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Genetic compatibility and hatching success in the sea lamprey (*Petromyzon marinus*)

Rolando Rodríguez-Muñoz and Tom Tregenza*

School of Biosciences, Centre for Ecology and Conservation, University of Exeter, Cornwall Campus, Tremough, Penryn TR10 9EZ, UK
**Author for correspondence* (*T.Tregenza@exeter.ac.uk*).

Recent discussion of genetic benefits of polyandry and female mate choice has distinguished between two potential factors influencing offspring quality: (i) some males carry higher quality genes and (ii) males and females differ in their degree of genetic compatibility. We examined evidence for effects of good genes and genetic compatibility on embryonic survival of sea lamprey (*Petromyzon marinus*), a fish species with external fertilization that spawns in North Atlantic rivers. Using *in vitro* fertilization, we made all possible crosses among 10 males and 5 females collected in the spawning grounds. Male identity did not have any significant effect on hatching success. However, female identity and male×female interactions had a highly significant effect on hatching success. Our results suggest that genetic compatibility between male and female genomes plays an important role in embryo survival during the early stages of development in the sea lamprey.

Keywords: genome interactions; inviability; non-additive genetic variance; external fertilization

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1. INTRODUCTION

Two of the main hypotheses explaining the evolution of female mate choice and polyandrous behaviour rely on the existence of either differences in genetic quality among males or variation in genetic compatibility among potential partners (Zeh & Zeh 1996, 1997; Jennions & Petrie 2000; Colegrave *et al.* 2002). Thus, females fertilizing their eggs with sperm from males carrying good genes or genes that are more compatible with their own genome, gain a fitness advantage for their offspring. Although most studies seek to explain their findings in terms of either good or compatible genes (Tregenza & Wedell 2000), both explanations are not necessarily mutually exclusive (Colegrave *et al.* 2002; Puurtinen *et al.* 2005). Inbreeding depression is an example of genetic incompatibility with widespread occurrence (Tregenza & Wedell 2000), but there is also good evidence for the coexistence of good and compatible genes that are not dependent upon consanguineous matings in a number of different taxonomic groups (Laurila *et al.* 2002; Ivy 2007; Wedekind *et al.* 2008).

Because they have external fertilization, lampreys provide an opportunity to study genotype by genotype interactions and good gene effects on viability in naturally occurring genomes. Lamprey social mating systems range from monogamy to promiscuity (Malmqvist 1986), and polyandrous behaviour has been described even in very territorial and socially monandrous species such as the sea lamprey (*Petromyzon marinus*; Scribner & Jones 2002). In this study, we carry out a cross-classified design to assess the genetic components of early embryo survival on the sea lamprey. This design allows for the calculation of male, female and male×female effects, and the estimation of the contributions of additive, maternal, dominance and environmental components to the total phenotypic variance (Lynch & Walsh 1998).

2. MATERIAL AND METHODS

We collected 15 female and 18 male adult lampreys at their spawning grounds in the river Sella basin (Northern Spain). The day after collection, we crossed 10 males and 5 females following a North Carolina II design (Lynch & Walsh 1998, p. 598). After being anaesthetized, we extracted approximately 1.3 ml of sperm from each of the males and stored it at low temperature (Rodríguez-Muñoz & Ojanguren 2002). Females were processed consecutively and one at a time. For each female, we first extracted a sample of eggs exceeding the total amount required for the 10 crosses. Then we took 10 subsamples using a teaspoon, and introduced them into 10 dry 100 ml plastic jars. Each subsample was mixed with 50 μl of sperm from one of the males just after adding 25 ml of river water. The mixture was stirred for a few seconds and then left in the dark for approximately 10 min. Between 10 and 30 min after fertilization, we washed the eggs gently with clean water and transferred three subsamples of 50 eggs each into independent plastic jars filled with 50 ml of water. We did not replicate fertilizations because previous experience showed that all eggs are usually fertilized in this species (Rodríguez-Muñoz & Ojanguren 2002). Our design (in common with studies in other species, e.g. Wedekind *et al.* 2008) makes the assumption that this brief period, during which eggs and sperm from a cross are together before being divided into three independent pots, does not exert a common influence over the subsequent viability of the eggs.

Jars containing eggs were incubated in darkness at 17–20°C for the first 4 days, and at 18.4±1°C for the remaining incubation period. Incubation temperature was selected to maximize egg survival (Piavis 1961). Replicates from the same family were randomly assigned to one of 300 available positions distributed among seven incubation trays. Tray position was changed daily along the vertical shelving of the incubator, so that each tray spent an equal amount of time at each shelf. Dead eggs were removed at intervals of 3–4 days.

Fertilization rate was estimated from the number of eggs with a visible perivitelline space, characteristic of fertilized eggs (Ciereszko *et al.* 2000), 10 min after sperm and egg mixing. Hatching success was calculated from the number of eggs hatching in each replicate as a proportion of the total number of eggs fertilized.

We analysed hatching success by including sire and dam as random factors in a two-way ANOVA. Variance components and the relative contribution of additive, dominance, maternal and environmental effects were estimated following Lynch & Walsh (1998, pp. 598–603).

3. RESULTS

Among the 50 families, 44 had 100 per cent fertilization rate, 3 had 98 per cent and only 1 each had 97, 87 and 83 per cent. Overall hatching success was high, with 42 per cent of families having more than 90 per cent and 60 per cent of families more than 80 per cent. Developmental abnormalities such as strong cellular asymmetries in developing embryos were common, but we did not see any evidence for pathogen attacks such as fungal infections on unhatched eggs.

Dam and dam×sire effects had significant effects on hatching success (table 1), and explained 31 per cent of the total variance. Sire effects were absent, and sire

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Table 1. Results from the two-way ANOVA, factor variances and estimated values of the causal components of hatching success in *P. marinus*. (Approximate 95% confidence intervals (CI) are estimated using the conservative approach proposed by Lynch & Walsh (1998). The coefficient of variation (CV) for variance components is also shown (Houle 1992). The negative value for the sire variance was set to zero (Graham & Edwards 2001) for the calculation of variance components. V_M, maternal effects; V_A, additive variance; V_D, dominance variance; V_E, environmental variance.)

source	d.f.	MS	*F*	*p*-value	var (CI)	comp	var	CV	per cent
dam	4	0.57	4.750	0.003	0.015 (0.050)	V_M	0.015	15.7	14.8
sire	9	0.07	0.583	0.802	−0.002 (0.012)	V_A	0.000	0.0	0.0
dam × sire	36	0.12	1.714	0.019	0.017 (0.044)	V_D	0.067	33.2	65.5
residual	100	0.07			0.070 (0.044)	V_E	0.020	18.1	19.7

variance was slightly negative (Graham & Edwards 2001; Evans *et al.* 2007). Overall, dominance and maternal components explained 80 per cent of the phenotypic variance. Assuming epistatic genetic variance to be negligible, we found that dominance variance showed the highest contribution to the observed variation in hatching success (table 1) and additive variance had no effect.

4. DISCUSSION

Our results suggest that dominance variance is the main factor explaining hatching success in the studied sea lamprey population. Maternal effects are also important, but we found no evidence for additive variance. Similar findings have been reported for some other species (Rudolfsen *et al.* 2005; Evans *et al.* 2007), although the relative contribution of additive and non-additive variances on early survival varies widely (Marshall & Evans 2007; Pitcher & Neff 2007; Wedekind *et al.* 2008).

In fishes, egg-mediated maternal effects have been related to differences in egg ripeness, size or composition (Kamler 2005). Our lampreys were processed just after natural maturation, and thus they are very unlikely to be affected by overripening. This points towards differences in egg size or composition as the main potential factors explaining the observed variance due to maternal effects.

Our observation of high levels of dominance variance, combined with a lack of additive effects, could reflect the existence of balancing selection (Charlesworth & Hughes 2000), and appears to be a robust finding even though our design does not allow for the estimation of variance due to epistasis. Considering epistatic interactions involving only two loci, there is no evidence for additive × additive epistasis, as a small fraction of it is expected in the sire variance (Lynch & Walsh 1998; Charlesworth & Hughes 2000). Although epistasis involving dominance (additive × dominance and dominance × dominance interactions) could be present in the dam × sire effects, dominance variance constitutes an important portion of that interaction (Lynch & Walsh 1998). The potential existence of co-adapted gene complexes is a second factor that could explain the importance of sire × dam interactions, and might help to explain the maintenance of epistatic variance. For alternative co-adapted complexes to persist (and not be lost by drift), there need to be alternative niches in space or time. It is not apparent what such niches might be for these fish, although a possibility is that differences in temperature within the river could be important. Finally, the possible existence of selfish genetic elements that might lead to incompatibilities among mates (Zeh & Zeh 1996) warrants further investigation.

To prevent misleading results due to compatibility, our results underline the importance of estimating male fertilization success at very early stages of egg development in sperm competition experiments (García-González 2008).

Irrespective of the source of the incompatibilities we observed, an outstanding question is whether or not wild lampreys can avoid incompatible mates through mate choice. Such avoidance would help to maintain the sources of incompatibility since they would not be exposed to such strong selection. Post-copulatory choice is impossible in sea lampreys, since fertilization is external and male territoriality enforces monandry on females while they are with a particular mate. However, females may mate with more than one male over a breeding season (Scribner & Jones 2002), so it is possible that they might lay a greater proportion of their eggs upon receipt of some cue indicating the compatibility of a particular male. Alternatively, they might approach particular males selectively: it has been shown that male lampreys release a pheromone that is detected by females during the breeding season (Li *et al.* 2002). This olfactory signal may carry information on compatibility as occurs in some major histocompatibility complex-mediated mate choice situations (Milinski 2006), but no evidence is currently available. Many of these questions appear amenable to future studies, which might for instance compare the egg viability seen in crosses between pairs of fish found spawning together with that of random crosses.

Genetic compatibility could be an important factor in promoting the communal spawning behaviour characteristic of most lamprey species (Malmqvist 1986), where females can mate to up to three males at once (Cochran *et al.* 2008). This behaviour could allow females to extend mate choice in relation to compatibility beyond gametes releasing, particularly if egg–sperm interactions prior to fertilization allow selection of the most compatible males. Increased fertilization success through polyandry has been shown in another species with external fertilization (Marshall & Evans 2005). Our results suggest that it would be fruitful to conduct similar studies in other species of lamprey and other communal spawners.

Lamprey collection and gamete extraction were carried out under authorization from the Environmental Council. After gamete collection, all adult lampreys were released into the hatchery river, a tributary of the source river.

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