



## Research

**Cite this article:** Schrader M, Fuller RC, Travis J. 2013 Differences in offspring size predict the direction of isolation asymmetry between populations of a placental fish. *Biol Lett* 9: 20130327.  
<http://dx.doi.org/10.1098/rsbl.2013.0327>

Received: 11 April 2013

Accepted: 1 July 2013

### Subject Areas:

evolution, ecology

### Keywords:

reproductive isolation, isolation asymmetry, offspring size, coadaptation

### Author for correspondence:

Matthew Schrader

e-mail: mschrade@illinois.edu

## Evolutionary biology

# Differences in offspring size predict the direction of isolation asymmetry between populations of a placental fish

Matthew Schrader<sup>1,2</sup>, Rebecca C. Fuller<sup>2</sup> and Joseph Travis<sup>1</sup>

<sup>1</sup>Department of Biological Science, Florida State University, Tallahassee, FL 32306-1100, USA

<sup>2</sup>Department of Animal Biology, University of Illinois, Urbana, IL 61801, USA

Crosses between populations or species often display an asymmetry in the fitness of reciprocal  $F_1$  hybrids. This pattern, referred to as isolation asymmetry or Darwin's Corollary to Haldane's Rule, has been observed in taxa from plants to vertebrates, yet we still know little about which factors determine its magnitude and direction. Here, we show that differences in offspring size predict the direction of isolation asymmetry observed in crosses between populations of a placental fish, *Heterandria formosa*. In crosses between populations with differences in offspring size, high rates of hybrid inviability occur only when the mother is from a population characterized by small offspring. Crosses between populations that display similarly sized offspring, whether large or small, do not result in high levels of hybrid inviability in either direction. We suggest this asymmetric pattern of reproductive isolation is due to a disruption of parent-offspring coadaptation that emerges from selection for differently sized offspring in different populations.

## 1. Introduction

Crosses between populations or species often result in hybrid progeny with reduced fitness. Such post-zygotic reproductive isolation plays an important role in speciation, because it prevents gene flow between parental populations that are diverging genetically [1]. In many cases of hybridization, there is an asymmetry in the fitness of reciprocal  $F_1$  hybrid crosses [2–4]. This asymmetry has been called isolation asymmetry or Darwin's Corollary to Haldane's Rule [4]. The pattern cannot be explained by Dobzhansky–Muller incompatibilities (DMIs) between autosomal loci because reciprocal hybrids have the same autosomal genotype [4]. Instead, isolation asymmetry is probably due to DMIs involving uniparentally inherited factors or interactions between the maternal and hybrid progeny's genomes [4].

Isolation asymmetry has been observed in taxa from plants to vertebrates [2,3]. However, we know little about which factors determine its magnitude and direction. Turelli & Moyle [4] suggested that the direction of isolation asymmetry (i.e. which direction of the reciprocal hybrid crosses (RHCs) has the lowest fitness) might vary predictably with the relative rates of mitochondrial and nuclear evolution in the parental species. If species differ in these rates, then the crosses with the lower rate of offspring viability would be those in which the maternal parent was from the species with the higher relative rate of mitochondrial evolution [2,4]. This prediction is met in centrarchid fish [2]. However, it is also true that in RHCs between centrarchid species, offspring viability is lower when the maternal parent is from the species with the smaller body size [2].

This result suggests that the direction of asymmetry might be predictable from a pattern of adaptive differentiation in an organismal feature in addition to the rate at which different genomes accumulate nucleotide substitutions [2]. In two previous studies, we observed isolation asymmetry in RHCs between populations of least killifish (*Heterandria formosa*) that display differences in

size at birth [5,6]. In both of these studies, crosses between a female from a population characterized by small offspring (SO populations) and a male from a population characterized by large offspring (LO populations) had a higher rate of aborted embryos than the reciprocal hybrid cross. The congruence between these two studies suggests that isolation asymmetry in *H. formosa* may have a systematic basis: in crosses between populations with differences in offspring size, LO females are the better maternal parent. However, these previous studies did not involve hybrid crosses between populations with similarly sized offspring. Such crosses control for the effects of hybridizing genetically distinct populations independent of differences in offspring size and are needed to rigorously test whether the direction of isolation asymmetry is predictable from the typical offspring size of each parental population.

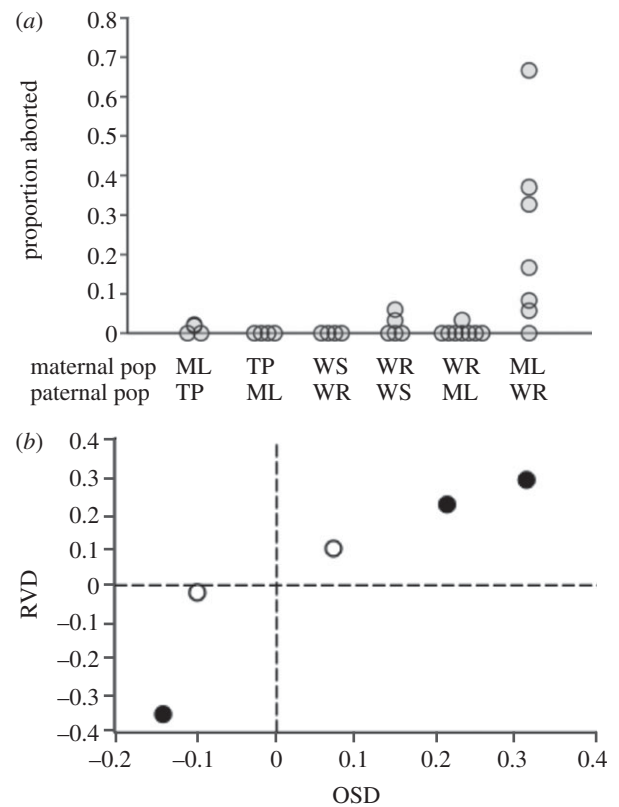
In this study, we conducted RHCs between individuals descended from two LO populations (Wacissa River (WR) and Wakulla Springs (WS)) and two SO populations (Moore Lake (ML) and Trout Pond (TP)). We also conducted RHCs between an SO population and an LO population that have not been previously hybridized (ML and WR). The results of these new crosses and those of our previous studies indicate that the direction of isolation asymmetry between *H. formosa* populations has a systematic basis: in crosses between populations with differences in offspring size, LO females are the better maternal parent.

## 2. Material and methods

*Heterandria formosa* is a poeciliid fish native to the southeastern United States. Females provision embryos between fertilization and birth via a placenta and give birth to fully developed, independent young. We conducted crosses between laboratory-born males and females descended from four *H. formosa* populations: ML, TP, WR and WS. Offspring from WR and WS are about 40% larger than those from ML and TP [6–9]. Previous studies indicate that offspring size in this species is determined by a combination of maternal effects and offspring genotype [6] and that differences between populations in offspring size have a genetic basis [6,10].

We conducted RHCs between TP and ML (TP<sub>female</sub> × ML<sub>male</sub>,  $n = 4$ ; ML<sub>female</sub> × TP<sub>male</sub>,  $n = 4$ ), WS and WR (WS<sub>female</sub> × WR<sub>male</sub>,  $n = 4$ ; WR<sub>female</sub> × WS<sub>male</sub>,  $n = 5$ ), and ML and WR (ML<sub>female</sub> × WR<sub>male</sub>,  $n = 8$ ; WR<sub>female</sub> × ML<sub>male</sub>,  $n = 8$ ). For each replicate cross, we paired a single virgin female with a single mature male in an 81 aquarium and measured offspring production for 60 days beginning with the birth of the first brood. For each cross, we recorded the total number of offspring produced and the number of these that were aborted embryos (data deposited in Dryad: doi:10.5061/dryad.277r4). Aborted embryos were born at a mid to late gestational stage and were not viable [5,6,10]. We compared the proportion of offspring that were aborted between each pair of RHCs using generalized linear models with a logit link and quasi-binomial error structure.

We also combined long-term data on offspring size and estimates of the viability of embryos from RHCs to test for a systematic basis in the direction of isolation asymmetry. This dataset consisted of data from five sets of RHCs involving four *H. formosa* populations (the three crosses described above and those described in two previous studies [5,6]) and long-term field data on offspring size from these populations [9]. We tested for a systematic bias in the direction of isolation asymmetry in a similar manner as that used by Bolnick *et al.* [2]. First, for each pair of RHCs, we arbitrarily assigned one population as A



**Figure 1.** (a) The proportion of offspring aborted in reciprocal hybrid crosses involving Moore Lake (ML), Trout Pond (TP), Wacissa River (WR), and Wakulla Springs (WS). Each data point represents the proportion of offspring aborted in a single replicate cross. (b) The relationship between differences in offspring size (offspring size difference, OSD) and the degree of asymmetry in F1 viability (relative viability difference, RVD) in crosses between *H. formosa* populations. Open circles represent crosses between populations with similarly sized offspring. Closed circles represent crosses between populations with differently sized offspring.

and one as B. We then calculated the average viability ( $V$ ) of offspring from each direction of the RHCs and the relative viability difference (RVD) between the RHCs. RVD was calculated as:

$$RVD = \frac{V_{AXB} - V_{BXA}}{\max(V_{AXB}, V_{BXA})}$$

We then calculated the difference between populations in the average dry-mass of offspring (offspring size difference, OSD), assigning populations as either A or B to match the assignments for the calculation of RVD. Data on offspring size were taken from [9]. Using this dataset, we tested whether RVD was correlated with OSD.

Populations that differ in offspring size may exhibit greater genetic divergence than populations with similarly sized offspring and any association between isolation asymmetry and differences in offspring size may be an outcome of hybridizing more genetically distinct populations. We examined this possibility by testing whether  $F_{ST}$  values between each pair of populations was correlated with OSD. We estimated  $F_{ST}$  values for each pair of populations using microsatellite genotypes for 35–74 females per population. These females were genotyped at three microsatellite loci as part of a previous study [11], and  $F_{ST}$  values were calculated using GENEPOP on the web.

## 3. Results

Crosses between LO populations (WR and WS) resulted in few aborted embryos, and there was no evidence that the

**Table 1.** Data from reciprocal hybrid crosses between *H. formosa* populations. Average viability is the average proportion of full term offspring for a given cross type. Relative viability difference (RVD), offspring size difference (OSD), and  $F_{ST}$  estimates are calculated as described in the text. Crosses between population pairs designated in bold letters were conducted in 2011–2012. All other crosses were conducted previously and superscripts designate the source of these data.

population pair		average viability (no. of replicate crosses, mean fecundity)		RVD	OSD	$F_{ST}$
<i>A</i>	<i>B</i>	<i>A</i> × <i>B</i>	<i>B</i> × <i>A</i>			
<b>TP</b>	<b>ML</b>	1.0 (4, 37.5)	0.99 (4, 37.5)	0.010	0.07	0.373 <sup>c</sup>
<b>WR</b>	<b>WS</b>	0.98 (5, 44.2)	1.0 (4, 29.3)	−0.020	−0.098	0.018 <sup>c</sup>
<b>WR</b>	<b>ML</b>	0.99 (8, 28.4)	0.76 (8, 30.5)	0.23	0.21	0.133 <sup>c</sup>
TP	WR	0.59 (9, 17.2) <sup>a</sup>	0.91 (9, 21.0) <sup>a</sup>	−0.35	−0.14	0.195 <sup>c</sup>
WS	ML	0.96 (22, 19.8) <sup>b</sup>	0.68 (25, 14.6) <sup>b</sup>	0.29	0.31	0.150 <sup>c</sup>

<sup>a</sup>Data from [5].

<sup>b</sup>Data from [6].

<sup>c</sup>Data from [11].

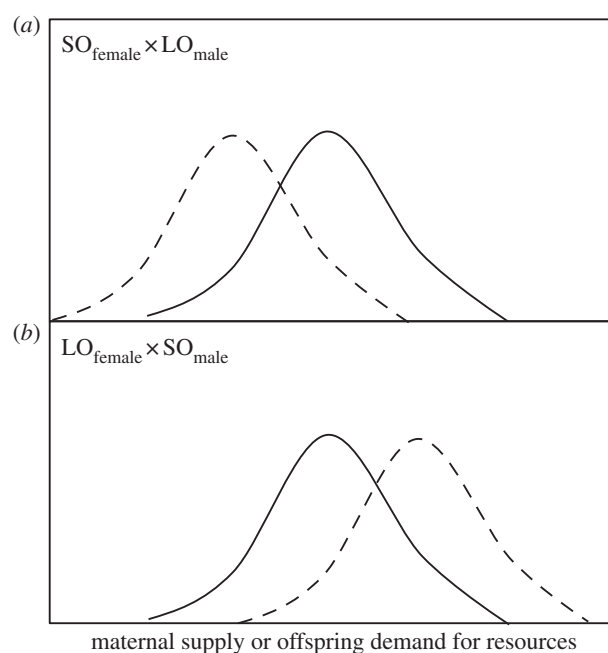
proportion of offspring that were aborted differed between the cross directions (figure 1;  $t = 0.003$ ,  $p = 0.98$ ). Similarly, crosses between SO populations (ML and TP) resulted in few aborted embryos, and the proportion of offspring that were aborted did not differ between the cross directions (figure 1;  $t = -0.005$ ,  $p = 0.99$ ). By contrast, the proportion of offspring that were aborted in crosses between LO and SO populations differed between the cross directions: crosses between an SO female and LO male ( $ML_{female} \times WR_{male}$ ) resulted in a higher frequency of aborted embryos than the reciprocal cross ( $WR_{female} \times ML_{male}$ ; figure 1;  $t = 2.57$ ,  $p = 0.011$ ).

When we combined data on offspring size with the results of RHCs involving these four populations, we found that when females from population *A* have large offspring relative to population *B*, they are the better maternal parents (table 1). This is reflected in the positive correlation between RVD and OSD (figure 1;  $r = 0.90$ ,  $p = 0.03$ ,  $n = 5$ ). To demonstrate that the positive association between RVD and OSD was not an artefact of which population was designated 'A' and which as 'B', we repeated this analysis using every possible assignment of populations as 'A' or 'B' (32 possible assignments). The correlation coefficient between RVD and OSD was always positive (range: 0.60–0.98), and the average correlation coefficient was significantly greater than 0 (mean  $r = 0.88$ ;  $t_{31} = 44.9$ ,  $p < 0.0001$ ).

Differences in offspring size were not confounded with genetic divergence between populations and do not contribute to the direction of isolation asymmetry.  $F_{ST}$  values varied considerably among pairs of populations (table 1) and were not correlated with OSD ( $r = 0.11$ ,  $p = 0.87$ ). In fact, both the highest and lowest  $F_{ST}$  estimates were between populations with similarly sized offspring.

## 4. Discussion

Isolation asymmetry is a common outcome of hybridization; however whether the magnitude or direction of isolation asymmetry can be predicted has received little attention from experimenters [2]. Here, we show that differences in offspring size predict the direction of isolation asymmetry observed in crosses between *H. formosa* populations: in crosses between populations with differences in offspring



**Figure 2.** A conceptual model for isolation asymmetry caused by mismatches of maternal supply (dashed lines) and offspring demand (solid line) for resources. (a) Illustrates crosses between a female from a SO population and a male from an LO population. (b) Illustrates the reciprocal hybrid cross. Both directions of the reciprocal hybrid cross result in hybrid progeny with the same autosomal genotype and the same level of demand for maternally supplied resources. SO mothers are assumed to supply embryos with lower levels of resources than LO mothers. In (a), maternal supply is less than offspring demand and this mismatch results in higher abortion rates. In (b), maternal supply is greater than offspring demand and embryos do not suffer reduced viability.

size, mothers from populations characterized by large offspring are the better maternal parent. Crosses between populations with similarly sized offspring do not result in decreased hybrid viability in either direction.

We suggest that the association between differences in offspring size and the direction of isolation asymmetry results from a disruption of parent–offspring coadaptation [12] over maternal supply and embryonic demand for resources. This could occur if offspring demand for resources is inherited as

an additive trait, while maternal supply is determined by the maternal genotype. Under this scenario, hybrid embryos created by crossing LO and SO populations, which have the same autosomal genotype, would exhibit intermediate demand for maternally supplied resources regardless of the cross direction. Previous studies indicate that mothers from SO populations invest less in individual offspring than mothers from LO populations [6]. Thus, when mothers of hybrid embryos are from an SO population, embryonic demand for resources may exceed the maternally determined supply resulting in high rates of aborted embryos. When mothers of hybrid embryos are from an LO population, maternal supply may exceed embryonic demand, allowing embryos to complete development normally (figure 2). Parent–offspring coadaptation is thought to play an important role in the evolution of parental care and possibly reproductive isolation [12–15]. However, the conditions under which a disruption of parent–offspring coadaptation over resource supply and demand influences the direction of isolation asymmetry has not been modelled explicitly. Our empirical results suggest that this is a fruitful avenue for future research.

In centrarchids, the direction of isolation asymmetry between species varies predictably with the relative rates of mitochondrial and nuclear evolution in the parental species, a pattern consistent with theoretical predictions [4]. Our data do not allow us to test whether differences between populations in nuclear and mitochondrial substitution rates contribute to isolation asymmetry between *H. formosa* populations. However, we think that this scenario is unlikely, considering that the experiments we have conducted involve conspecific populations that display limited genetic divergence compared with the centrarchid species studied by Bolnick *et al.* [2]. Instead, we suggest that divergence in a life-history trait that is subject to parent–offspring coadaptation (offspring size) predicts the direction of isolation asymmetry.

**Acknowledgements.** H. Rodd, K. McGhee, L. Kwan and two anonymous reviewers provided comments that greatly improved the manuscript. This work was performed under IACUC protocols 9321 (Florida State University) and 10171 (University of Illinois).

**Funding statement.** This work was supported by US NSF awards DEB 08-22547, DEB 09-53716 and DEB 11-20804.

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