b i o l o g y
letters

Body size differences do not arise from divergent mate preferences in a species pair of threespine stickleback

Megan L. Head, Emily A. Price and Janette W. Boughman

Supplementary data	"Data Supplement" http://rsbl.royalsocietypublishing.org/content/suppl/2009/05/20/rsbl.2009.0216.DC1.html
References	**This article cites 22 articles, 5 of which can be accessed free** http://rsbl.royalsocietypublishing.org/content/early/2009/05/22/rsbl.2009.0216.full.html#ref-list-1
P<P	Published online 27 May 2009 in advance of the print journal.
Subject collections	Articles on similar topics can be found in the following collections behaviour (508 articles) ecology (623 articles) evolution (724 articles)
Email alerting service	Receive free email alerts when new articles cite this article - sign up in the box at the top right-hand corner of the article or click **here**

Advance online articles have been peer reviewed and accepted for publication but have not yet appeared in the paper journal (edited, typeset versions may be posted when available prior to final publication). Advance online articles are citable and establish publication priority; they are indexed by PubMed from initial publication. Citations to Advance online articles must include the digital object identifier (DOIs) and date of initial publication.

To subscribe to *Biol. Lett.* go to: **http://rsbl.royalsocietypublishing.org/subscriptions**

Body size differences do not arise from divergent mate preferences in a species pair of threespine stickleback

Megan L Head[†,*]**, Emily A. Price**[‡]
and Janette W. Boughman

Department of Zoology, University of Wisconsin, Madison, WI 53706, USA
Author for correspondence (mlh21@le.ac.uk).
[†]*Current address: Department of Biology, University of Leicester, Leicester LE1 7RH, UK*
[‡]*Current address: Department of Environment and Society and the Ecology Center, Utah State University, Logan, UT 84322, USA*

Ecological speciation can be driven by divergent natural and/or sexual selection. The relative contribution of these processes to species divergence, however, is unknown. Here, we investigate how sexual selection in the form of male and female mate preferences contributes to divergence of body size. This trait is known be under divergent natural selection and also contributes to sexual isolation in species pairs of threespine sticklebacks (*Gasterosteus aculeatus*). We show that neither female nor male size preferences contribute to body size divergence in this species pair, suggesting that size-based sexual isolation arises primarily through natural selection.

1. INTRODUCTION

Ecological speciation occurs when divergent selection reduces gene flow between populations that exploit different ecological niches (Schluter 2000, Rundle & Nosil 2005). Natural selection can drive ecological speciation when adaptation to different environments alters traits also used in mate recognition, leading to assortative mating among populations (Schluter 2000). Alternatively, sexual selection can cause divergence when different environments alter sexual selection on male sexual displays and/or female preferences for these displays (Endler 1992, Boughman 2002).

While it is clear that both natural and sexual selection play a role in ecological speciation, their relative importance remains a major outstanding question in speciation research (Nosil *et al.* 2007). They may interact to determine the direction and magnitude of evolutionary response in reproductive isolation (Blows 2002), and sexual selection may be most effective when operating alongside natural selection (Ritchie 2007). Most studies investigating how sexual selection causes speciation have focused on secondary sexual traits where natural selection can be excluded as

a primary cause of divergence. However, we need to explore how natural and sexual selection operate on the same traits to produce reproductive isolation, to fully understand this process.

Species divergence in body size can result from natural selection due to differences in predator regime (Nosil & Crespi 2006), foraging resources (Schluter 1993) or parasites (Maccoll 2009). However, sexual selection may also influence the evolution of body size (Andersson 1994). Body size is often an important criterion in mate choice, and can influence success during intrasexual mate competition (Hunt *et al.* 2009). Thus, both sexual and natural selection may be important in driving species divergence in body size, and the resulting size divergence can lead to sexual isolation.

We examine the role of mate choice in body size divergence in a species pair of threespine stickleback (*Gasterosteus aculeatus*). Limnetic and benthic sticklebacks co-occur in a number of lakes throughout British Columbia (McPhail 1994). Both natural and sexual selection have been implicated in the evolution of sexual isolation between these species pairs (Rundle *et al.* 2000; Boughman 2001). Natural selection for improved feeding efficiency has led to divergence in body size and shape. Limnetics have small, slender bodies suited to foraging in open water, and benthics have large deep bodies suited to foraging on prey attached to the sediment or vegetation (Schluter 1993). Body size differences between species are important to reproductive isolation; the probability of hybridization is higher between similar-sized individuals (Nagel & Schluter 1998; Boughman *et al.* 2005). This pattern is also seen in stream-anadromous species pairs of sticklebacks (McKinnon *et al.* 2004), indicating the importance of body size in maintaining reproductive isolation throughout the stickleback species complex. However, whether sexual selection contributes to size-based sexual isolation is unknown. Variation in body size preference among threespine stickleback populations exists with some populations showing no size preference (Kraak *et al.* 1999; Baube 2008). Such differences in size-based mate choice between species could cause both divergent selection on body size and size-based sexual isolation. Here, we ask whether limnetic and benthic sticklebacks differ in male or female size preferences. Because benthics are larger than limnetics, we expect benthics to prefer larger mates and limnetics to prefer smaller mates. We infer the direction of evolutionary change by including the anadromous ancestors.

2. MATERIAL AND METHODS

Size preferences of limnetic, benthic and anadromous sticklebacks were assessed in no-choice mating trials. No-choice trials are standard for measuring mating preferences in sticklebacks (Nagel & Schluter 1998) and are useful for assessing the strength of preferences (Wagner 1998). For details on experimental setup, see the electronic supplementary material.

Courtship behaviours recorded included male zig-zagging, biting and leading and female following (described in Rowland 1994). We calculated female preference as the proportion of male leads that a female followed to account for variation in male courtship behaviour. For male courtship intensity, we calculated the rate of zig-zagging and biting per second. To estimate the probability of spawning, we recorded whether the female entered the male's nest.

Standard length of males and females was measured with vernier calipers accurate to 0.2 mm. Males were measured before and after
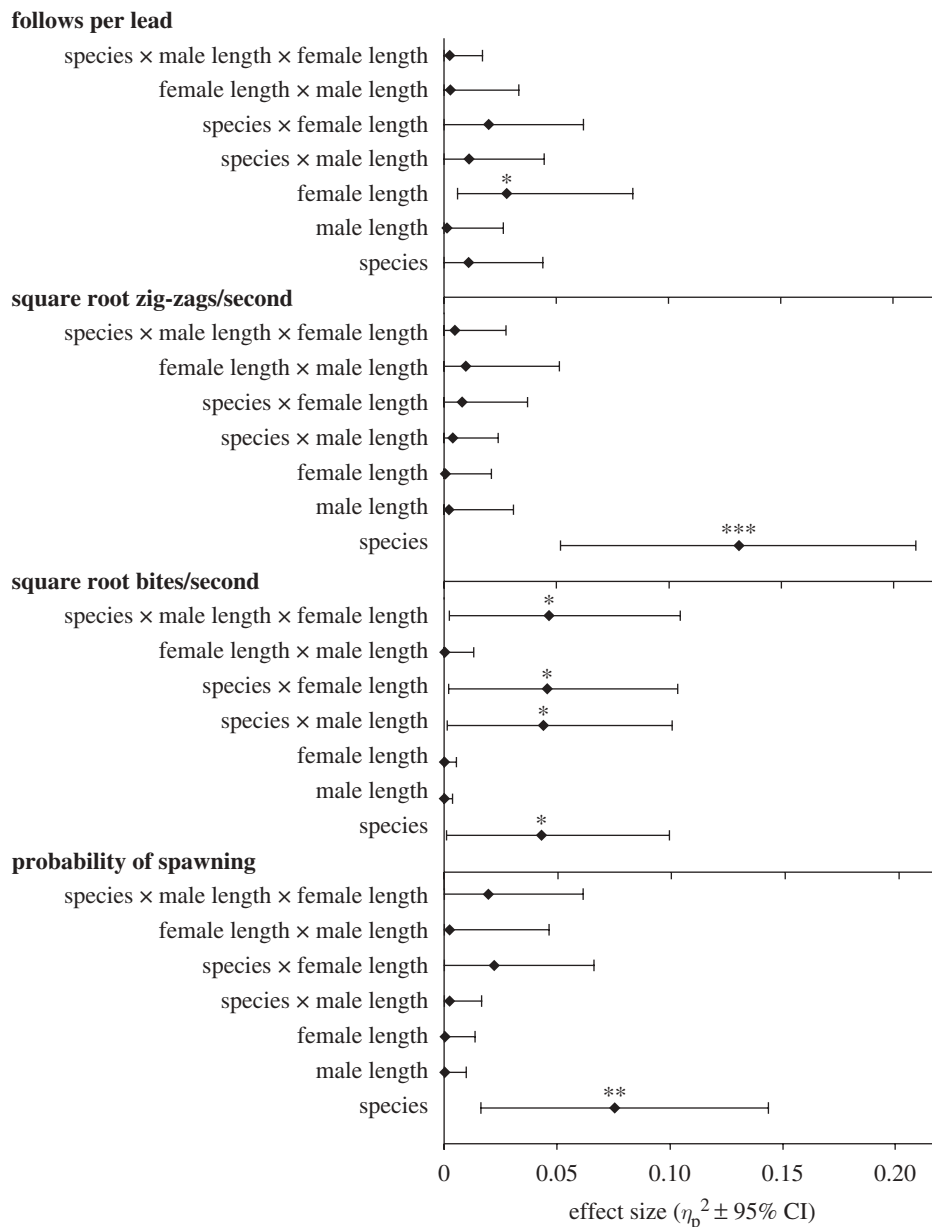
Figure 1. Effect sizes and 95 per cent confidence intervals for the relationship between all model terms and female preference for males (follows per lead), male courtship of females (rate of zig-zags and bites) and overall mating success (probability of spawning). $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

behavioural trials and measurements were averaged for analyses. Females were measured after trials.

(a) *Data analysis*

We measured courtship, preferences and mating success of 60 male and female pairs from each population, but excluded trials where females or males were unresponsive. Consequently, we obtained a sample size of 56 anadromous, 45 benthic and 48 limnetic trials. We used only one observation for each fish.

All analyses were performed using SPSS 16.0. Male and female mate preferences were square root transformed when appropriate to ensure normality and analysed using ANCOVA. The probability of spawning was analysed using logistic regression; we estimated effect sizes in the same manner as for other variables (see below). We investigated the effects of species, male length, female length and all interaction terms on male and female mate preferences and on spawning probability. For all models, non-significant terms were sequentially removed until only significant sources of variation remained. Divergent sexual selection based on differences in male and female size preferences would be evident as an interaction between the species and the size of the chosen fish. To examine mate preferences within species when such interactions exist, we visualize data using thin-plate splines and conduct separate regressions for each species.

To determine effect sizes, we calculated partial eta squared and its confidence intervals ($\eta_p^2 \pm 95\%$ CI) using ESCI (http://psychology.anu.edu.au/people/smithson/details/CIstuff/CI.html) for non-significant and significant terms in the models. Effect sizes were calculated from F values either when the term was removed from the model (non-significant terms) or from the final model (significant terms).

3. RESULTS

Female response to males did not depend on male size for any species. The probability of a female following a male did depend on her own size (figure 1), with larger females more likely to follow males. However, this effect accounted for less than 3 per cent of the total variability in female follows per lead ($\eta_p^2 = 0.0278$).

Male zig-zag courtship did not depend on either male or female size (figure 1) despite differences in the overall rate of zig-zagging between species (figure 1). Limnetic and benthic males zig-zagged at a

higher rate (untransformed mean $\pm$ s.e.: limnetic $=$ 0.079 $\pm$ 0.014; benthic $=$ 0.043 $\pm$ 0.007) than anadromous males (0.025 $\pm$ 0.004) (Tukey's test: limnetic versus benthic $p = 0.091$, limnetic versus anadromous $p < 0.001$ benthic versus anadromous $p < 0.000$).

Male biting courtship showed a significant three-way interaction between species, female length and male length (figures 1 and 2). This effect is due to a weak but significant interaction between male and female length for limnetics ($\beta = -0.002$, t $= -2.182$, $p = 0.035$), but not for benthic or anadromous sticklebacks (benthic: $\beta = -0.000$, t $= -0.237$, $p = 0.814$; anadromous: $\beta = 0.002$, t $= 1.123$, $p = 0.266$).

Despite size-related differences in male and female courtship behaviour, the probability of spawning was not dependent on male length, female length or their interaction. Spawning probability did, however, depend on species (figure 1); anadromous pairs spawned more often (50%) than benthics (20%) or limnetics (27%) (logistic regression: anadromous versus benthic $p = 0.003$, anadromous versus limnetic $p = 0.019$, benthic versus limnetic $p = 0.423$).

4. DISCUSSION

Benthic and limnetic sticklebacks differ markedly in size and this size difference is critical to sexual isolation between species (Nagel & Schluter 1998; Boughman *et al.* 2005). We find no evidence for size-based assortative mating within species, indicating that mate preferences within species are not responsible for size-based sexual isolation.

Neither benthic nor limnetic females showed size-based mating preferences. The strongest size effect was increased male biting with increased female size, which was especially pronounced for small limnetic males. However, this is unlikely to contribute to divergence in body size. First, this effect is too small to explain large size differences between species. Second, previous research has shown that males tailor their courtship to the species of female. Limnetic males court benthic females more aggressively than limnetic females (Kozak *et al.* 2009). Our results show that this adjustment in male behaviour extends to limnetic females that are in the size range of benthic females. This could be caused by mate recognition errors or by males tailoring courtship towards female display preferences. Either way, spawning probability does not depend on either male or female size, indicating that mate preferences do not drive size divergence between species.

This contrasts with earlier findings on another key contributor to sexual isolation in this system: male breeding coloration. Sexual selection has caused divergence in male breeding coloration due to varying conspicuousness of this trait in different light environments, and limnetic and benthic females differ in colour preference (Boughman 2001). The correlation between preference strength and male colour suggests that divergent colour preferences drive differences in male colour between species, and differences in both preference and colour cause sexual isolation (Boughman 2001). It appears that within species mate preferences are integral to colour-based sexual isolation (Boughman *et al.* 2005). Thus, sexual
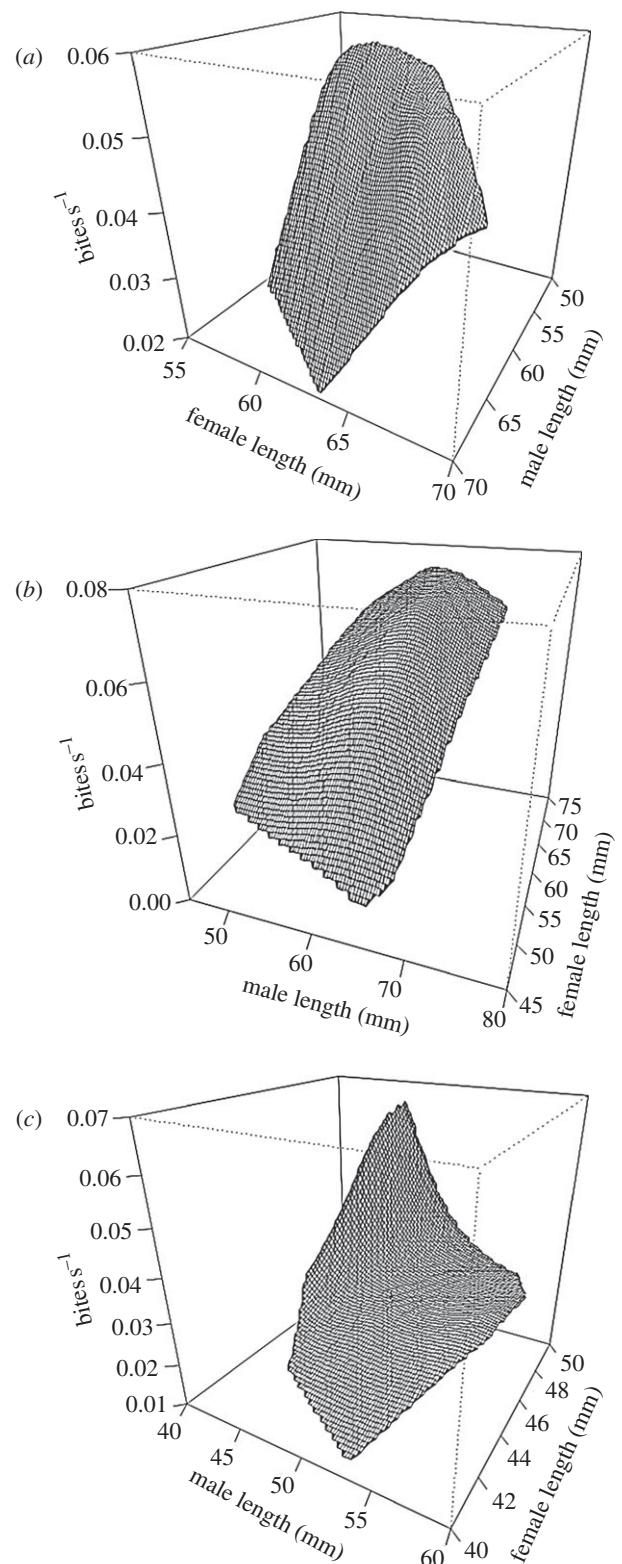

Figure 2. Thin-plate splines showing the relationship between male length, female length and male bite rate for (*a*) anadromous, (*b*) benthic and (*c*) limnetic sticklebacks. Thin-plate splines were obtained using a smoothing parameter $\lambda = 0.1$. Note different scales and orientation of axes.

selection plays a different role in colour divergence from that in size divergence.

While we can rule out divergent size preferences, sexual selection may contribute to size differences and size-based sexual isolation through two other mechanisms: male–male competition or mate preferences for a correlated

trait. Male–male competition may play a role in speciation (Seehausen & Schluter 2004), but is often overlooked. Body size has been shown to influence success in male–male competition in many taxa (Hunt *et al.* 2009) including sticklebacks (Kraak *et al.* 1999). Differences between benthic and limnetic breeding habitats (McPhail 1994) may influence the intensity of male–male competition; limnetics are known to be more aggressive towards rivals than benthics (Ridgway & McPhail 1987). Thus, differences in male–male competition could exert divergent selection on body size in the two species.

Mating preferences may, however, influence body size divergence if they act on correlated traits. A likely correlated trait is body shape. Shape differences between these species are well established (McPhail 1994) and could provide a reliable cue to species identity. Whether body shape is used in mate choice is unknown, but is worth investigating, especially given theoretical predictions that preferences for locally adapted mates should evolve and contribute to sexual isolation (Servedio 2004).

Theoretical predictions and empirical observations highlight the interplay between sexual and natural selection and their dual roles in the evolution of sexual isolation. It appears natural selection is key in determining body size divergence in this threespine stickleback species pair. However, we need more work on male–male competition and preferences for correlated traits before sexual selection can be completely ruled out. An interesting avenue for further work is why natural and sexual selection act so differently on the two primary traits that isolate these species.

Andersson, M. (ed.) 1994 *Sexual selection*. Princeton, NJ: Princeton University Press.

Baube, C. L. 2008 Body size and the maintenance of reproductive isolation in stickleback, *Gasterosteus*. *Ethology* **114**, 1122–1134. (doi:10.1111/j.1439-0310.2008.01564.x)

Blows, M. W. 2002 Interaction between natural and sexual selection during the evolution of mate recognition. *Proc. R. Soc. Lond. B.* **269**, 1113–1118. (doi:10.1098/rspb.2002.2002)

Boughman, J. W. 2001 Divergent sexual selection enhances reproductive isolation in sticklebacks. *Nature* **411**, 944–948. (doi:10.1038/35082064)

Boughman, J. W. 2002 How sensory drive can promote speciation. *Trends Ecol. Evol.* **17**, 571–577. (doi:10.1016/S0169-5347(02)02595-8)

Boughman, J. W., Rundle, H. D. & Schluter, D. 2005 Parallel evolution of sexual isolation in sticklebacks. *Evolution* **59**, 361–373.

Endler, J. A. 1992 Signals, signal conditions and the direction of evolution. *Am. Nat.* **139**, S125–S153. (doi:10.1086/285308)

Hunt, J., Breuker, C. J., Sadowski, J. A. & Moore, A. J. 2009 Male–male competition, female mate choice and their interaction: determining total sexual selection. *J. Evol. Biol.* **22**, 13–26. (doi:10.1111/j.1420-9101.2008.01633.x)

Kozak, G. M., Reisland, M. & Boughman, J. W. 2009 Sex differences in mate recognition and conspecific preference in species with mutual mate choice. *Evolution* **63**, 353–365. (doi:10.1111/j.1558-5646.2008.00564.x)

Kraak, S. B. M., Bakker, T. C. M. & Mundwiler, B. 1999 Sexual selection in sticklebacks in the field: correlates of reproductive, mating, and paternal success. *Behav. Ecol.* **10**, 696–706. (doi:10.1093/beheco/10.6.696)

Maccoll, A. D. C. 2009 Parasites may contribute to 'magic trait' evolution in the adaptive radiation of three-spined sticklebacks, *Gasterosteus aculeatus* (Gasterosteiformes: Gasterosteidae). *Biol. J. Linn. Soc.* **96**, 425–433. (doi:10.1111/j.1095-8312.2008.01123.x)

McKinnon, J. S., Mori, S., Blackman, B. K., David, L., Kingsley, D. M., Jamieson, L., Chou, J. & Schluter, D. 2004 Evidence of ecology's role in speciation. *Nature* **429**, 294–298. (doi:10.1038/nature02556)

McPhail, J. D. 1994 Speciation and the evolution of reproductive isolation in sticklebacks (Gasterosteus) of south-western British Columbia. In *Evolutionary biology of the threespine stickleback*. (eds M. A. Bell & S. A. Foster). Oxford, UK: Oxford University Press.

Nagel, L. & Schluter, D. 1998 Body size, natural selection, and speciation in sticklebacks. *Evolution* **52**, 209–218. (doi:10.2307/2410936)

Nosil, P. & Crespi, B. J. 2006 Experimental evidence that predation promotes divergence in adaptive radiation. *Proc. Natl Acad. Sci. USA* **103**, 9090–9095. (doi:10.1073/pnas.0601575103)

Nosil, P., Crespi, B. J., Gries, R. & Gries, G. 2007 Natural selection and divergence in mate preference during speciation. *Genetica.* **129**, 309–327. (doi:10.1007/s10709-006-0013-6)

Ridgway, M. S. & McPhail, J. D. 1987 Rival male effects on courtship behavior in the Enos lake species pair of sticklebacks (*Gasterosteus*). *Can. J. Zool.* **65**, 1951–1955. (doi:10.1139/z87-297)

Ritchie, M. G. 2007 Sexual selection and speciation. *Annu. Rev. Ecol. Evol. Syst.* **38**, 79–102. (doi:10.1146/annurev.ecolsys.38.091206.095733)

Rowland, W. J. 1994 Proximate determinants of stickleback behaviour. In *The evolutionary biology of the threespine stickleback*. (eds M. A. Bell & S. A. Foster). Oxford, UK: Oxford University Press.

Rundle, H. D. & Nosil, P. 2005 Ecological speciation. *Ecol. Lett.* **8**, 336–352. (doi:10.1111/j.1461-0248.2004.00715.x)

Rundle, H. D., Nagel, L., Boughman, J. W. & Schluter, D. 2000 Natural selection and parallel speciation in sympatric sticklebacks. *Science* **287**, 306–308. (doi:10.1126/science.287.5451.306)

Schluter, D. 1993 Adaptive radiation in sticklebacks—size, shape, and habitat use efficiency. *Ecology* **74**, 699–709. (doi:10.2307/1940797)

Schluter, D. 2000 *The ecology of adaptive radiation*. Oxford, UK: Oxford University Press.

Seehausen, O. & Schluter, D. 2004 Male–male competition and nuptial-colour displacement as a diversifying force in Lake Victoria cichlid fishes. *Proc. R. Soc. Lond. B* **271**, 1345–1353. (doi:10.1098/rspb.2004.2737)

Servedio, M. R. 2004 The evolution of premating isolation: local adaptation and natural and sexual selection against hybrids. *Evolution* **58**, 913–924.

Wagner, W. E. 1998 Measuring female mating preferences. *Anim. Behav.* **55**, 1029–1042. (doi:10.1006/anbe.1997.0635)