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Genetic and plastic contributions to trait divergence between parapatric habitats: female life-history traits in threespine stickleback within the Misty Lake system

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ABSTRACT

Question: How do genetic and plastic effects on maternal investment influence divergence between parapatric populations that do or do not show high gene flow? How might these patterns influence adaptation and progress towards ecological speciation?

Organisms: Wild-caught and laboratory-reared lake, inlet stream, and outlet stream threespine stickleback (*Gasterosteus aculeatus*) from the Misty Lake system, northern Vancouver Island, British Columbia, Canada. In nature, the inlet–lake pair shows low gene flow, whereas the outlet–lake pair shows high gene flow.

Methods: Analysis of covariance was used to compare egg size (dry mass), clutch size (number of eggs = fecundity), and clutch mass (dry mass = reproductive effort) among habitats (lake, inlet, outlet) and between rearing environments (wild, laboratory). Body size was used as a covariate to consider life-history variation relative to body size.

Results: In the wild, inlet females had greater reproductive effort and higher fecundity than did lake females, both before and after correction for body size. Outlet females were intermediate but closer to lake females, and showed clines in life-history traits with distance from the lake. In the laboratory, differences in these traits were in a similar direction but smaller. Differences between habitats in reproductive effort and clutch size are thus shaped by complementary (co-gradient) contributions from genetic differences and plasticity. Egg size did not vary between the habitats and was not plastic.

Conclusions: Outlet females were estimated to have a 32–71% decline in reproductive output in the wild – but this maladaptation would have been greater in the absence of plasticity. These modifying effects of plasticity on maladaptation will influence gene flow and progress towards ecological speciation.

Keywords: ecological speciation, gene flow, parapatry, plasticity, stickleback.

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INTRODUCTION

How new species form, or why they fail to do so, remains a key question in evolutionary biology (Darwin, 1859; Dobzhansky, 1937; Mayr, 1963; Schluter, 2000; Coyne and Orr, 2004). One prominent speciation model invokes the phenomenon now known as ecological speciation: adaptation of populations to different environments can cause the evolution of reproductive barriers that reduce gene flow (Schluter, 2000; McKinnon *et al.*, 2004; Nosil, 2012). Ecological speciation, although powerful, does not proceed unopposed. In particular, theory suggests that dispersal between populations can lead to gene flow that reduces the effectiveness of selection in adapting each population to its local environment (Lenormand, 2002; Bridle and Vines, 2006; Garant *et al.*, 2007; Räsänen and Hendry, 2008; Cristescu *et al.*, 2012). Fitting this expectation, numerous studies have shown that gene flow can constrain adaptive divergence among populations (Reichert, 1993; Sandoval, 1994; Hendry and Taylor, 2004; Nosil and Crespi, 2004; Moore *et al.*, 2007; Moore and Hendry, 2009), which can sometimes lead to strong maladaptation (Diaz and Blondel, 1996; Spitzer, 2006; Nosil *et al.*, 2009) and can place major limits on ecological speciation (Nosil, 2007; Berner *et al.*, 2009).

Phenotypic plasticity can modify the above interactions between dispersal, gene flow, adaptive divergence, and speciation (Crispo, 2008; Pfennig *et al.*, 2010; Fierst, 2011; Thibert-Plante and Hendry, 2011; Fitzpatrick, 2012) for at least three reasons. First, when dispersal is high, selection will often favour the evolution of trait plasticity (Sultan, 2003; Thibert-Plante and Hendry, 2011) because it allows individuals to adaptively adjust their phenotypes for the environment in which they find themselves, and because high gene flow makes adaptive divergence more difficult. Second, after adaptive plasticity evolves, it can hamper adaptive divergence – because phenotypes are brought closer to their local optima, which weakens divergent selection (Price *et al.*, 2003; Ghalambor *et al.*, 2007). Third, plasticity can alter the reproductive success of dispersers, which can thereby modify gene flow and hence influence divergence and speciation. In particular, plasticity that occurs before dispersal can reduce gene flow, whereas plasticity that occurs after dispersal can increase gene flow (Thibert-Plante and Hendry, 2011).

Our first goal in the present paper is to assess how dispersal that causes high gene flow can hamper local adaptation in nature. We do so through the common approach of asking whether populations subject to high gene flow from other habitats manifest trait values that deviate from those expected to be optimal (e.g. Reichert, 1993; Sandoval, 1994; Hendry and Taylor, 2004; Nosil and Crespi, 2004; Moore *et al.*, 2007). These earlier studies mostly focused on morphology, whereas here we focus on female maternal investment, which should be strongly related to fitness. We specifically compare two populations in similar habitats, one of which experiences high gene flow from a different habitat and the other of which does not. If gene flow is constraining adaptation, we expect female reproductive investment traits in the high gene flow population to differ from those in the low gene flow population, and to differ in a manner that suggests they are suboptimal. Our second goal is to assess the extent to which divergence in maternal investment among habitats is phenotypically plastic or genetically determined, thus informing the above roles that plasticity might play in divergence and speciation. We perform this analysis in threespine stickleback (*Gasterosteus aculeatus* L.) from lake and stream habitats.

STUDY SYSTEM AND SPECIFIC OBJECTIVES

The threespine stickleback species complex shows extensive phenotypic variation across its circumboreal range, making it a valuable model system in evolutionary ecology (Bell and

Foster, 1994; McKinnon and Rundle, 2002; Gibson, 2005; Ravinet *et al.*, 2013; Reimchen *et al.*, 2013). Specifically, colonization of a variety of freshwater habitats following the most recent glacial period led to the establishment of many new populations in which distinct phenotypes rapidly evolved (Bell and Aguirre, 2013). For life-history traits relevant to maternal investment, freshwater populations have nearly always evolved reduced clutch size and reduced reproductive effort compared with their marine ancestors (reviewed in Baker *et al.*, 2008). Beyond this generalization, considerable variation is present among freshwater populations in egg size, reproductive effort, fecundity, reproductive size and age, and allometries with female size (Baker, 1994; Baker *et al.*, 1998, 2008). In Cook Inlet, Alaska, for example, average egg size (dry mass) in freshwater populations varies among populations by at least 2.5-fold (Baker *et al.*, 2008). It has also been shown that maternal investment traits can evolve very rapidly (Baker *et al.*, 2011) and can differ between habitats within a single population (Baker *et al.*, 2005; Karve *et al.*, 2013). To date, however, the potential contribution of this variation to reproductive isolation during ecological speciation has not been considered.

Stickleback are useful for studying ecological speciation because populations can be found that represent almost every stage in the process (Bell and Foster, 1994; McKinnon and Rundle, 2002; Berner *et al.*, 2009; Hendry *et al.*, 2009). One particularly informative situation occurs when replicate population pairs are found in different habitats between which dispersal is still possible, such as in the case of parapatric lake–stream pairs (Lavin and McPhail, 1993; Aguirre, 2009; Berner *et al.*, 2009; Roesti *et al.*, 2012; Hendry *et al.*, 2013). Lake and stream habitats differ in a number of ecological attributes that impose divergent selection on stickleback (Moore and Hendry, 2005; Moore *et al.*, 2007; Berner *et al.*, 2008, 2009; Kaeuffer *et al.*, 2012). This selection has caused lake and stream stickleback to differ in a wide range of morphological traits associated with swimming performance, foraging mode, and predator avoidance (Moodie, 1972; Reimchen *et al.*, 1985; Taylor and McPhail, 1986; Lavin and McPhail, 1993; Walker, 1997; Hendry *et al.*, 2002, 2011; Berner *et al.*, 2009; Kaueffer *et al.*, 2012). And yet lake and stream pairs do not always differ strongly in these traits (Hendry and Taylor, 2004; Berner *et al.*, 2011; Kaeuffer *et al.*, 2012; Hendry *et al.*, 2013), at least partly because high gene flow between the habitats can severely hamper adaptive divergence (Hendry and Taylor, 2004; Moore *et al.*, 2007; Roesti *et al.*, 2012).

Our study focuses on stickleback populations in three habitats in the Misty Lake system, northern Vancouver Island, British Columbia, Canada. Misty Lake (50°36′32″N, 127°15′46″W) is a small (surface area = 35.6 ha) and shallow (mean depth = 1.7 m; maximum depth = 6.1 m) lake located in the Keogh River system. Two streams are connected to the lake: the inlet stream, which enters the lake at its southeastern end, and the outlet stream, which exits the lake at its northern end. The environments in both streams are divergent from the lake environment and selection should therefore favour adaptive divergence of both stream populations from the lake (Moore *et al.*, 2007; Berner *et al.*, 2008, 2009; Kaeuffer *et al.*, 2012). The adaptive divergence that is actually attained, however, is highly modified by gene flow (Lavin and McPhail, 1993; Hendry *et al.*, 2002; Moore and Hendry, 2005, 2009; Moore *et al.*, 2007; Roesti *et al.*, 2012). Gene flow is low between the lake and inlet populations, which are correspondingly highly divergent in adaptive traits. In contrast, gene flow is high from the lake to the outlet, and the outlet fish are correspondingly intermediate between typical lake and stream fish.

Previous work on adaptive divergence in the Misty system has focused on morphology, behaviour, and swimming performance, all of which are presumably tied to fitness. Here we consider three maternal investment traits (clutch mass, clutch size, and egg size) that are likely to be even more closely linked to fitness (Roff, 1992; Kingsolver *et al.*, 2001). First, we analyse data for wild-caught females to estimate variation within and between the three

habitats; hereafter referred to as lake, inlet, and outlet. Second, we combined data from wild-caught fish and those raised under common-garden conditions to explore the potential role of phenotypic plasticity in driving the observed patterns of variation. Our overall goal was to understand the role that life-history traits – and their plasticity – might play in promoting divergence between the stream and lake stickleback within the Misty Lake system.

METHODS

We studied the lake, three sites in the inlet stream, and five sites in the outlet stream. These sites are a subset of those sampled by Moore and Hendry (2005; see their Figure 1). All sites were at least 10 km from the ocean and no anadromous stickleback have ever been collected despite long-term study of this system. Both wild-caught (inlet, $n = 42$; lake, $n = 42$; outlet, $n = 27$) and laboratory-reared (inlet, $n = 28$; lake, $n = 48$; outlet, $n = 33$) fish were used in our study. For the field assessment of wild-caught fish, we captured fish in May–June using un-baited minnow traps soaked overnight. All captured fish were assessed for reproductive condition and only females that were conspicuously gravid were retained. Within 24 h of capture, all retained fish were killed by an overdose of tricaine methane sulphonate (MS-222), weighed to the nearest 0.01 g, and then immediately preserved in 10% buffered formalin. All preserved females were dissected to assess their reproductive stage as described previously (Heins and Baker, 1993; Baker *et al.*, 1998). Only females with fully developed, ovulated eggs were used in our study.

For the laboratory assessment of common-garden fish, we first collected females and males (as described above) from the lake, from inlet site 4, and from outlet site 4 (see the map in Moore and Hendry, 2005). Standard procedures were then used to generate artificial crosses between randomly selected males and females within each site. These crosses were the same as those described in our previous studies (Delcourt *et al.*, 2008; Sharpe *et al.*, 2008; Hendry *et al.*, 2011; Räsänen *et al.*, 2012). The fertilized embryos were shipped to McGill University and reared until about 18 months of age, at which time manipulation of the light/dark cycle was used to bring them into breeding condition. When a female was ready to spawn ['RE stage' – eggs fully developed and ovulated as described in Baker *et al.* (1998)], clutches were manually stripped from females, and both clutches and females were preserved in buffered 10% formalin.

Data for egg size (dry mass, g), clutch size (number of eggs), clutch mass (dry mass, g), and female somatic mass (blotted wet mass, g) were obtained following the methods described in Baker *et al.* (1998). Briefly, all developed eggs in a clutch were counted, and the eggs were dried for 24 h at 40°C. The entire dried clutch was weighed to 0.0001 g, and the mass of an individual egg was estimated by dividing dry clutch mass by clutch size. Thus, data for egg size are presented as dry egg masses. Females were thoroughly blotted to remove excess moisture, and weighed to the nearest 0.01 g, which provides highly repeatable measurements (J.A. Baker, personal observation). Several points attend these metrics. First, individual eggs were not weighed because precision is low and existing data suggest negligible within-clutch variation in stickleback (J.A. Baker, personal observation). Second, dry mass rather than wet mass was used for eggs because the former is more repeatable (J.A. Baker, personal observation), whereas wet mass rather than dry mass was used for the soma because the latter allows subsequent use of the body for other purposes. Overall, dry mass estimates are ~23% of 'wet' mass estimates for clutches of stickleback eggs (Baker and Foster, 2002). Third,

when scaled for body size, clutch mass is a commonly used indicator of the level of female reproductive 'effort' per clutch (Roff, 1992).

The data for wild-caught females were analysed for differences among the three habitats (inlet, lake, outlet) using analyses of covariance (ANCOVAs) with female somatic mass as the covariate. All data were $\log_{10}$-transformed so as to homogenize variances, improve normality, and achieve linearity. Each trait was analysed independently following confirmation (Levene and Shapiro-Wilks tests) that the assumptions of ANCOVA were met. Interactions between habitat and the covariate (i.e. slope inequality) were evaluated via *F*-tests within a preliminary ANCOVA. If a significant interaction was detected, the ANCOVA used the common within-groups slope (Reist, 1986). Subsequent to the above habitat-level analyses, which grouped all five outlet sites together, we computed site-level least squares means from the ANCOVAs to assess trait change with distance from the lake in the outlet. Although size-standardization (as above) is typical, reproductive output is a function of actual clutch size, not size-standardized clutch size. We therefore also analysed $\log_{10}$ clutch size for the wild-caught fish in a simple one-way analysis of variance (ANOVA; i.e. no covariate).

Plastic and genetic contributions to phenotypic divergence were evaluated by analysing wild-caught and laboratory-reared females together in a two-way, fixed-effects ANCOVA. The fixed effects were habitat (inlet, lake, outlet) and environment (field, laboratory). The interactions between these effects were also considered and female somatic mass was used as a covariate. As before, data were $\log_{10}$-transformed prior to analysis, assumptions of normality and variance equality were evaluated, and covariate interactions tested and removed from analyses.

RESULTS

Wild-caught females

Clutch mass was positively related to female mass in all three habitats, and log-log slopes of the relationships (inlet: $\beta = 0.873$, S.E. $= 0.123$; lake: $\beta = 0.811$, S.E. $= 0.102$; outlet: $\beta = 0.975$, S.E. $= 0.174$) did not differ significantly among habitats when assessed by ANCOVA ($F_{2,101} = 1.37$, $P = 0.27$). Females from the three habitats differed in clutch mass at a common body size ($F_{2,103} = 17.4$, $P < 0.001$) (Fig. 1A), with each habitat being distinct from all others (pair-wise comparisons via Tukey's HSD test: all $P < 0.001$). Specifically, inlet females had relatively heavier clutches than did lake females, which had the lightest clutches, and outlet females, which had intermediate clutch masses.

Clutch size patterns generally tracked clutch mass patterns, as was to be expected given the lack of differences in egg size among habitats (clutch mass is the product of egg size and clutch size). Clutch size was positively related to body size ($F_{1,107} = 52.88$, $P < 0.001$), and the relationships (inlet: $\beta = 0.757$, S.E. $= 0.148$; lake: $\beta = 0.729$, S.E. $= 0.131$; outlet: $\beta = 1.063$, S.E. $= 0.175$) did not differ significantly among habitats when analysed by ANCOVA ($F_{2,105} = 1.02$, $P = 0.37$). Clutch size at a common body size differed considerably among the three habitats ($F_{2,107} = 19.6$, $P < 0.001$), but not all habitats were statistically distinct from each other (Fig. 1B). In particular, inlet females had relatively larger clutches than either lake or outlet females ($P < 0.01$), but relative clutch sizes did not differ significantly between lake and outlet females ($P > 0.10$). Patterns for absolute clutch size (i.e. not adjusted for female size) closely mirrored those for relative fecundity ($F_{2,107} = 8.33$, $P < 0.001$), with inlet

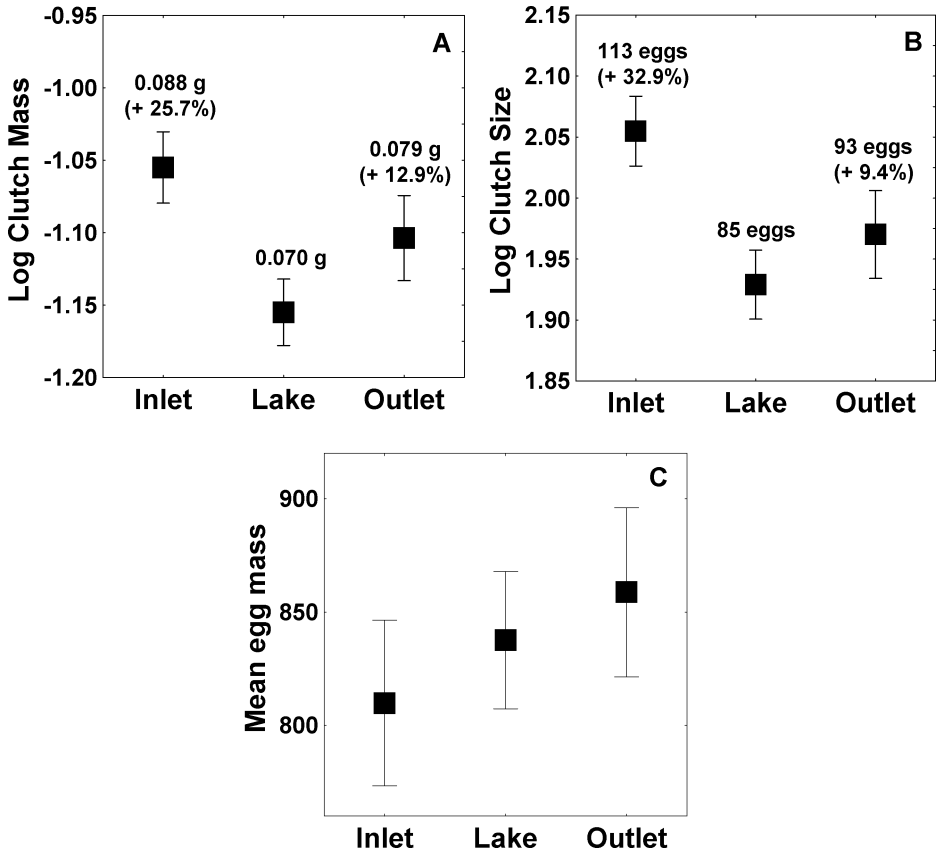

Fig. 1. Comparative life-history traits of wild-caught female threespine stickleback from three habitats within the Misty Lake system. Plot points indicate means and 95% confidence limits derived from ANCOVA, with values adjusted to a common female somatic mass ($\log_{10}$ mass = 0.279; 1.90 g). (A) Relative clutch masses (g). Values above each plot point indicate back-transformed clutch masses and the proportional difference relative to the clutch mass of lake females. (B) Relative clutch size. Values above each plot point indicate back-transformed clutch sizes and proportional difference relative to the clutch size of lake females. (C) Mean dry egg masses (μg).

females producing an average of 108 eggs, lake females 85 eggs, and outlet females 98 eggs (all pair-wise Tukey HSD tests were $P < 0.03$).

Egg mass was positively related to female mass ($F_{1,103} = 9.28$, $P = 0.003$), and this relationship was not significantly different among habitats ($F_{2,100} = 1.25$, $P = 0.25$). Despite statistical non-significance, visual inspection of the data suggested the overall relationship was driven primarily by inlet females. Habitat-specific slope estimates were $\beta = 0.208$ (inlet: S.E. = 0.066, $P = 0.002$), $\beta = 0.08$ (lake: S.E. = 0.076, $P = 0.288$), and $\beta = 0.040$ (outlet: S.E. = 0.107, $P > 0.50$). ANCOVA using a common slope showed no evidence for divergence in relative egg size among the habitats ($F_{2,103} = 0.67$, $P > 0.50$; Fig. 1C). ANOVA without a covariate produced the same conclusion (data not shown).

The mean relative clutch mass of outlet females increased with distance downstream from the lake ($\beta = 0.000043$, S.E. = 0.000013; $F_{1,67} = 10.85$, $P < 0.002$) (Fig. 2A). The same

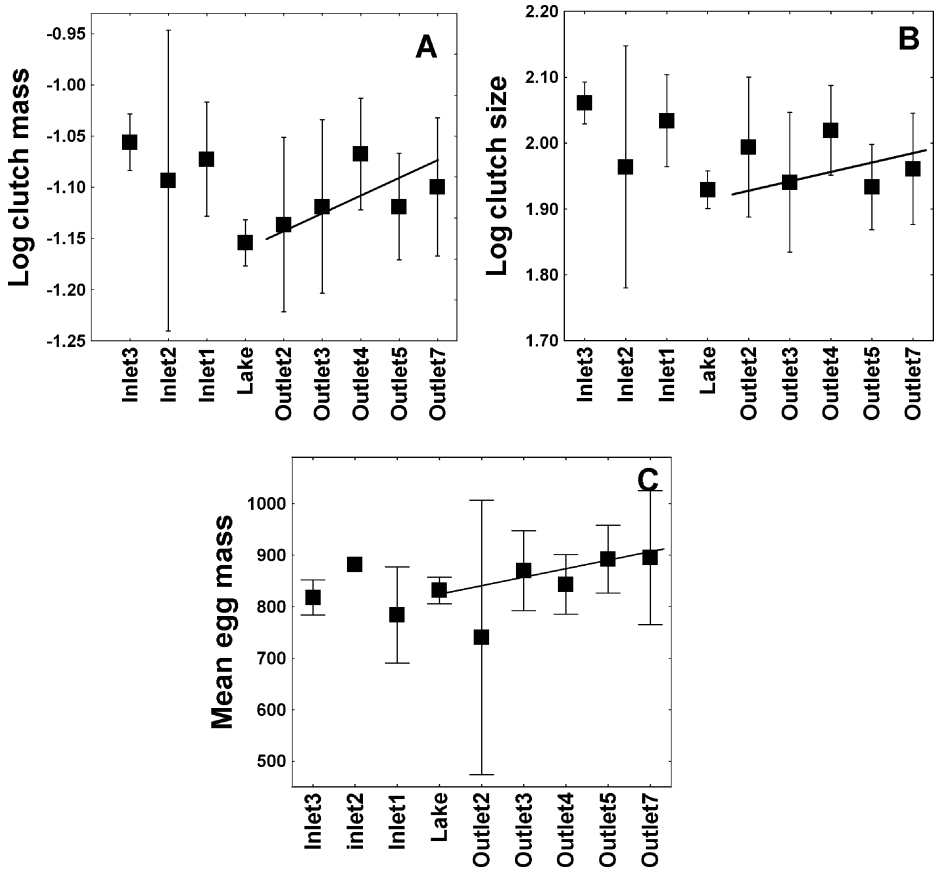

Fig. 2. Variation in clutch mass (A), clutch size (B), and egg mass (C) of wild-caught female threespine stickleback across collecting sites within the three habitats of the Misty Lake system. Values are adjusted to a common female size of 1.90 g somatic mass. Sites are arranged in order of increasing distance from the lake. Trend lines are shown for the outlet sites.

was true for mean relative clutch size ($\beta = 0.000048$, S.E. = 0.000017; $F_{1,67} = 7.729$, $P < 0.007$) (Fig. 2B). Part of the increase in clutch mass with distance from the lake might be due to a modest, although non-significant, trend towards increasing egg mass with distance ($\beta = 0.000013$, S.E. = 0.000010; $F_{1,67} = 1.641$, $P = 0.205$) (Fig. 2C).

The above trends might be influenced by variation in age composition. Although sample sizes are too small to be definitive, size-frequency plots (Fig. 3) indicated that most inlet females were age 1 (with some age 2), whereas most lake and outlet females were ages 2 and 3, with some perhaps age 4. After adjustment to a common female size, the estimated age-classes of females showed no differences in either clutch mass or clutch size. As shown above, egg size did increase with female size in the inlet, but at present it is unclear whether the relationship is due to size or age.

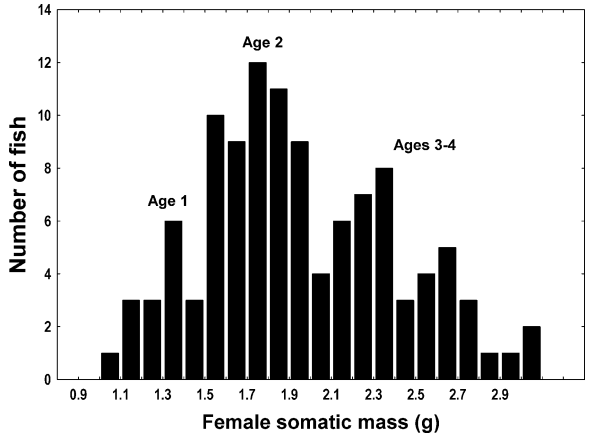

Fig. 3. Size-frequency of female stickleback dissected in this study to determine female investment characteristics. Suggested ages of each size mode are indicated.

Laboratory-reared females

ANCOVAs that included both wild-caught and laboratory-reared females indicated that plasticity contributed to clutch mass and clutch size but not egg size. Relative clutch mass was substantially lower in the laboratory than in the field for females from all three habitats ($F_{1,196} = 49.1$, $P < 0.001$) (Fig. 4A). A significant interaction ($F_{2,196} = 3.94$, $P = 0.021$) arose because the differences between habitats were much larger in the wild than in the laboratory. Nevertheless, some genetic differences between habitats were still suggested in that laboratory-reared inlet females had significantly larger relative clutch masses ($P < 0.05$) than laboratory-reared lake and outlet females, which did not differ from each other ($P > 0.25$). Results for relative clutch size were qualitatively similar to those for relative clutch mass (Fig. 4B) in that clutch sizes were smaller in the laboratory than in the wild ($F_{1,200} = 13.4$, $P = 0.0003$). However, no significant differences in clutch size were evident between laboratory-reared females from the three habitats (all pair-wise $P > 0.50$). Egg sizes for females from all three habitats were similar in the wild and in the laboratory (Fig. 4C) and no significant effects were detected (all $P > 0.25$).

DISCUSSION

For wild-caught females, two of the three aspects of maternal investment differed across habitats (inlet, lake, outlet), a result that mirrored previous studies of morphology (Hendry *et al.*, 2002; Moore and Hendry, 2005; Moore *et al.*, 2007; Sharpe *et al.*, 2008) and behaviour (Delcourt *et al.*, 2008). In particular, inlet females had higher reproductive effort (clutch mass) and larger clutches (more eggs) than did lake females, whereas outlet females were intermediate and closer in phenotype to lake females – especially so for outlet females near the lake. These patterns were evident both before and after standardization for body size. These parapatric inlet–lake differences match allopatric stream–lake differences documented for stickleback in Alaska (Baker *et al.*, 1998, 2008; Baker and Foster, 2002) and elsewhere (Moser *et al.*, 2012), suggesting that stream environments generally select for higher reproductive effort and more eggs than do lake environments.

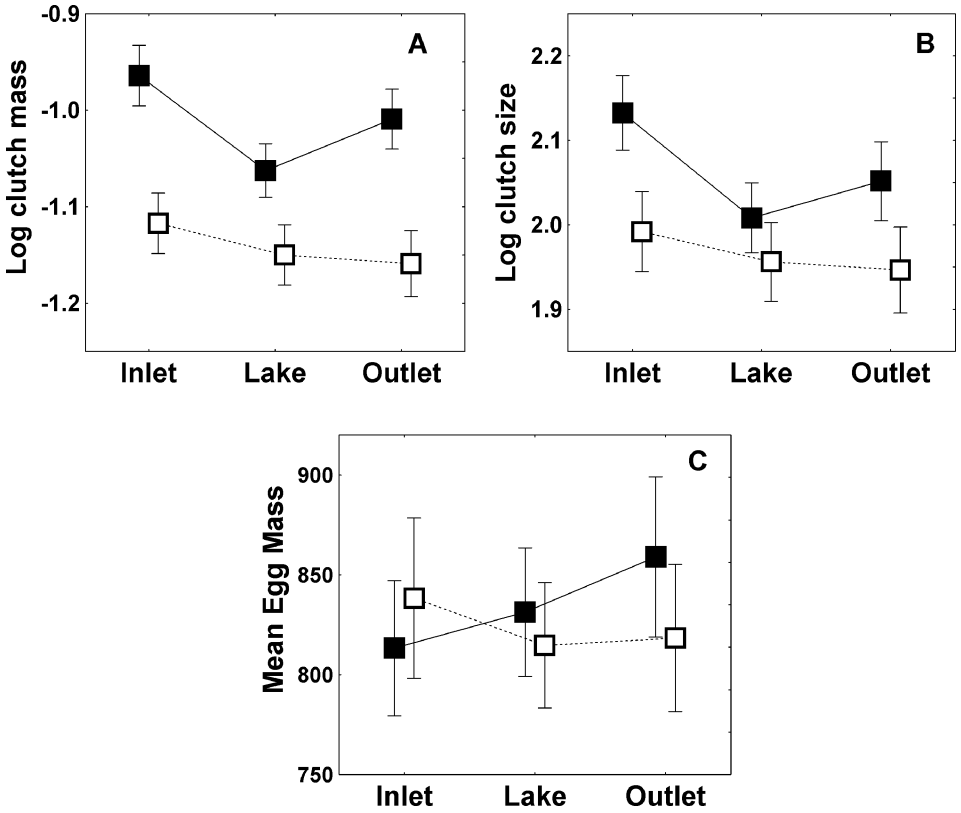

Fig. 4. Comparison of clutch mass (A), clutch size (B), and egg mass (C) for wild-caught and laboratory-reared females from three habitats within the Misty Lake system. Values are adjusted means and 95% confidence bounds from an ANCOVA using female somatic mass as the covariate (covariate mean size is 2.45 g). Solid squares indicate data for wild-caught fish; open squares indicate data for laboratory-reared fish. Note that the data for wild-caught females will differ slightly from Fig. 1 due to the least-squares means being estimated from a different overall set of females (includes laboratory-reared fish).

One possible explanation for higher reproductive effort and clutch size in streams is divergence in age at reproduction. According to one of the basic tenets of life-history theory, high reproductive effort is expected in populations in which the probability of survival to a second breeding season is low [high survival cost of reproduction or high natural mortality (Roff, 1992)]. Body size frequency distributions of stickleback in the three Misty habitats (Fig. 3) (J.-S. Moore, unpublished data) suggest that most breeding inlet fish are one-year-old individuals, with some age 2 breeders. In contrast, lake and outlet fish survive and breed over a greater range of ages, with fewer maturing at age 1. Similar results have been recorded for stream–lake contrasts in Europe (Moser *et al.*, 2012), suggesting that post-reproductive survival is lower in streams. Despite this repeatable *direction* of stream–lake divergence, the *magnitude* of divergence seems sometimes to be constrained by gene flow, as seen here in the intermediate life-history traits of outlet females, where gene flow from the lake is very high (Hendry *et al.*, 2002; Moore *et al.*, 2007; Roesti *et al.*, 2012).

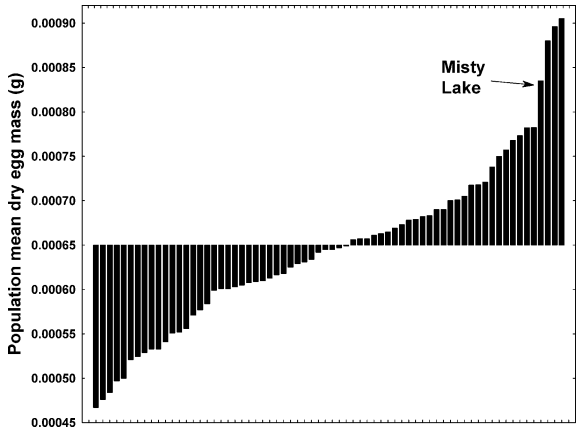

Fig. 5. The egg size of Misty system stickleback compared with 67 south-central Alaskan populations (updated from Baker *et al.*, 2008). The horizontal line at 0.000650 g dry mass indicates the egg size of oceanic stickleback in Alaska.

In contrast to clutch mass and clutch size, egg size did not differ substantially across habitats, a pattern also found in Europe (Moser *et al.*, 2012). In contrast to this egg size similarity among habitats, Misty stickleback differ from virtually all other measured stickleback populations in making comparatively enormous eggs (cf. Baker *et al.*, 1998, 2005, 2008; Baker and Foster, 2002; Heins and Baker, 2003) (Fig. 5). These patterns suggest the action of some selective factor favouring large offspring (Perez and Munch, 2010) in all Misty habitats. The alternative – constraints arising from a common ancestor with large eggs – is less likely because egg size in ancestral oceanic stickleback is consistently smaller than that seen in the Misty system (Baker, 1994; Baker *et al.*, 2008; R.W. King, unpublished data). Why selection favours such large eggs within the Misty system is not known. In one Alaskan lake with very large eggs, a rapid decrease in egg size was associated with an increase in lake productivity (Baker *et al.*, 2011), suggesting that fry size is sensitive to food supply, at least very early in life. However, in south-central Alaska generally, egg size variation across more than 75 populations shows no significant correlation with water chemistry, lake productivity, or predator regime (J.A. Baker, unpublished data). Similarly, a comparison of 43 Haida Gwaii populations that measured many of the same environmental variables (Oravec and Reimchen, 2013) found only pH to be a significant predictor of egg size, and that effect was quite weak. In contrast to morphological traits related to predator deterrence or feeding efficiency, it is possible that no single selective factor underlies the evolution of large eggs in stickleback.

Our combined analysis of wild-caught and laboratory-reared females revealed that both clutch mass and clutch size (but not egg size) show considerable phenotypic plasticity. First, clutch mass and clutch size were both lower in the laboratory than in the wild for females from all three habitats, a result consistent with comparisons of wild-caught and laboratory-raised stickleback from eight Alaskan populations (J.A. Baker, unpublished data). Second, differences among habitats were greater in the wild than in the laboratory, suggesting that plasticity enhances divergence between habitats in nature. Third, the magnitude of the difference between wild-caught and laboratory-reared females differed among the habitats, being greatest in the inlet and lowest in the lake. This result suggests that plasticity has evolved to be different between the habitats (e.g. Morin *et al.*,

1999; Torres-Dowdall *et al.*, 2012), although the reason (whether selective or not) remains to be elucidated.

Despite the above plastic effects, differences in reproductive effort between lake and inlet females persisted in the laboratory, indicating genetic divergence in this trait. Evidence of genetic divergence in reproductive effort in Misty system stickleback also matches findings from Alaskan populations (J.A. Baker, unpublished data), and it indicates that female life-history traits undergo evolutionary divergence between stickleback populations in different environments. Interestingly, the genetic and plastic effects were in the same direction, i.e. inlet females had genetically larger clutch masses and clutch sizes than did lake females and plasticity further increased this difference – the differences were larger for wild-caught fish than for laboratory-reared fish. This pattern indicates co-gradient variation, as opposed to counter-gradient variation (*sensu* Conover and Schlutz, 1995), and it suggests that plasticity might weaken selection for genetic divergence (Price *et al.*, 2003; Ghalambor *et al.*, 2007).

Implications

Across many types of traits, the Misty inlet–lake comparison indicates strong genetically based and putatively adaptive divergence in the absence of appreciable gene flow (Hendry *et al.*, 2002, 2011; Moore *et al.*, 2007; Sharpe *et al.*, 2008; Kaueffer *et al.*, 2012). By contrast, the Misty outlet–lake comparison indicates minor or non-existent genetically based divergence in the same traits in the presence of high gene flow (Moore *et al.*, 2007; Sharpe *et al.*, 2008; Berner *et al.*, 2009; Roesti *et al.*, 2012). We now consider what life-history traits might tell us about maladaptation and what role plasticity might play in adaptation and maladaptation.

Based on environmental data, we have previously argued that selection in the outlet – at least at a reasonable distance from the lake – should favour stickleback traits similar to those seen in the inlet (Moore and Hendry, 2005, 2009; Moore *et al.*, 2007). This conclusion was based on similarity in habitat characteristics, including water depth, stream width, and current speed (see Table 2 in Moore and Hendry, 2005). In addition, the diet of Misty outlet stickleback contains almost no zooplankton (Berner *et al.*, 2009) – just as in the inlet. Selection should therefore favour similar traits in the inlet and outlet, yet this is not the case: outlet fish are instead more lake-like. The implication is that outlet fish are maladapted and should suffer reduced fitness. Pooling data for the three outlet sites (4, 5, and 7) that have the most stream-like habitat, the estimated clutch size corrected for female body size is 93 eggs, compared with 85 eggs for lake females. This outlet–lake difference of only 8 eggs represents a 71% reduction in clutch size divergence relative to the inlet–lake difference of 20 eggs. Using only outlet site 4 (from which our laboratory-reared fish came), the corresponding reduction is 32%. Interestingly, the former value is similar to the estimate of migration load provided for morphology (Moore *et al.*, 2007).

Overall, then, the present data for female life-history traits provide additional evidence that gene flow can cause strong maladaptation in nature. Of course, this estimate is still only crude given that the fitness of female stickleback depends on many factors in addition to those we studied. These factors include the total number of clutches that a female produces in her lifetime (Fletcher and Wootton, 1995; Brown-Peterson and Heins, 2009; Wootton and Fletcher, 2009), the survival and/or fecundity cost of reproduction (Hutchings, 1993; Kuparinen *et al.*, 2011), and the relationship between egg size and offspring fitness (Dziminski *et al.*, 2009). We do not currently have estimates of how these factors influence maladaptation in the Misty outlet.

Plasticity could theoretically increase or decrease the negative influence of gene flow on adaptive divergence, and might thereby constrain or promote progress towards ecological speciation (Thibert-Plante and Hendry, 2011). Misty outlet and lake fish diverge more in an adaptive direction in nature than in the laboratory. This co-gradient effect of plasticity enhancing adaptive trait divergence could have both negative and positive consequences for the outlet population. On the positive side, plasticity that increases the reproductive output of dispersers will increase the total reproductive output of the outlet population within a generation. On the negative side, the same effect could decrease the total reproductive output of the outlet population across generations by increasing gene flow and hampering local adaptation. This last effect will favour plasticity over local adaptation (Sultan, 2003; Thibert-Plante and Hendry, 2011) and can reduce progress towards ecological speciation. Interestingly, this expectation matches what we see in the Misty system: outlet fish show high plasticity (Sharpe *et al.*, 2008; present study) and little or no progress towards ecological speciation (Berner *et al.*, 2009; Raeymaekers *et al.*, 2010; Räsänen *et al.*, 2012; Roesti *et al.*, 2012).

In summary, we find apparently complementary (co-gradient) genetic and plastic contributions to phenotypic divergence in reproductive effort (clutch mass) and fecundity (clutch size) between parapatric populations that exchange few genes (lake and inlet). At the same time, we find lower levels of divergence in these traits for parapatric populations that exchange many genes (lake and outlet). This latter situation likely means that dispersers from the lake into the outlet have lower fitness than residents in the outlet. However, plasticity appears to reduce this disadvantage faced by dispersers and thus enhances gene flow and presumably further limits adaptive divergence. Plasticity here seems likely to place constraints on progress towards ecological speciation.

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