2005), provide the basis for mate choice, and hence reproductive isolation. Phenotypic divergence of the Loberg Lake population has occurred within only a few generations (Bell 2001; Bell et al. 2004; Arif et al. 2009; Aguirre and Bell 2012), and our results show that substantial reproductive isolation has also evolved quickly, possibly as a correlated effect of this phenotypic divergence.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Mean (± 1 SE) of: (A) SL of each group by sex. Kruskal-Wallis H-test, $\chi^2 = 477.03$, $df = 3$, $P < 0.001$. RS = large Rabbit Slough, RSs = small Rabbit Slough, L = Loberg, and C = Corcoran. (B) SL of successful (spawned) and unsuccessful males (t-test, $t = 1.67$, $df = 173$, $P = 0.097$) and females (t-test, $t = 4.18$, $df = 671$, $P < 0.001$). Asterisks denote significant differences (** $P < 0.001$).

Figure 2.

Measures of assortative mating propensity. L = Loberg, C = Corcoran, RS = large Rabbit Slough, and RSs = small Rabbit Slough. The error bars are one standard error, and letters above the error bars indicate trials from which that trial is significantly different (Dunnett T3 multiple comparison, $P < 0.05$). Trial types differed in the mean number of females to spawning (Kruskal-Wallis H-test, $\chi^2 = 43.12$, $df = 9$, $P < 0.001$) and the frequency of failed matings (Kruskal-Wallis H-test, $\chi^2 = 50.20$, $df = 9$, $P < 0.001$). Differences in the percent of successful mating with the first female were nearly significant (Kruskal-Wallis H-test, $\chi^2 = 50.20$, $df = 9$, $P = 0.053$).

Table 1

The number of trials completed for each trial type and the number of successful trials in parentheses. L = Loberg, C = Corcoran, RS = large Rabbit Slough, and RSs = small Rabbit Slough.

Male population	Female Population			
	L	RS	RSs	C
L	13 (11)	26 (18)	13 (13)	13 (12)
RS	19 (4)	30 (28)		
RSs	19 (14)		14 (14)	
C	12 (9)			18 (16)

Results of Kruskal-Wallis test for assortative mating within and between lake populations (LxL, LxC, CxC, and CxL; left), and differences in assortative mating measures between trials involving L fish with RS or RSs fish (right). A Mann-Whitney test was used to compare the large vs. small RS to Loberg trials for both males and females. Note the asymmetric mating for number of females to success and percent success.

Table 2

Measure of assortative mating in LxL, LxC, CxC and CxL trials	χ^2	df	P	L σ^2 RS-L vs. RSs-L	L ϕ L-RS vs. L-RSs
# of ♀♀ to Success	2.30	3	0.511	U = 133 n = 38 P = 0.377	U = 77 n = 37 P = 0.004
% Success	1.72	3	0.633	U = 117 n = 38 P = 0.168	U = 83 n = 37 P = 0.007
% Success 1 st ♀	2.68	3	0.445	U = 152 n = 38 P = 0.761	U = 144 n = 37 P = 0.425