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The potential additive and non-additive benefits of mate choice in the threespine stickleback (*Gasterosteus aculeatus*)

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ABSTRACT

Hypothesis: The genetic benefits of female mating preferences can be additive (good alleles'), non-additive ('compatible alleles') or both, and the importance of each may be population dependent.

Organism: Threespine stickleback (*Gasterosteus aculeatus*) from two independent natural populations.

Location: Two adjacent sites in Scotland, one a pond with high stickleback density and high parasite prevalence, the other a river with low stickleback density and fish predators.

Methods: *In vitro* fertilizations in a North Carolina Type II breeding design to estimate the contribution of additive and non-additive genetic variation to early offspring survival.

Results: In both populations we detected significant additive maternal effects. These effects were largely explained by variance in egg size, though not entirely so. In the pond population there were significant male additive genetic effects on offspring survival, implicating a role for 'good alleles', but not non-additive effects. In the river population there were significant additive and non-additive effects, implicating a role both for 'good alleles' and genetic compatibility. Different populations of the same species may vary in the mechanism by which sexual selection operates.

Keywords: additive genetic benefit, genetic compatibility, good genes, major histocompatibility complex, mating system, non-additive genetic benefit, olfactory cues, sexual selection, teleost, stickleback.

INTRODUCTION

The genetic or indirect benefits of female mate choice have been a contentious feature of sexual selection theory (Parker, 1992; Kirkpatrick and Barton, 1997; Qvarnström *et al.*, 2006; Kempenaers, 2007). 'Good genes' (more correctly 'good alleles') models of mate choice predict additive benefits of female mating decisions, with some males representing a superior partner for all

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females in a population, and leading to directional selection (Kempenaers, 2007). ‘Compatible genes’ (‘compatible alleles’) models predict that genetic benefits are non-additive, with only a particular combination of complementary parental alleles generating fitness benefits in the offspring (Zeh and Zeh, 1996, 1997; Neff and Pitcher, 2005; Piálek and Albrecht, 2005). These two models of mate choice evolution are not mutually exclusive, and male genetic quality can be considered as the combined effects of good and compatible alleles (Neff and Pitcher, 2005).

In populations in which female mate choice selects for good alleles, females are predicted to show mating preferences for the same males, and selection is directional. Good alleles are associated with signals of male quality, typically in the form of ornamentation or display (Iwasa and Pomiankowski, 1994; Maynard Smith and Harper, 2003). In contrast, mate choice for compatible alleles is predicted to show non-additive variation, and in populations where compatible allele effects operate, females are not expected to share mate preferences, with the outcome that selection will not be strongly directional (Tregenza and Wedell, 2000; Neff and Pitcher, 2005). How genetic compatibility functions as the basis to a mate choice system is not completely clear, although it is likely to be limited to specific sets of genes, since complex interactions of male and female genotypes across many genes place severe constraints on any such system (Puurtinen *et al.*, 2009). One such genetic system comprises the genes of the major histocompatibility complex (MHC) (Jordan and Bruford, 1998; Tregenza and Wedell, 2000). MHC genes have a primary role in the vertebrate immune response, but can also influence individual odour (Penn and Potts, 1999; Yamazaki and Beauchamp, 2007). The MHC potentially enables an individual to compare self with non-self using olfactory cues, thereby providing a mechanism underlying mate choice decisions (Milinski, 2006; Gerlach *et al.*, 2008). There is evidence that MHC genes function in mate choice decisions in a range of taxa and, therefore, as a key element of mating system evolution (Tregenza and Wedell, 2000; Roberts and Gosling, 2003; Milinski, 2006; Piertney and Oliver, 2006; Kempenaers, 2007; Baratti *et al.*, 2012; Reichard *et al.*, 2012).

Sexual selection has the potential to underpin evolutionary divergence among populations (Ritchie, 2007). Despite its significance, little is known about spatial or temporal variation in the strength of sexual selection and its impact on mating system evolution (Mobley and Jones, 2007). Here we use the threespine stickleback (*Gasterosteus aculeatus*) model to investigate inter-population variation in the potential genetic benefits of female mate choice decisions in two contrasting environments. *Gasterosteus aculeatus* is a small teleost fish that occurs in both marine and freshwater habitats in the temperate and boreal northern hemisphere (Wootton, 1976, 1984). Fertilization is external and males perform parental care of eggs and larvae in a specially constructed nest, while females show male mating preferences (Wootton, 1976, 1984). A striking feature of the mating system of *G. aculeatus* is the extent of variation reported in the basis to female mating decisions. A role for non-genetic, direct benefits of mate choice for females has been described in this species, through females selecting males on the basis of nest quality (Barber *et al.*, 2001a; Rushbrook and Barber, 2008), territory size and quality (Candolin and Voigt, 2001; Le Comber *et al.*, 2003), and parental care (Künzler and Bakker, 2000). There is also evidence for indirect benefits, both additive (Barber *et al.*, 2001b) and non-additive (Milinski, 2006). A role for arbitrary mate choice (i.e. not contingent on male quality) has also been implicated through Fisherian selection (Bakker, 1993) and sensory bias (Smith *et al.*, 2004). This unusual level of variation in the basis to female mate choice in *G. aculeatus* suggests that several mechanisms may operate in this species, perhaps reflecting different selective environments. Notably, several studies have implicated a central role of mate choice for MHC in this species (Milinski, 2006).

Our aim was to investigate whether variation in the genetic benefits of female mate choice could be detected among populations. To do this, we quantified the relative magnitude of potential good and compatible allele benefits of mate choice in two recently diverged populations from contrasting environments. We used a North Carolina Type II breeding design (Lynch and Walsh, 1998), utilizing *in vitro* fertilizations (IVF) to generate crosses to test for potential additive (good alleles) and non-additive (compatible alleles) genetic variation on the survival of young stages.

METHODS

Experimental fish

Gasterosteus aculeatus were collected from two sites using minnow traps at the start of the spawning season in April 2012. The first site, the Kinness Burn, St. Andrews, Scotland (latitude 56°33′51″N, longitude 2°78′94″W), is a short river that drains an area of arable farmland and coastal woodland in East Fife (hereafter referred to as the river population). Fish were also caught from Loches Pool, a man-made pond in the University of St. Andrews botanic gardens (latitude 56°33′59″N, longitude 2°80′75″W) (Spence *et al.*, 2012) (hereafter referred to as the pond population). The pond is adjacent (<10 m) to the Kinness Burn, which was the source of the pond population when it was filled from the river approximately 20 years ago. Fish from both populations were between 38 and 55 mm body length (BL; measured from the tip of the snout to the base of the tail fin). There was no significant difference in the body length of fish samples collected from each population (unpaired *t*-test, $t_{62} = 0.88$, $P = 0.140$). The mean (±S.E.) body length of river fish was 47.3 ± 1.0 mm and of pond fish 45.2 ± 0.9 mm.

Despite their close proximity, conditions in the two collection sites were different. The river was a lotic site that supported a small population of brown trout (*Salmo trutta*) and *G. aculeatus* densities were relatively low. The pond was a lentic site from which other fish were absent. The density of *G. aculeatus* was high, and there was a high prevalence of *Glugea anomala*, with approximately 42% of the population exhibiting at least one externally visible xenoma, with some fish hosting >20 xenomas. Avian predators, principally grey herons (*Ardea cinerea*) and kingfishers (*Alcedo atthis*), occurred at both sites.

In vitro fertilizations

To measure the relative contribution of additive and non-additive genetic effects on offspring fitness traits, a North Carolina Type II breeding design (Lynch and Walsh, 1998) using *in vitro* fertilizations (IVF) was adopted to generate a series of replicated half-sib families. Eight blocks, each with a set of 2 × 2, male × female factorial crosses were used. Within each block, each male was crossed with two females, with a replicate of each cross using the same males and females. Therefore, this design generated two replicates of four families of maternal and paternal half-siblings in each block, with eight families in the final combined design for each complete replicate; a total of 64 families for each population.

To generate crosses, gravid females were isolated, with gravidity ascertained by the degree of abdominal swelling and dilation of the genital opening. Eggs were gently stripped from gravid females and the clutch divided into four approximately equal batches in separate 70 mm diameter Petri dishes containing fresh water (Barber and Arnott, 2000). The mean

(±S.E.) number of eggs per batch for the river population was 24.8 ± 1.2 and for the pond population 23.4 ± 1.1. Males were humanely sacrificed and their testes dissected out. Both testes were transferred to a 70 mm diameter Petri dish containing 10 mL of teleost saline (Yokoi *et al.*, 2008) and disrupted with a dissecting needle to release spermatozoa (Le Comber *et al.*, 2004) for each male separately. A 1 mL subsample of this sperm solution was pipetted over two batches of eggs from each of the two females, and the covered Petri dishes were left on the laboratory bench for 30 min. The fertilized eggs were washed with fresh water and photographed alongside a scale bar under standard light conditions using a Canon EOS 300D camera with 60 mm macro lens. The procedure was repeated using the sperm from both males. Egg size was subsequently estimated from digital images. Fertilized eggs were transferred to an aerated egg incubator at 17°C (Barber and Arnott, 2000), with four families of maternal and paternal half-siblings housed in separate incubators. Eggs were not chemically treated to prevent fungal or protozoal infection. A record was kept of embryo survival after 24 h and 96 h, with dead embryos recognized when they turned opaque.

Statistical analysis

Dependent variables were embryo survival after 24 h and 96 h. Survival rates at hatching were too low to permit analysis in a balanced factorial design (Lynch and Walsh, 1998). Because the amount of yolk invested in offspring can significantly affect offspring fitness (Wootton, 1998), egg size was used as a covariate in all analyses. For each 2 × 2 factorial block, analysis of covariance (ANCOVA) was used to compare the effects of sire, dam, and their interaction on each dependent variable. Sums of squares were combined to calculate mean squares and degrees of freedom for all blocks combined (Lynch and Walsh, 1998).

RESULTS

For the river population there was a significant effect of egg size on offspring survival, which explained 46% and 40% of variance in survival after 24 h and 96 h respectively. There was no significant additive male effect on offspring survival after either 24 h or 96 h (Table 1); variance attributable to males was typically zero or negative (and hence set to zero). Additive maternal effects, excepting egg size, were significant after 24 h, although they accounted for only a minor component of variance (Table 1). No significant additive maternal effects were detected after 96 h. There was no significant effect of male × female interaction after 24 h, although there was after 96 h. Non-additive interaction effects accounted for 18% and 27% of variance in offspring survival after 24 h and 96 h respectively (Table 1).

For the pond population there were highly significant egg size effects on offspring survival after 24 h, which explained 51% of variance in survival at this stage, and significant effects after 96 h, explaining 41% of variance in survival. There were no significant additive male effects on survival after 24 h, although there were after 96 h, accounting for 15% of variance after this period (Table 2). Additive maternal effects had a significant effect on survival after 24 h, but not after 96 h, explaining 17% and 10% of variance in survival respectively (Table 2). There were no significant non-additive interaction effects on survival in this population.

Table 1. Summary of results of factorial crosses for embryo survival after 24 h and 96 h for *in vitro* fertilizations of *G. aculeatus* from the river population

Source	d.f.	SS	MS	*F*	*P*	Variance (%)
Egg survival over 24 h (0.75 ± 0.047)						
Egg size	8	1.468	0.184	3.96	**0.004**	46
Male	8	0.613	0.077	1.65	0.162	0
Female	8	0.993	0.124	2.68	**0.030**	4
Male × Female	8	0.787	0.098	2.12	0.074	18
Error	24	1.879	0.078			32
Egg survival over 96 h (0.14 ± 0.028)						
Egg size	8	1.257	0.157	3.35	**0.010**	40
Male	8	0.779	0.097	2.08	0.079	0
Female	8	0.677	0.085	1.81	0.125	0
Male × Female	8	0.965	0.121	2.58	**0.035**	27
Error	24	0.682	0.028			34

Note: Variance components for each factor were calculated following Lynch and Walsh (1998). *P*-values < 0.05 are shown in bold. Mean (±s.e.) of variables in parentheses.

Table 2. Summary of results of factorial crosses for embryo survival after 24 h and 96 h for *in vitro* fertilizations of *G. aculeatus* from the pond population

Source	d.f.	SS	MS	*F*	*P*	Variance (%)
Egg survival over 24 h (0.91 ± 0.018)						
Egg size	8	0.183	0.023	6.10	<0.001	51
Male	8	0.088	0.011	2.00	0.091	7
Female	8	0.148	0.019	3.36	**0.010**	17
Male × Female	8	0.044	0.006	1.47	0.221	5
Error	24	0.090	0.004			20
Egg survival over 96 h (0.17 ± 0.028)						
Egg size	8	0.288	0.036	3.67	**0.006**	41
Male	8	0.253	0.032	2.61	**0.033**	15
Female	8	0.196	0.025	2.02	0.087	10
Male × Female	8	0.097	0.012	1.24	0.320	4
Error	24	0.235	0.010			30

Note: Variance components for each factor were calculated following Lynch and Walsh (1998). *P*-values < 0.05 are shown in bold. Mean (±s.e.) of variables in parentheses.

DISCUSSION

The goal of this study was to compare the potential benefits of female mate choice, which represents a key element of the mating system of *G. aculeatus*, in two populations of the stickleback with a common origin but from contrasting environments. Using a North Carolina Type II breeding design and *in vitro* fertilizations, we identified common features of parental effects on offspring survival during early development, but also key differences.

In both populations we detected significant additive maternal effects. These effects were largely explained by variance in egg size, though not entirely so. Additive maternal effects, both including and excluding egg size, explained a greater proportion of variance in survival after 24 h than after 96 h (Tables 1 and 2). Egg size is a major determinant of egg quality, and is a highly heritable trait in teleost fishes (Brooks *et al.*, 1997). Egg size is a reflection of female investment and correlates with the quantity of yolk allotted to each offspring. A change in egg size faces two potential trade-offs. A trade-off between egg size and clutch size, within the limits of a given reproductive allotment, means that selection for greater egg or embryo size will necessarily result in a decline in clutch size. A second trade-off is between egg size and mortality. Across a range of species, larger eggs have greater hatching success and produce larger larvae (Pepin, 1991; Trippel, 1998). Larger larvae tend to have reduced susceptibility to starvation, lower risk of predation, and greater food capture abilities (Blaxter and Hempel, 1963; Trippel, 1998; Tamada and Iwata, 2005), and consequently better growth rates (Kamler, 2008). Egg size may also correlate negatively with survival. This effect can arise because larger eggs tend to take longer to hatch and reach independence, making them susceptible to predators for longer (Steele, 1977). Additive maternal effects excluding egg size were significant, but relatively short-lived and of smaller magnitude than egg size effects (Tables 1 and 2). These effects may relate to variation in egg quality parameters such as the content of fatty acids or yolk proteins (Brooks *et al.*, 1997).

We also detected differences in the potential benefits of female mate choice between the two study populations. In the river population there were significant non-additive interaction effects on offspring survival, while in the pond population there were significant male additive effects. These results suggest divergent selection in the two populations. The environmental conditions at these two sites differed in terms of flow regime, fish density, the range of predators, but most strikingly in the context of mate choice in this species, in the prevalence of a pathogen. Pond populations of *G. aculeatus* are typically exposed to a greater range of parasites than river populations (Wegner *et al.*, 2003), and the pond population we used in this study exhibited a 42% prevalence of the microsporidian parasite *Glugea anomala*. This parasite increases conspicuousness to predators (Milinski, 1985) and causes tissue (Dyková and Lom, 1978) and nerve damage (Dezfuli *et al.*, 2004), with an overall negative impact on the fitness of *G. aculeatus* (Barber, 2007). MHC allelic diversity correlates with protection against the effects of *Glugea anomala* in *G. aculeatus* (Kurtz *et al.*, 2004), potentially selecting for mate choice for MHC, and thereby driving divergence in the mating system of the two study populations.

In *G. aculeatus*, the genes of the MHC are well characterized and have been shown to both play a key functional role in parasite resistance and to influence mate choice decisions, with both additive and non-additive genetic benefits (Milinski, 2006; Barber, 2007). Mate choice for MHC in *G. aculeatus* can select for optimum, rather than maximum heterozygosity, suggesting that a specific level of MHC heterozygosity in the offspring will maximize fitness. A prediction is that females should prefer mates that will generate optimum genetic diversity in the offspring (Aeschlimann *et al.*, 2003), with selection for non-additive genetic compatibility effects. In populations exposed to high parasite diversity, *G. aculeatus* were shown to express high MHC variability (Wegner *et al.*, 2003). Thus, where *G. aculeatus* are exposed to a broad range of parasites, but without strong selection exerted by any one pathogen, a compatibility effect is predicted. However, specific MHC alleles can also confer additive benefits through greater protection against particular pathogens (Arkush *et al.*, 2002; Pitcher and Neff, 2006). In a population with a high prevalence of a specific pathogen, there should be strong selection for specific MHC alleles that confer resistance primarily to that pathogen, which would be manifested as additive genetic benefits (Pitcher and Neff, 2006). The rapid evolution of MHC alleles against specific pathogens has been demonstrated experimentally in *G. aculeatus* (Eizagguire *et al.*, 2012), also implicating a role for additive genetic benefits. These predictions broadly matched our findings, with significant additive effects in the pond population of *G. aculeatus* exposed to a high prevalence of *Glugea anomala*, and presumably strong selection for resistance to this pathogen, but non-additive effects in the river population where overall pathogen prevalence was expected to be lower (Wegner *et al.*, 2003). This apparent divergence in the mating systems of these two populations appears to have evolved quickly. Contemporary evolution in *G. aculeatus* is recognized to occur at a rapid pace (Bell *et al.*, 2004; Wootton, 2009; Bell and Aguirre, 2013), although a change to the mating system of this species over such a short period has not previously been demonstrated. An additional possibility is that the differences we detected between the two study populations reflect plastic responses. Epigenetic control of gene expression potentially permits a rapid response to selection and could provide the link between phenotypic plasticity and longer-term adaptation (Richards, 2006). Epigenetic effects have been linked to pathogen infections in Atlantic salmon (*Salmo salar*) (Kjoglum *et al.*, 2005), and while research on the genetics of adaptation in *G. aculeatus* has been comprehensive and wide-ranging, a role for epigenetic control of gene expression in adaptation is hitherto lacking.

While these results imply selection for different mate choice mechanisms in the two study populations, a weakness of the study is that female mate preferences were not tested. A prediction for these populations is that females from the river population should fail to show congruent mate preferences, reflecting non-additive benefits of mate choice. In contrast, those from the pond population should tend to show mate preferences for the same males (Spence and Smith, 2006; Casalini *et al.*, 2009; Agbali *et al.*, 2010); that is, those with MHC alleles provide additive benefits through resistance to *Glugea anomala*. Thus a more refined design would be to present females of known MHC genotype with males of known genotype, with specific mate choice outcomes based on the functional dissimilarity between the MHC alleles of males and females (Reichard *et al.*, 2012). Further circumstantial evidence for divergence in mate choice mechanisms could be obtained from investigating the relationship between parasite abundance and diversity with allelic variability of the MHC genes (Froeschke and Sommer, 2005; Oliver *et al.*, 2009; Fraser *et al.*, 2010; Smith *et al.*, 2011).

A further caveat to our conclusions is that effects were measured over a short time scale, from fertilization to hatching, and it is questionable whether these effects would translate into longer-term fitness effects. Comparable studies that have examined the fitness benefits of additive and non-additive effects have shown that egg and larval performance in fish and amphibians do have longer-term implications for fitness, and that survival and growth rates during the early stages of development are important (Dziminski *et al.*, 2008; Agbali *et al.*, 2010). Thus, while these conclusions are tentative, high variance in mortality rates early in development inevitably has implications for overall reproductive success. A weakness of studies of this type is that they are, by practical necessity, laboratory based. Consequently, the effects of fluctuations in environmental parameters, or the impact of pathogens on survival can be limited. In the present study, eggs were not chemically treated to control fungal and protozoal infection prior to incubation so as to permit the potential impact of pathogens. Fungal infection of eggs was regularly observed during the study and egg mortalities were relatively high overall. Mortalities were presumed to be a function of pathogen infection and parental compatibility, although the ultimate cause of mortalities was uncertain. Benign environmental conditions generated in laboratory studies can mask underlying genetic effects (Reznick, 1985). Thus high mortality rates in the present study may have served to illustrate that significant indirect effects were in operation. However, this raises the possibility that with a different laboratory microenvironment an alternative outcome might have been obtained, though this is a potential weakness of many laboratory-based evolutionary experiments.

An additional consideration is that data were collected for a single breeding season. While spatial variation among populations in the intensity of sexual selection, and its implication for mating systems is recognized (e.g. Cruzan *et al.*, 1994; Mobley and Jones, 2007), temporal variation has been largely overlooked (Magurran and Henderson, 2010), although both may be significant and merit further research. Similarly, only a single replicate of each habitat type (pond vs. river) was investigated and attributing our findings to the impact of a single variable is, at best, tentative. Fuller replication would permit more broad generalizations to be drawn from these results.

In conclusion, this study provides evidence for population differences in the basis to mate choice in response to prevailing environmental conditions. Inter-population variation in the basis to female mate choice has been proposed, though there is currently limited empirical support (Milinski, 2006). Rapid adaptive responses to changing selection environments are a feature of *G. aculeatus*, which offers an unusually tractable model for such studies (Milinski, 2006; Huntingford and Ruiz-Gomez, 2009; Wootton, 2009).

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