Abrupt changes in defence and trophic morphology of the giant threespine stickleback (*Gasterosteus* sp.) following colonization of a vacant habitat

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To evaluate the first stages of adaptation and differentiation following colonization into a vacant and ecologically divergent habitat, 100 melanistic and low-plated adult threespine stickleback (*Gasterosteus aculeatus*) exhibiting gigantism in body size and robust spines were transferred in 1993 from a large dystrophic lake (Mayer Lake, Haida Gwaii, British Columbia, Canada) with a diverse vertebrate predator regime, into an adjacent fishless eutrophic pond with macroinvertebrate piscivores. Eight generations were examined as a test of multiple functional hypotheses on the morphological traits that differentiate lake and pond stickleback throughout their distribution. Measurements of 20 defence and trophic traits were made on 275 wild-caught fish collected from the source and transplant populations over multiple years. There have been significant reductions (males: females) in plate count (8%: 0.5%), lateral plate 2 frequency (46%: 17%), lateral plate 3 height (17%: 18%), pelvic spine length (12%: 6%), dorsal spine length (21%: 16%), gill raker number (5%: 1%), and gill raker length (37%: 43%), and an increase in gill raker spacing (+5%: 0%), jaw length (+5%: +4%), and eye diameter (+8%: +7%). These changes within eight generations represent one-third of the morphological differences observed between naturally established large lake and pond populations in these archipelago and are all in the direction predicted from the change in habitat. Field samples indicate strong selection in the colonists although lab-reared individuals implicate an additional role of plasticity for dorsal spine and gill raker lengths, which may contribute to the rapid adaptation into novel and highly divergent selective landscapes.   © 2012 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2012, **107**, 494–509.

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INTRODUCTION

Adaptive radiation has received particular attention on archipelagos where vacant habitats have allowed evolutionary experimentation in morphology and behaviour (reviewed by Cain, 1954; Mayr, 1963; Grant, 1999). Ecological release facilitates radiation in these new habitats because the normal competitive constraints are removed. This has resulted in differentiation, for example, in bill morphology of Galapagos finches (Grant & Grant, 2005), body shape in Caribbean anoles (Losos, 1992) and trophic structures of arctic whitefish (Østbye *et al.*, 2006). The early phases

of these natural experiments such as the number of colonists, evolutionary rates and relative contribution of phenotypic plasticity are largely lost to history. Potentially, significant shifts in niche space in the new habitat would substantially modify trait fitness curves and result in rapid adaptation of the phenotype. Alternatively, if the phenotype is highly integrated, morphological change to these considerable shifts in niche could be very gradual as successive generations of colonists slowly adapt to the new habitats or possibly engage in a 'random walk' of the phenotype (Bell, Travis & Blouw, 2006). Despite the extensive insight already achieved in ongoing studies of adaptive radiation (reviewed by Futuyma, 2009), there remain few investigations on the early responses of the colonizing populations to divergent adaptive landscapes.

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The postglacial colonization of temperate freshwaters by ancestral marine threespine stickleback has become a useful research model for investigations of population differentiation (Wootton, 1976, 1984; Bell & Foster, 1994; Schluter, 2000). One of the striking examples of adaptive radiation in this taxon occurs in lakes of the Haida Gwaii archipelago off the coast of British Columbia, western Canada. These fish have diversified in multiple morphological, physiological, and life-history traits and the differences are generally predictable in relation to habitat. The major differences across a gradient from large lakes through to small ponds in these archipelago are a reduction in adult body size (Moodie & Reimchen, 1976), increase in relative body depth (Spoljaric & Reimchen, 2007), reduction in the number and size of the anterior plates (Reimchen, 1983; Reimchen, Stinson & Nelson, 1985), reduction in the size or number of dorsal and pelvic spines (Reimchen, 1980), reduction in number of gill rakers (Moodie & Reimchen, 1976; Reimchen *et al.*, 1985), and an increase in relative gape (Spoljaric & Reimchen, 2007). The predictive differences in this morphological complex are replicated among different watersheds (Moodie & Reimchen, 1976), within watersheds where both lakes and ponds are present (Reimchen *et al.*, 1985), and across lake–stream interfaces (Moodie, 1972a; Reimchen *et al.*, 1985), and in the last-named comprise multiple loci positioned across the genome (Deagle *et al.*, 2012). Similar trends are observed in other regions from coastal zones in British Columbia (Reimchen & Nosil, 2006), Alaska (Francis *et al.*, 1986; Bourgeois *et al.*, 1994), and Western Europe (Gross, 1977). The majority of morphological changes in the Haida Gwaii stickleback appear to reflect adaptive responses to the regular shifts in predation and trophic communities (Reimchen, 1994). Large lakes are dominated by multiple predator species (salmonids and diving birds) with zooplankton as the dominant food base while the smallest ponds, which do not have predatory fish or resident diving birds, have macroinvertebrate piscivores and benthic prey such as dipteran larvae. Differences in pursuit and manipulation behaviour of these predators influence defence morphology (Reimchen, 1994) while shifts in trophic communities are associated with gill raker and head morphology (Schluter & McPhail, 1992; Reimchen & Nosil, 2006).

The lengths of time required for evolution of these divergent morphotypes are probably highly variable. Transitions from the completely plated to the low-plated morph, which segregates as a simple Mendelian trait, can occur within decades (e.g. Bell, Aguirre & Buck, 2004) but the rates of change for complexes of traits such as body shape, number, size and position of plates, number and length of spines, and number and size of gill rakers have not been determined. These could be influenced by the strength of selection, the amount of additive genetic variance and covariation among traits, as well as phenotypic plasticity, which might facilitate the earliest stages of colonization to novel selective landscapes (Price, Qvarnstrom & Irwin, 2003; Pigliucci, 2005; West-Eberhard, 2005; Spoljaric & Reimchen, 2007).

Based on the multiple instances of adaptive responses of stickleback to the specific ecological conditions that differentiate large lakes and small ponds in the Haida Gwaii archipelago, a field experiment was initiated in 1993 involving a transplant of a limnetic morphotype adapted to a large dystrophic lake with a diverse predator regime into a small shallow eutrophic pond lacking fish to evaluate the extent of change, if any, in defence and trophic traits of successive generations of the transplant population. The source population is the endemic giant black stickleback from Mayer Lake, which is the largest bodied stickleback known throughout the circumboreal distribution of *Gasterosteus* (Moodie, 1972a). The transplanted individuals successfully reproduced during the year of introduction, resulting in population expansion that levelled off (based on trap success) in several years (T. E. Reimchen, pers. observ.). No a priori predictions as to rate of change were possible. Changes, if present, could be stochastic from founder effects or deterministic (genetic and/or plasticity) assuming a functional response of the lake morphotype to the pond habitat. If the latter, we predict: (1) a reduction in number or size of the anterior plates (LP 1–3) (as the pond lacks predatory fish (Reimchen, 1983), (2) a reduction in size of the dorsal and pelvic spines (because the pond is lacking predatory fish and has minimal avian piscivory) (Reimchen, 1994), (3) a shift in trophic structures including a reduction in number and size of gill rakers and an increase in relative gape (as the shallow pond has few zooplankton and is dominated by benthic taxa), and (4) an increase in relative body depth. Here we evaluate defence and trophic traits throughout the early stages of the colonization process.

METHODS

STUDY LOCALITY, TRANSPLANT AND COLLECTION DETAILS

Mayer Lake (ML; 53.642°N, 132.057°W) and Mayer Pond (MP; 53.628°N, 132.044°W) are located on the north-east region of the Haida Gwaii archipelago on the west coast of Canada (Fig. 1). General descriptions are provided elsewhere (Moodie, 1972a; Moodie, 1984; Spoljaric & Reimchen, 2007). The source locality (Mayer Lake) is large (~490 ha), with low conduc-
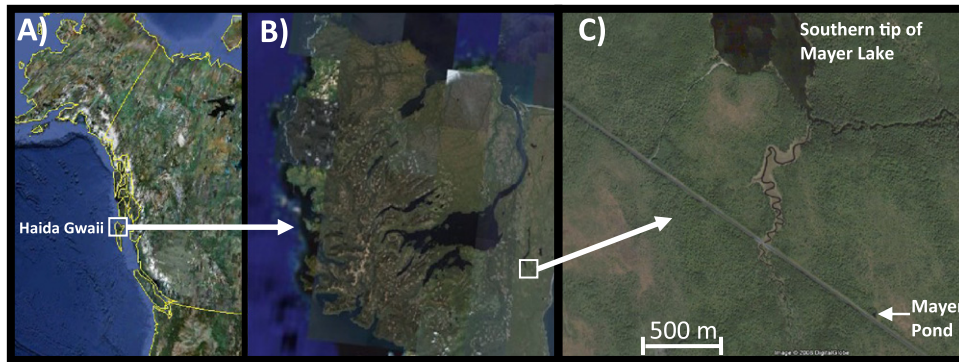
Figure 1. A, Haida Gwaii, British Columbia, Canada (A). The two study sites, located on the north-east corner of Graham Island (B), include Mayer Lake and the nearby Mayer Pond (C). Source: 'Mayer Lake and Mayer Pond.' 53.634°N and 132.048°W. Google Earth. Image compiled 8 August 2005. Image accessed 29 October 2008.

tivity (64 µS cm^{-1}), dark tannin staining, sparse littoral macrophytes, zooplankton (Cladocera, Copepoda) and multiple predators including cutthroat trout (*Oncorhynchus clarki*), Dolly Varden (*Salvelinus malma*), prickly sculpin (*Cottus asper*), loons (*Gavia* spp.), grebes (*Podiceps* spp.), mergansers (*Mergus* spp.) and kingfisher (*Ceryle alcyon*). The sticklebacks from the source lake have one of the most divergent morphologies from throughout the circumboreal distribution of threespine stickleback. They are highly melanistic and characterized by exceptionally large adult body size [80–100 mm standard length (SL)], robust vertebrate predator defences including 7–8 lateral plates (positions 1–8), strong dorsal and pelvic spines, a streamlined body shape for a limnetic niche, and a high gill raker number (Moodie, 1972b) and breed in their third summer (Gambling & Reimchen, 2012). The fish exhibit low phenotypic variability in traits relative to other populations, suggesting strong directional selection (Moodie & Reimchen, 1976), yet show average genetic variability (h) based on mitochondria (O'Reilly *et al*., 1993) and genome-wide single nucleotide polymorphic markers (Deagle *et al*., 2012). The pond used for the transplant is found in the same watershed and was chosen as it lacked resident fish and was small enough (0.25 ha, 1 m maximum depth) to exclude surface diving birds. Belted kingfisher and great blue heron are present in the archipelago and are potential predators in the pond although none has been seen at the pond in 15 years of field observations. The pond has low conductivity (70.5 µS cm^{-1}), lacks an inlet and outlet stream, is eutrophic with 90% cover of pond lily (*Nuphar luteum*), and has a well-developed macroinvertebrate benthos (Diptera, Ostracoda, Odonata, and Coleoptera). Following the transplant in May 1993, adults reproduced and first generation young of the year (30–40 mm SL) were captured in September 1993. In September 1994 and subsequent years collections comprised predominantly 40–75 mm SL individuals and small numbers of young of the year. The source population has a 2-year generation time although Mayer Pond fish may have transitioned to a 1-year generation time by the year 2000 (T.E.R., unpubl. data). Based on relative trap success, population size reached asymptotic values by summer 1994 and remained similar until about 2005. At that time, a non-native frog (*Rana* sp.) became established in the pond and from 2007 to 2011, tadpoles outnumbered stickleback 50:1 in the traps. In 2011, capture and marking of 370 sticklebacks (50–80 mm SL) from the pond and subsequent recapture after 2 weeks indicated a population of ~1000 reproductive and subadult fish. Sources of mortality on the stickleback in the pond are primarily macroinvertebrates piscivores (e.g. adult *Dytiscus* and larval *Aeshna*), which target young of the year fish (Reimchen, 1980). Additional fry are probably taken by adult and subadult stickleback, which can be cannibalistic (Foster, Garcia & Town, 1988; Reimchen, 1990).

Stickleback used for morphological analyses were collected from multiple time periods. We used samples from Mayer Lake in 1929 and 2003 (one additional Mayer Lake 1968 sample is from Moodie & Reimchen, (1976) and provides added total plate count data) and from Mayer Pond in 1994, 1995, 1998, 2003, 2007, and 2009. We analysed both sexes (total $N = 272$; 74 from Mayer Lake, 198 from Mayer Pond), present results for both but only graph males ($N = 142$; 48 from Mayer Lake, 94 from Mayer Pond) as females ($N = 130$; 26 from Mayer Lake, 104 from Mayer Pond) show comparable trends. The majority of fish were between 50 and 70 mm SL. The 1929 Mayer Lake sample was collected with a beach seine, while all recent collections were made with standard (8-mm mesh) G-minnow traps [the 1968 sample documented in Moodie & Reimchen, (1976) was also collected by beach seine]. Fish were killed and stored in
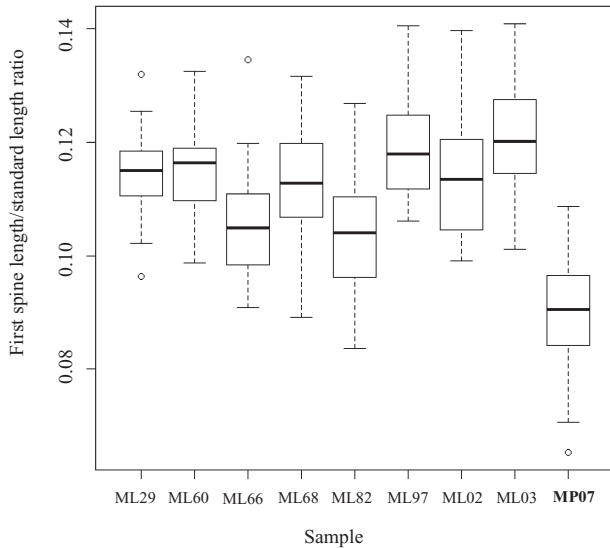
Figure 2. Box plot of relative first dorsal spine length for eight Mayer Lake samples and a recent 2007 Mayer Pond sample. Mayer Lake symbols from 1997, 2002, and 2003 represent collections from the same locality in the lake as that used for the transplant population (MP).

Figure 3. Morphological measurements include: A, from the side: left jaw length (JL), left eye diameter (ED), plate presence (positions 3–7 present on drawing), plate height (e.g. LP3H) of plate positions 1, 2, and 3 when present (plate width perpendicular to height also measured but not displayed), the length of the first and second dorsal spine (D1 and D2, respectively), and the height and area of the ascending process (APH and APA, respectively); B, from the extracted and stained first left gill arch: count of gill rakers on the upper and lower branch of the gill arch, the length of calcified and stained portion of the first, third, and fifth gill raker below the joint between the upper and lower branch, as well as the spacing between them; C, from the underside: length of the pelvic plate (PPL) and left pelvic spine (PS), as well as the area of the pelvic plate (PPA).

ethanol while earlier samples (< 1970) were first preserved in formalin and then transferred to alcohol. Moodie (1972b) observed no differences in body shape or phenetic differences between 1929 and large samples in the late 1960s, concluding that the stickleback in the lake are highly conserved in morphology. To further evaluate this, we also compared size-standardized dorsal spine length of males among Mayer Lake samples from eight samples (1929, 1960, 1966, 1968, 1982, 1997, 2002, and 2003; total $N = 189$). There was a maximum 15% difference in spine length among years and all of these were significantly higher than a recent Mayer Pond sample (Fig. 2). Samples prior to 1997 were obtained from separate localities in the lake relative to those from 1997, 2002, and 2003 which were obtained from the same site as that used for the transplant population.

We collected fertilized egg masses from five nests at Mayer Lake and from two nests at Mayer Pond in July 2007 and reared the offspring at the University of Victoria (UVic) Aquatic Facility. Only one Mayer Pond nest survived but based on the size (> 300 eggs) it comprised at least two separate clutches (Baker *et al.*, 2008). The egg masses were raised in separate partitions of the same tank with common circulating system water and subject to a natural daylight cycle (fluorescent lights turned on and off by a light sensor on the roof). Newly hatched fry were fed brine shrimp nauplii two to three times daily ad libitum. Once the fry were large en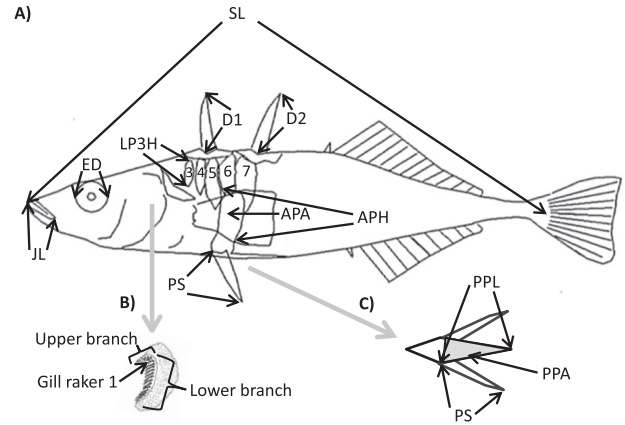ough, they were fed bloodworms and occasionally 0.2-mm trout pellets and frozen krill for nutritional variety. When the fish were equivalent in size to the wild caught counterparts, they were killed with an overdose of tricainemethanesulfonate (MS-222 or TMS), according to the UVic Aquatic Unit facility Standard Operating Procedure OA2003, and preserved in 70% ethanol for further analysis. Although the daily mortality rates in both source and transplant populations were low (~1%) over the 6-month rearing period, the cumulative mortality was considerable (~84%) and the potential for selective mortality is clear. However, we include the data as these provide insight into morphology of two groups when raised under constant conditions. We raised and analysed 106 fish ($N = 77$, 24 males and 53 females, from Mayer Lake and 29 fish, 14 males and 15 females, from Mayer Pond).

MEASUREMENTS

Twenty metrics were obtained from each specimen (Fig. 3). We measured fish in hand for standard length and maximum depth (depth immediately anterior to the first dorsal spine) using vernier callipers (accurate to 0.1 mm), and used a dissecting scope to

obtain counts and position of left and right lateral plates (according to landmarks detailed in Reimchen, 1983) as well as to sex the fish by examining the gonads. The first gill arch on the left side was removed and stained with an alizarin red solution for ~30 min and then left overnight in 95% ethanol. We used a 10-megapixel camera (Pentax K10d) with a macro lens (Pentax 100 mm f2.8) and an accessory flash (Sigma EF – 500 DG Super) to take detailed photos from: the left and right sides of the fish, the underside, the front (specifically showing the width of the first spine), and the extracted/stained gill arch which had been pressed into one plane using a slide and coverslip. Vernier callipers provided an equivalent scale in all photos. Using this scale and ImageJ software (Rasband, 2009) we were able to obtain measurements representing trophic structures (left eye width and height, left jaw length, gill raker number, gill raker length and gill raker spacing) as well as defence structures (if present, the width and height of LP1, LP2 and LP3), the length and width of the first dorsal spine, the length of the second dorsal spine, the length of the pelvic spine, the length and area of the pelvic plate, and the height and total area of the ascending process.

To assess the repeatability of morphological measurements we randomly selected 16 wild caught fish (eight each from Mayer Lake and Mayer Pond) and re-measured all continuous morphological traits. The average difference between the original and replicated values (original minus replicate/average of the two) for all traits was 2.7%, and all features had less than 5% difference with the exception of the right ascending process area measurement. This had 11.2% difference and was excluded from further analyses.

ANALYSES

We undertook a principal component analysis (PCA) using size-standardized measurements. As the continuous trait measurements are correlated with SL, we standardized each datum to that as would be found in a 60-mm fish (the average size of fish in the transplant population). We used residuals from the trait vs. SL regression for each sex and sample separately. We applied an SL correction to the residual (residual*60/SL) to remove proportional effects and added these corrected residuals to the average value of a 60-mm fish in each sample. PCs (26 from ML29, 41 from ML03, 20 from MP94, 21 from MP95, 31 from MP98, 30 from MP03, 44 from MP07, 43 from MP09) were computed using traits that had the highest repeatability, including: body depth, second dorsal spine length, left LP3 height, left ascending process height, left pelvic spine length, pelvic plate length, gill raker 5 bone length, and gill raker

spacing (PCA $N = 256$ as some of the 272 individuals did not have values for one or more of the included traits). For all bilateral traits, both sides were measured, but for simplicity only the left measurements are included as right side values show similar trends. For both the PCA and individual assessment of these size-corrected continuous measurements, we used an ANOVA with Tukey's post-hoc comparisons to identify any differences between the Mayer Lake baseline and each Mayer Pond sample. Size-corrected jaw length and eye diameter, which could not be included in the PCA due to previous destructive sampling of the heads of fish from the 1929 Mayer Lake sample, were analysed with univariate statistics.

The two Mayer Lake samples (1929 and 2003) were statistically equivalent and pooled for the majority of features: total lateral plates (general linear model – GLM, Poisson error structure, male: $P = 0.37$, female: $P = 0.59$); individual lateral plate presence (GLM, Binomial error structure, male: $P > 0.3$, female: $P > 0.17$); PC1 (ANOVA, male: $P = 0.16$, female: $P = 0.11$); pelvic spine lengths (ANOVA, male: $P > 0.1$, female: $P > 0.45$); gill raker lengths (ANOVA, male: $P = 0.2$, female: $P > 0.5$). The two features that were not equivalent in the two Mayer Lake samples (dorsal spine length in males: $P < 0.04$ comparing ML29 and ML03; left LP3 height in females: $P < 0.01$) were compared individually with each Mayer Pond sample.

We used a GLM to determine if there are differences in the total number of plates (GLM with Poisson error structure), and presence of specific lateral plates (GLM with Binomial error structure) in Mayer Pond fish compared with Mayer Lake fish over time since the transplant. All statistical analyses were completed using SPSS version 14 or R version 2.9.2 (Team, 2009).

We also measured evolutionary rates of change in Mayer Pond using Haldanes, a more appropriate measure than Darwins for such a short time frame (Gingerich, 1993). We present two scenarios: (1) assuming our Mayer Lake samples are representative of the original colonists founding Mayer Pond we compare Mayer Lake with the most recent 2009 Mayer Pond sample to estimate rates of evolutionary change; (2) in case the Mayer Lake samples are not representative, and to account for the potential of plasticity that may have resulted in immediate changes, we compare the first (1994/1995) and most recent (2009) generations within Mayer Pond as a more conservative estimate of evolutionary rates. As the population probably transitioned from a 2-year generation time (eight generations between colonization and the 2009 sample) to a 1-year generation interval (maximum 16 generations) we use 12 generations in our calculations. We calculate Haldanes

Table 1. PCA loadings for the first two PCs generated from size-corrected values (transformed for differences in body size to represent features of a 60-mm fish) of continuous measurements taken from 256 fish representing Mayer Lake and Mayer Pond populations

Feature	Principal component (males)		Principal component (females)	
	1	2	1	2
Maximum body depth	0.171	**0.854**	0.497	**0.676**
Second dorsal spine length	**0.857**	−0.138	**0.760**	−0.428
Left lateral plate 3 height	**0.704**	0.119	**0.733**	−0.146
Left ascending process height	0.216	**0.703**	0.344	**0.551**
Left pelvic spine length	**0.861**	−0.155	**0.673**	−0.446
Pelvic plate length	**0.666**	0.237	**0.604**	0.327
Gill raker 5 bone length	**0.666**	−0.114	**0.553**	−0.238
Gill raker 1 to 5 distance	−0.348	**0.608**	0.212	**0.663**

The main contributing features are in bold type. PC1 = 38.2% in males, 33.1% in females; PC2 = 21.5% in males, 22.1% in females, of the variation in the data**.**

using ln-transformed values according to the procedure and equations outlined in Gingerich (1993).

RESULTS

PRINCIPAL COMPONENT ANALYSIS

The source and transplant populations segregated in multivariate space. PC1 primarily represents defence traits such as dorsal and pelvic spine length (38.2 and 33.1% of the explained variance in males and females, respectively), while PC2 is primarily trophic traits such as gill raker size and body depth (21.5 and 22.1% explained variance in males and females, respectively; Table 1). Mayer Pond fish have lower values and exhibit almost complete separation from Mayer Lake fish along PC1 (Tukey's post-hoc test, all $P < 0.001$ for males, $P < 0.04$ for females) but on PC2 there is major overlap with marginally higher values for Mayer Pond (Fig. 4). When partitioned for the multiple years, the centroids for the transplant population differ primarily on PC2 with more recent years having higher values (greater body depth and greater gill raker spacing) relative to earlier transplant samples.

UNIVARIATE ANALYSES

The number of lateral plates differed between Mayer Pond and Mayer Lake populations in males, but not in females. In males, the first generation (1994) after transplant had a 6% increase from Mayer Lake ($P = 0.03$), and this declined by 14% (7.4 to 6.4 plates) over the subsequent seven generations to the most recent sample (comparison of Mayer Lake to MP09, $P = 0.003$; Fig. 5). Although there is modest fluctuation, this yields a negative regression slope in the Mayer Pond samples (GLM with Poisson error struc-
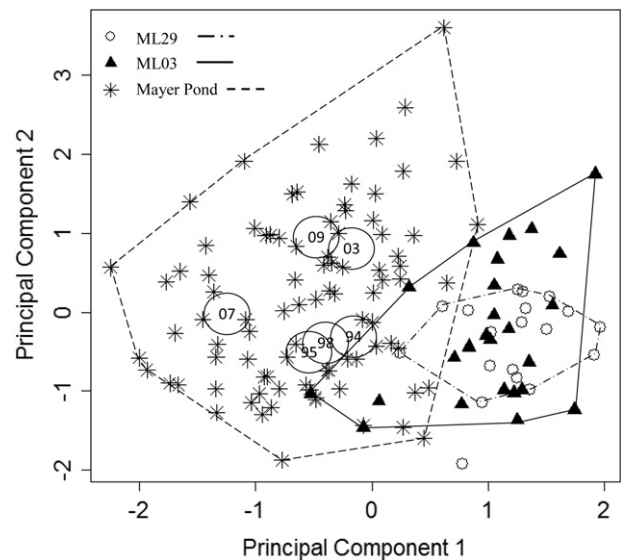

Figure 4. PC1 and PC2 for males of Mayer Lake and Mayer Pond samples of stickleback. Mayer Lake (ML29 and ML03 represented by circle and triangle, respectively); Mayer Pond (asterisk). Bounding polygons show the range of each. The centroids (mean PC1 and mean PC2) for each individual Mayer Pond sample are shown by a circle containing the two-digit year of the sample (e.g. Mayer Pond 1994 = 94).

ture, comparison of slope with zero; $P < 0.001$), but no differences in the two Mayer Lake samples separated by 73 years (comparison of slope to zero, $P = 0.37$). There were no significant changes in plate count in females in Mayer Pond over successive generations (GLM with Poisson error structure, comparison of slope with zero; $P = 0.84$).

Analysis of plate positions provides additional insight into the changes in plate number. In males,
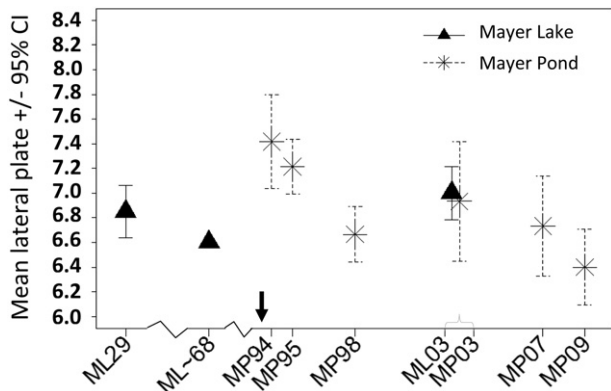
Figure 5. Mean total left lateral plate count in Mayer Lake (ML; solid triangles and lines) and Mayer Pond (MP; asterisks and dotted line) male samples over time. The ML ~68 mean plate count data are from earlier analyses (Moodie & Reimchen, 1976).

there is a progressive reduction in the frequency of LP2 (96–50%) over 16 years since the original transplant (GLM, binomial error structure, effect due to time $P < 0.001$). LPs at positions 3–7 are present in all fish in both localities. LP8 undergoes a general, but non-significant reduction (comparison of Mayer Pond samples with Mayer Lake, all $P > 0.3$). LP9 is more common in Mayer Pond 1994 and 2007 samples (both $P < 0.05$), but not in other years ($P > 0.4$). LP1 and LP10 are infrequent but variable (all $P > 0.12$; Fig. 6). Female plate presence is quite similar between Mayer Pond and Mayer Lake females (all comparisons $P > 0.08$, other than MP07 LP1 where $P < 0.001$). However, like males, Mayer Pond females show a significant decrease (–17%) in llp2 presence over time ($P = 0.04$). Neither Mayer Lake males nor females show this reduction between the 1929 and 2003 samples ($P > 0.9$ and $P = 0.7$, respectively).

Height of lateral plates determines the area of epidermis covered on any segment. In males, the first generation after transplant showed no change in LP height compared with Mayer Lake fish, but in the second generation, there was a 20% reduction in height which has persisted to the present, apart from an apparent recovery and subsequent reduction in 2003 (Fig. 7A). Similarly in females, plate height is significantly reduced in all years except the first two samples from 1994 and 1995 (both $P > 0.1$) compared with the ML29 sample (other $P < 0.04$). Compared with ML03, it is significantly smaller in 2003 and 2007 ($P < 0.03$).

Standardized pelvic and dorsal spine lengths are reduced in the transplant population compared with Mayer Lake fish (Fig. 7B, C). In males, about half of the reduction in pelvic spines occurred in the first few generations, with differences being further accentu-

ated in recent years (all $P < 0.02$). Females show a similar reduction over time, although the differences are only significant in the more recent samples (2003 and earlier samples: $P > 0.08$; later samples $P < 0.05$). Both sexes show significant reductions in dorsal spine length ($P < 0.02$), with the full extent of the reductions manifesting within the first few samples taken following transplant. Size of the male dorsal spines in the 1929 Mayer Lake samples is slightly smaller than those from the 2003 Mayer Lake sample (9.0 versus 9.3, $P < 0.001$).

We examined number, size, and spacing of gill rakers. In males, gill raker numbers changed very little (Fig. 7D). Size-standardized raker lengths were ~30% shorter in the transplant population (1.20–0.85 mm, all $P < 0.001$) and the difference was most accentuated (40%) in the first generation (Fig. 7E). Spacing of rakers in the transplant population was similar to the source population in the first generation but thereafter these were marginally higher in each generation, two of which (2003, 2009) were significant (Tukey; $P < 0.01$ for both; Fig. 7F). Similar to males, females display no difference in raker number (all $P > 0.7$), and show a reduction in gill raker length with all samples significantly smaller in the transplant population (all $P < 0.001$). Although females show no change in raker spacing from 1994 to 1998 ($P > 0.2$), they have a similar, marginal increase over the last 6 years with a significant increase by 2009 ($P < 0.001$).

Estimates of gape were compared between the populations using standardized size of the left jaw. There was a general increase in jaw length following the first generation after transplant. However, none of the individual comparisons was significant apart from the most recent sample which showed significantly larger jaw size in males of the transplant population (Tukey $P < 0.05$; Fig. 7G).

Standardized eye diameter differed between the source and transplant population. In males in all years, including the first generation after transplantation, the eye was about 8% larger in the pond relative to the lake population ($P < 0.001$) with the largest differences (12%) occurring in the most recent sample (Fig. 7H). Females show no change in the first two samples ($P > 0.06$), but subsequently also have an increased eye diameter ($P < 0.01$).

Relative body depth varied over the duration of the field observation. There is evidence of a reduction of body depth in the transplant population in the earliest samples (1995 and 1998 samples in males, 1994, 1995, and 1998 samples in females; $P < -0.03$) followed by an increase to the most recent 2009 sample, which shows a significant increase in body depth compared with the source population ($P < 0.001$ for males and females).
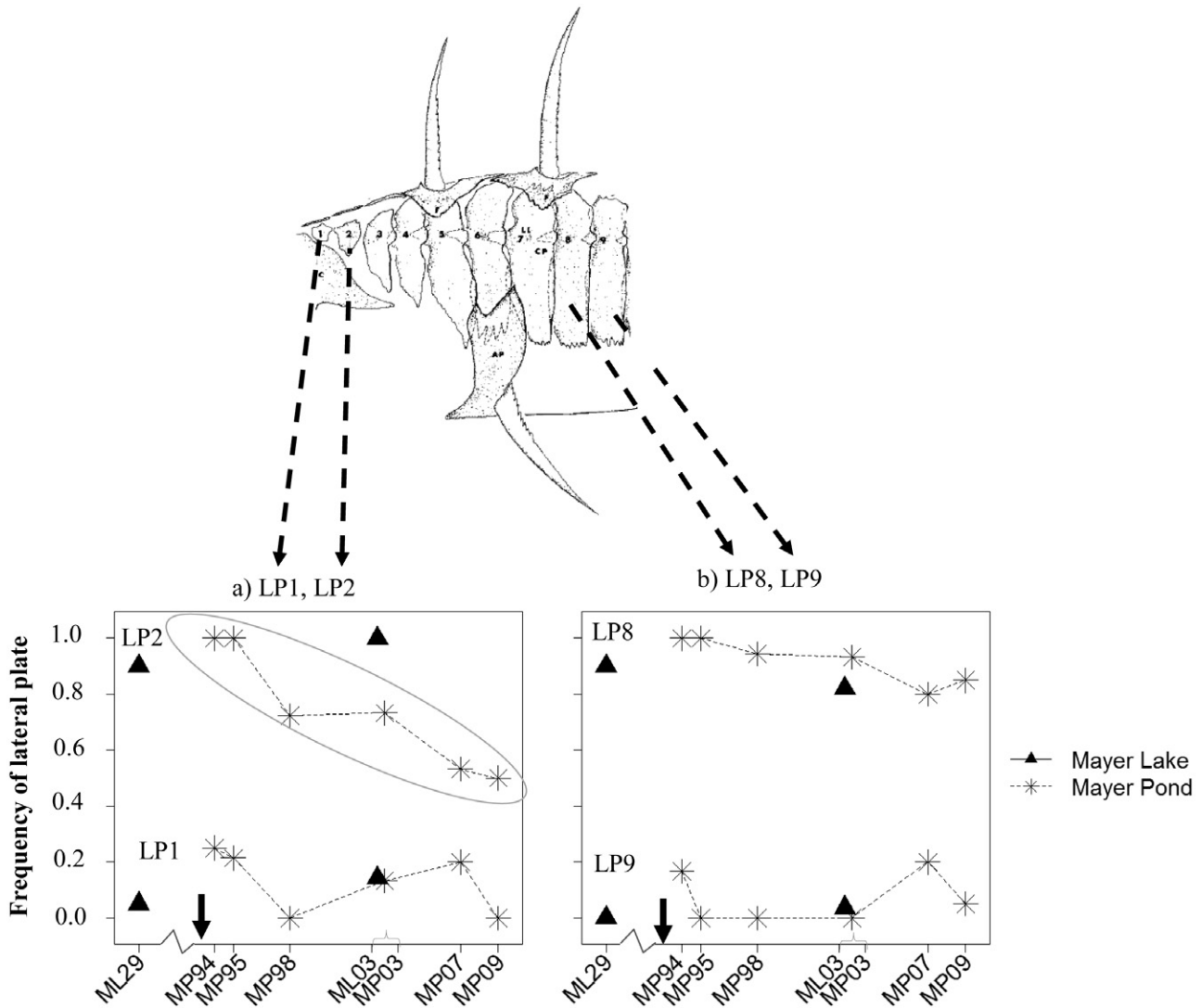
Figure 6. Mean plate presence in Mayer Lake (triangles) and Mayer Pond (asterisk) male samples for LP1, LP2, LP8, and LP9, (LP3–7 are present in all fish). Panels show two groups: (a) anterior plates LP1–LP2, and b) mid-body, buttressing plate LP8 and posterior plate position LP9. Trends between Mayer Pond samples over time since transplant are illustrated with a dotted line connecting each successive sample. Significant trend (GLM) over time is circled (LP2).

In summary, following 16 years of habitation (8–12 generations) in the novel habitat, there are directional changes relative to the Mayer Lake source population, including: total plate count: males, –3%; females, –4%; lp2 frequency: males, –42%; females, –30%; lateral plate 3 height: males, –16%; females, –13%; pelvic spine length: males, –10%; females, –8%; dorsal spine length: males, –18%; females, –18%; gill raker number: males, –10%; females, –5%; gill raker length: males, –49%; females, –29%; gill raker spacing: males, +10%; females, +7%; jaw length: males, –3%; females, +17%; eye diameter: males, +6%; females, +12%; body depth: males, +5%; females, +6%). In four of these 11 traits, at least half of the changes occurred in the first generation following transplant.

We estimated evolutionary rates (Table 2). Over the 16 years (12 generations) since colonization, the average rate of change between Mayer Lake and Mayer Pond fish for six traits is 0.15 Haldanes (males = 0.17; females = 0.12), and was highest for dorsal spine length and lowest for the frequency of LP8. Restricting the estimates between the first and last generations of Mayer Pond fish yields average rates of 0.09 Haldanes (males = 0.11; females = 0.07).

LAB-RAISED FISH

Stickleback hatched and raised in the laboratory differed in PC space from those captured from the field (Fig. 8). On PC1, the centroids for the two populations
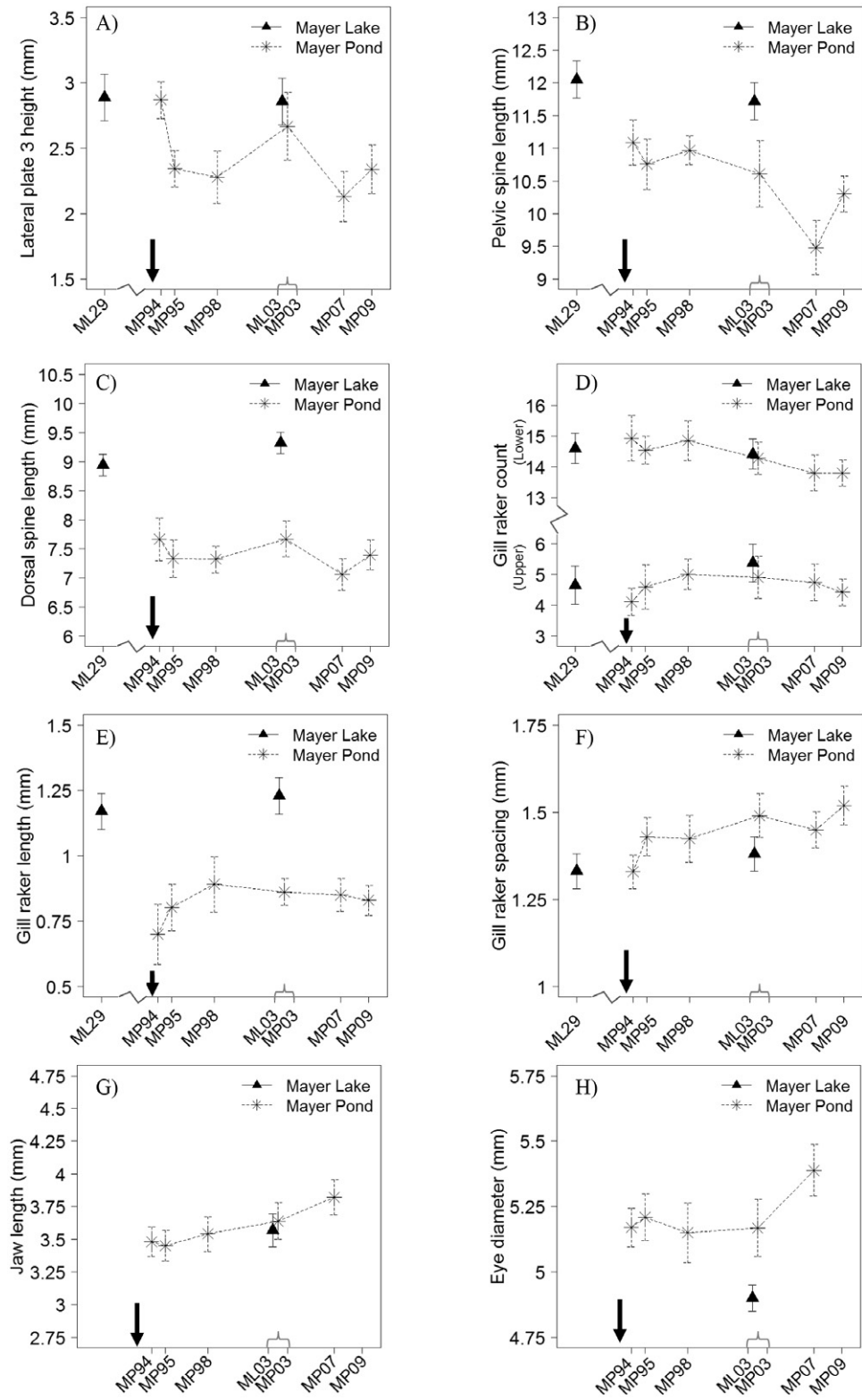
Figure 7. Means and 95% confidence intervals for measurements of Mayer Lake (triangles) and Mayer Pond (asterisks) male samples: A, lateral plate height; B, pelvic spine length; C, dorsal spine length; D, number of rakers; E, gill raker length; F, gill raker spacing; G, jaw length; H, eye diameter. Mayer Pond samples are joined by a dotted line to illustrate trends over time.

Table 2. Evolutionary rates of change in Haldanes for continuous traits and lateral plate presence for males/females

Trait	Comparison of Mayer Lake to Mayer Pond after 12 generations (Haldanes)	Change within Mayer Pond after 11 generations (Haldanes)
Pelvic spine length	–0.19/–0.07	–0.09/–0.09
Dorsal spine length	–0.25/–0.20	–0.01/–0.06
Gill raker length	–0.22/–0.17	0.05/0.03
Eye diameter	0.23/0.21	0.22/0.08
Average evolutionary rates (of absolute values) for continuous measurements	0.22/0.16	0.06/0.06
Lateral plate 1–3 presence	–0.12/–0.06	–0.15/–0.08
Lateral plate 4–8 presence	0.00/–0.03	–0.05/–0.05

Values compare Mayer Lake to the 2009 Mayer Pond sample, potentially representing the change from the original colonists after 16 years or 12 generations, and the change within Mayer Pond between the first generation in 1994/1995 and the most recent 2009 sample 11 generations later.
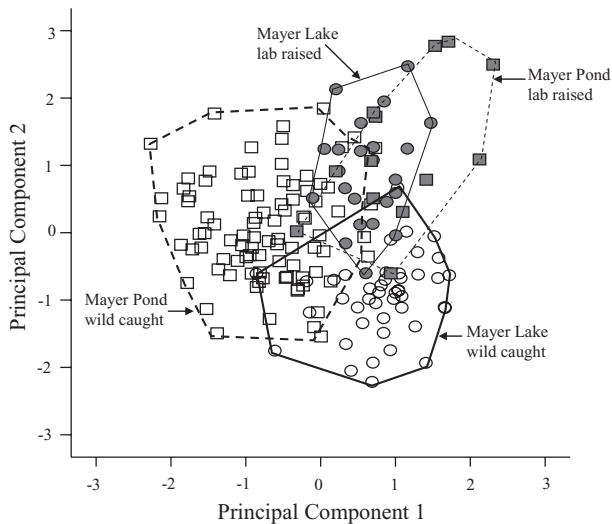

Figure 8. PC1 and PC2 for wild caught (open symbols) and lab-raised (solid symbols) Mayer Lake (circle symbols and solid lines) and Mayer Pond (square symbols and dotted lines) male samples. Bounding polygons show the range of each.

of lab-reared fish differ by 0.43 units, one-third the separation evident in wild-captured fish (1.56 units), similar to that for PC2 (0.31 vs. 1.12 respectively).

For univariate comparisons, Mayer Pond differed from Mayer Lake in multiple traits (Table 3). Among the ten traits in males, only one was significantly different between the source and transplant population (dorsal spine length), and it is in the opposite direction observed in wild caught fish. Two traits, total plate count and LP2 presence, are close to significance. Females exhibit five traits with significant differences between the source and transplant populations. Three of these (pelvic spine length, gill raker length, and gill raker spacing) were in the same direction as field-captured fish while two (jaw length and eye diameter) were in the opposite direction.

DISCUSSION

Following the transfer of a giant and highly divergent freshwater stickleback from a large lake to a small pond, we found that over 16 years (8–12 generations) for each defence and trophic trait, there was about a 10% shift in expression in the direction predicted from widespread geographical trends that differentiate large lake and pond habitats. Analysis of individual traits provides a context for these changes and furthers insight into the first stages of adaptation and differentiation operating in this novel population.

Temporal shifts in frequencies of number of lateral plates on stickleback have been identified following recent colonization events. Most common is the loss of the posterior plates as this reduction can occur within several decades (Klepaker, 1993; Bell *et al.*, 2004; Kristjansson, 2005; Le Rouzic *et al.*, 2011) and is facilitated by multiple selection mechanisms including reduced calcium availability (Giles, 1983; Bell *et al.*, 1993), reduced mass for increased buoyancy (Myhre & Klepaker, 2009), and increased trunk flexibility for evasive behaviour (Reimchen, 1992; Bergstrom, 2002). Temporal variability in the anterior plates has received less attention. An 8% reduction in number of anterior plates (from 6.8 to 6.3) has been observed over a three-decade period in an Alaskan lake (Bell *et al.*, 2004) and a 14% cyclical change (from 5.2 to 4.5) between summer and winter in a Haida Gwaii lake, associated with shifts in propotion of avian and salmonid piscivores (Reimchen, 1995).

Table 3. Summary of percentage differences between Mayer Lake and Mayer Pond lab-raised fish for males and females for ten morphological traits (P-value of t-test in parentheses)

Trait	Male	Female
Total plate count	–8.7% ($P = 0.06$)	–7.3% ($P = 0.09$)
LP2 frequency	–74.5% ($P = 0.06$)	+24% ($P = 0.4$)
LP3 height	–0.6% ($P = 0.91$)	5.3% ($P = 0.3$)
Pelvic spine length	+2.4% ($P = 0.20$)	–4.2% ($P = 0.03$)
Dorsal spine length	+9.4% ($P < 0.01$)	+3.6% ($P = 0.03$)
Gill raker number	–0.6% ($P = 0.3$)	–4% ($P = 0.3$)
Gill raker length	+0.7% ($P = 0.86$)	–26% ($P < 0.001$)
Gill raker spacing	+6% ($P = 0.14$)	+10% ($P < 0.001$)
Jaw length	–0.8% ($P = 0.74$)	–16% ($P < 0.001$)
Eye diameter	–0.2% ($P = 0.86$)	–2.7% ($P = <0.01$)

Sign of percentage difference represents Mayer Pond minus Mayer Lake.

We observed a 3% reduction in number of lateral plates over eight generations. Although this reduction is in the direction we predicted given the shift in predation regime (Reimchen, 1994; Marchinko, 2009), the loss of plates cannot be readily intepreted without understanding the functional context of the missing plate positions.

We found that the general decrease in total plate count was driven almost entirely by a significant and progressive loss of LP2. These anterior plates (LP1–3) do not support spines but rather cover the naked supracleithral region and protect the underlying epidermis and lateral line nerve against puncturing by toothed fish predators (Reimchen, 1983, 1992). Localities such as ponds that are too small to support vertebrate piscivores have stickleback that typically lack these anterior plates (Reimchen, 1994). As such, our results are consistent with our expectations of the changes that would occur in the tranplant pond without vertebrate piscivores. Previous evidence for heritable effects in total plate number (Hagen, 1973; McPhail, 1992; Hatfield, 1997; Bell, 2001; Colosimo *et al.*, 2004, 2005; Knecht, Hosemann & Kingsley, 2007), as well as our data indicating that that the loss of LP2 persists in lab-raised fish, is consistent with genetic changes in the Mayer Pond population.

Yet the actual selective mechanism for the loss of LP2 remains obscure. The anterior plates have less biomechanical importance compared with the more posterior buttressing plates (Reimchen, 1983) and typically have high levels of fluctuating asymmetry consistent with reduced functionality (Bergstrom & Reimchen, 2000). Loss of LP2 is in the same sequence to that observed among populations, i.e. LP2 before LP3 (Reimchen, 1983), but as well is in the same sequence to the ontogenetic addition of the plates (LP2 after LP3: Bell, 1981). Sticklebacks which vary by a single anterior plate differ in their timidity (Moodie, 1972a; Moodie, McPhail & Hagen, 1973; Huntingford, 1981), as well as in their individual isotopic trophic niche (Reimchen, Ingram & Hansen, 2008). Marchinko (2009) observed a frequency reduction in the complete-plate EDA allele of juvenile stickleback with exposure to macroinvertebrate predation and this occurred prior to the ontogenetic expression of lateral plates, indicating potential linkage effects between specific plates and behaviours.

In addition to loss of the anterior lateral plates in natural pond populations without vertebrate piscivores, there is also a reduction in the vertical height of plates with a resulting loss of buttressing effects between the plates and the dorsal and pelvic spines (Reimchen, 1983). Shape of lateral plates appear to be greatly influenced by three genetic markers that explained a significant amount of variability in plate size and which map to the same region of the chromosome controlling plate number (Colosimo *et al.*, 2004). We predicted a reduction in height of the lateral plates and measured LP3 as this was present in all fish. Several of the Mayer Pond samples (1994, 2003) are equivalent to the source population but the majority of years showed a significant reduction in the height of this plate consistent with prediction. However, the yearly variability in the pond samples suggests either very strong fluctuating selection or environmentally induced plasticity. We favour the latter interpretation as the lab-raised individuals from the two localities did not differ from each other in plate size. Our data indicate that for anterior plates, size reduction precedes loss of the plates, possibly due to the production costs of structures that are no longer required for defence (Giles, 1983).

Spines are important antipredator defences in stickleback as these lead to a greater escape probability after capture by a predator (Hoogland, Morris & Tinbergen, 1956) and their relative sizes are

smaller where gape-limited piscivores are uncommon (reviewed by Reimchen, 1994). Spines are occasionally reduced in number in association with habitats dominated by macroinvertebrate piscivores (Reimchen, 1980) or those with low conductivity and calcium-deficient waters (Giles, 1983; Bell, 1987; Bell *et al*., 1993). Invertebrate predators may use the spines to restrain captured stickleback (Reimchen, 1980), leading to temporal and spatial trends in spine loss dependent on the proximity to invertebrate predation within the lake (Reimchen & Nosil, 2002). Marchinko (2009) found that invertebrate predators produced significant selection on genes controlling spine length in stickleback, resulting in an overall reduction in spine length in several generations. We predicted a reduction in size or number of spines in our transplant population because of the predominance of macroinvertebrate piscivores. There was no reduction in number but an approximate 15% reduction in size of dorsal and pelvic spines relative to the source population. Differences in spine sizes are known to have a heritable component (Hatfield, 1997; Peichel *et al*., 2001; Colosimo *et al*., 2004), suggesting that at least some of the reduction in spine length we observed represents genetic changes. Yet, we suspect a contributory role for plasticity as the changes in spine length occurred in the first generation following transplant and stickleback raised in common conditions in the lab showed no differences in spine length relative to the source population. Calcium deficiency has also been proposed as a mechanism for loss of armour (Giles, 1983) but this would not readily account for our results as the conductivities are similar between the source and transplant habitats. Other research indicates that multiple attributes of stickleback life history including egg size, hormone levels in eggs, growth rate and adult body size can be influenced by olfactory, visual, and other cues (Bell *et al*., 2011; Frommen *et al*., 2011) and it is likely that such cues differ sharply between Mayer Lake, dominated by predatory fish in dystrophic waters, and Mayer Pond, dominated by macrobenthos in eutrophic waters.

Population differences in stickleback feeding structures have been primarily investigated for 'benthic' and 'limnetic' species pairs that occur in several lakes of southern British Columbia (McPhail, 1992; Schluter & McPhail, 1992; Cresko & Baker, 1996; Hatfield, 1997; Peichel *et al*., 2001). Gill rakers are one of the best discriminators of trophic differences (Schluter & McPhail, 1992; Bolnick, 2004; Berner *et al*., 2008; Bolnick & Paull, 2009). In our transplant population on Haida Gwaii, we found only marginal differences in gill raker number but a highly significant decrease in gill raker length compared with those in the source population. This probably reflects a combination of genetic and plastic responses. Approximately one-third of the variability in raker length is heritable (Schluter, 1996) while ontogenetic changes in diet also influence gill raker length probably facilitated by novel mechanical stress imposed during critical periods of development (Lavin & McPhail, 1986; Day, Pritchard & Schluter, 1994; Day & McPhail, 1996; Wund *et al*., 2008). Cichlids demonstrate a striking shift in jaw development dependent on the extent of hard parts in the diet (Meyer, 1987). Our results, showing a first generation reduction in raker length following transplant and which exhibit no further reduction over the 16 years, implicates plasticity rather than strong selection. This inference is supported in lab-raised males (but not females) which show no differences between the source and colonizing populations when raised on a common diet. This result suggests caution in estimating strength of selection for differences in rakers among populations (Bolnick, 2004; Berner *et al*., 2008).

Body depth, eye diameter, and jaw length, which increased in our transplant population, also reflect dietary niche in stickleback (Schluter & McPhail, 1992). Our univariate data show only marginal evidence for increased body depth but geometric body shapes of these colonists indicate a shift towards a deeper body (Spoljaric & Reimchen, 2007, 2011). For both sexes, we found evidence for an increase in eye diameter and jaw length in the transplant population, some of which may be phenotypic plasticity based on trends in our lab-raised samples. Whether selection or plasticity, the prey available in Mayer Pond are more 'benthic' than Mayer Lake (Leaver, 2010) and the reduction in gill raker length, increase in jaw length, and increase in eye diameter mirrors the 'benthic' morphotypes observed in other localities (Schluter & McPhail, 1992).

How large are these predictive differences we observed over eight generations in this experimental colonization relative to the differences that exist between large lakes and natural pond populations in the archipelago? We compared morphometrics of males from the source population (Mayer Lake) with previously collected data for stickleback from three intact ponds located in the Sangan watershed in the northern region of the Haida Gwaii archipelago as these exhibit the typical pond adaptations including major reduction in armour and a shift to benthic trophic traits (Reimchen *et al*., 1985; T. E. Reimchen, unpubl. data). The average number of lateral plates in the intact pond populations was about 70% lower than Mayer Lake stickleback (7.1 vs 2.2) compared with a 3% reduction in our transplant population. Therefore, the change observed in Mayer Pond over 16 years represents about 4% of the potential reduction in the lateral plate numbers that we would expect to see, assuming the

stickleback in the three intact ponds represent an equilibrium for these habitats. For individual lateral plates, there was a 96% reduction of LP2 in the three ponds, compared with a 50% reduction in Mayer Pond. LP3, LP4, and LP5 are lost in 91, 65, and 43%, respectively, of the fish in the three pond populations but there are no losses of these plates over the eight generations in the Mayer Pond individuals. We also compared data on spine and trophic morphology. Pelvic spine length was reduced 34% (Mayer Lake = 8.9 mm, Intact Ponds = 5.9 mm) compared with a 9% reduction in the transplant population. The second dorsal spine was reduced by 37% (Mayer Lake = 5.6 mm, Ponds = 3.5 mm) compared with 18% reduction in Mayer Pond. Gill raker number was reduced by 10% (Mayer Lake = 20.2, Ponds = 18.1) compared with 7.5% in Mayer Pond. Jaw length was increased by 35% (Mayer Lake = 3.1 mm, Ponds = 4.2 mm) compared with a 7% increase in Mayer Pond. Eye diameter increased by 23% (Mayer Lake = 4.1 mm, Ponds = 5 mm) compared with 9% for Mayer Pond. Consequently, the changes we observed in Mayer Pond spine and trophic morphology after only eight generations, and for many traits after one generation, represent approximately one-third of the potential differences that exist between intact large lake and small pond populations in the archipelago.

Evolutionary rates following our transplant are among the higher ones reported in general meta-analyses of rapid evolution in a broad diversity of taxa (Gingerich, 1983; Hendry & Kinnison, 1999; Kinnison & Hendry, 2001; Gingerich, 2009) and are comparable with the rapid shifts (11 years) observed in lateral plate morph frequencies of Alaskan stickleback (Bell *et al.*, 2004). If we use an eight-generation period rather than the more conservative 12 generations, our rates are 1.5 times higher. However, our estimates, in addition to previous published rates, comprise some unknown contribution of phenotypic plasticity to the observed changes and therefore will overestimate the evolutionary rates. Restricting our analysis to changes within the transplant population following successful colonization reduces this issue and still yields rates three times the average evolutionary rates listed by Kinnison & Hendry (2001) in their meta-analysis of 434 studies of populations with fewer than 80 generations.

Our results are consistent with other studies evaluating evolutionary changes in colonizing populations. Introduction of guppies to new habitats results in shifts in multivariate morphological and life-history attributes (Reznick & Bryga, 1987). Transplantation of Caribbean anoles to biophysically variable habitats showed that the extent of changes was directly related to the magnitude of habitat differences, and that these changes were probably facilitated by plas-

ticity (Losos, Warheit & Schoener, 1997; Losos *et al.*, 2000). Recent but natural colonization of islands by passerines indicates the efficacy of microevolutionary changes in morphology in response to the new habitats (Clegg *et al.*, 2002).

Stickleback are a model species for evolutionary studies (Bell, 1995; Foster & Baker, 2004), and those from the Haida Gwaii archipelago exhibit exceptional differentiation among and within populations. Our introduction of one of the most divergent populations of freshwater stickleback into an ecologically opposite habitat shows evidence for rapid adaptation in univariate and multivariate space each in the direction predicted from the shifts in the selective landscape. Parts of these shifts appear to be accentuated by phenotypic plasticity and may be representative in the widespread dispersal, colonization, and differentiation of this taxon across freshwaters in the northern hemisphere. Recent genetic analyses (B. E. Deagle *et al.*, unpubl. data) using a genome-wide SNP array for stickleback (Jones *et al.*, 2012) indicate no reduction in genetic variability in the transplant population and shifts in allele frequencies predicted from comparisons across sharp ecological gradients (Deagle *et al.*, 2012). The integration of the genetic data with the continued temporal shifts in the transplant population towards the pond morphotype will allow improved resolution of the evolutionary changes during the early stages of population differentiation.

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