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Offspring genetic diversity increases fitness of female Atlantic salmon (*Salmo salar*)

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Abstract Inbreeding has negative effects on individual and population performances. Therefore, enhancement of offspring genetic diversity is believed to play a major role in shaping mating systems. However, no study has clearly separated the direct effect of having multiple partners from the indirect effect of having more outbred offspring on the resulting reproductive success of individuals in the wild. In this study, we report significant associations between both multiple mating and within-individual genetic diversity of offspring, and an increased reproductive success of wild female Atlantic salmon, *Salmo salar*. Specifically, we found that females with a higher number of mates also have more outbred offspring (within-individuals), and that both of these characteristics increased their reproductive success expressed in terms of offspring surviving when combining all freshwater juvenile stages. Our findings also indicate that determinants of fitness are different among sexes as within-individual offspring genetic diversity was not a strong predictor of male reproductive success, while the number of mates was important. We also show that females mated with more outbred males than on average, which potentially increased their chances of producing outbred offspring. These results therefore suggest that there could be more important indirect genetic benefits of multiple mating for females than for males.

Keywords Multiple paternity · Genetic benefits · Reproductive success · Internal relatedness · Mate choice

Introduction

Inbreeding has negative effects on both individual and population performances (reviewed in Crnokrak and Roff 1999; Keller and Waller 2002). Offspring with limited genetic diversity commonly show reduced fitness (Brown and Brown 1998; Kruuk et al. 2002), while there has been increasing evidence of positive associations between outbreeding and offspring probability of survival (Rossiter et al. 2001). Therefore, maximization of offspring diversity should play a major role in shaping mating systems in order for individuals to increase their offspring survival. It has been suggested that increased mean progeny diversity resulting from multiple mating reduces inbreeding (Brown 1997), sibling competition (Griffiths and Armstrong 2001), and predisposes offspring to a wider array of environmental conditions (Yasui 1998). Mating with different partners could also provide indirect genetic benefits, as an individual could improve its probability of finding a partner that would provide a higher within-individual genetic diversity in offspring and thus potentially increase his fitness (Blomqvist et al. 2002; Tregenza and Wedell 2002). However, few studies have attempted to establish if the increase in fitness resulting from multiple mating is dependent on the greater number of partners that an individual has (direct effect) or if the benefits are related to the potential advantage of having more individually outbred offspring (indirect effect), with a higher within-individual genetic diversity (IR_{O} ; see Methods).

Available evidence for genetic and phenotypic diversity of salmonids at the watershed scale (Taylor 1991), combined with their precise homing to natal streams (Stabell 1984; Garant et al. 2000), provide ample opportunity for mating to occur between close relatives (Wang et al. 2002). This may have promoted both mechanisms of inbreeding avoidance and of maximization of offspring

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genetic diversity by mating multiply with different partners. Indeed, previous work has shown that multiple mating is common in both sexes in Atlantic salmon (Garant et al. 2001; Taggart et al. 2001), but the resulting within-individual offspring genetic diversity, and its link with survival, has never been investigated.

Recently, sensitive measures have been developed to estimate individual genetic diversity and these were used to correlate multi-locus heterozygosity of individuals with traits related to fitness in natural populations of mammals and birds (reviewed in Coltman and Slate 2003). Specifically, the internal relatedness (IR), a relative measure of inbreeding/outbreeding based on individual allelic identities at several neutral markers has proven to be very efficient (Amos et al. 2001; Acevedo-Whitehouse et al. 2003). However, considerably less attention has been devoted to measuring the effects of reduced genetic diversity in wild fishes. Most studies on fishes have been carried out for aquaculture purposes or under controlled conditions. Nevertheless, such studies have shown a negative influence of reduced genetic diversity on fitness-related traits (reviewed in Wang et al. 2002; see also Heath et al. 2002).

Here we explore both the effects of multiple mating and of within-offspring genetic diversity on the resulting reproductive success of adults in a population of wild Atlantic salmon (*Salmo salar* L.). Specifically, we tested for a positive effect of an increased number of partners for a given individual on its resulting reproductive success. At the same time we tested if there was an influence of the within-individual offspring genetic diversity (quantified with IR) on the reproductive success. By using a model that included both factors, and controlling for other potential determinants of reproductive success, we were able to establish the main determinants of fitness for each sex separately. Finally, we investigated if individuals of each sex mated preferentially with relatively outbred partners by comparing observed within-adult mean IR at the population level and the observed within-partner averaged IR.

Methods

Study system

The details of the study system and of the parentage assignment procedures have been previously published (Garant et al. 2001, 2003). Briefly, we caught 76 adult wild salmon (*Salmo salar*), 41 males and 35 females, and introduced them in a river stretch previously uninhabited by salmon. In the years 1996–1998, we lethally sampled as many offspring as possible from each of the freshwater age classes. A total of 1,029 offspring were sampled over the 3 years. All individuals (adults and offspring) were scored at five highly polymorphic microsatellite DNA markers (Garant et al. 2003). Parentage assignment was conducted using a maximum likelihood procedure implemented in PAPA v1.1 (Duchesne et al. 2002; program available at: <http://www.bio.ulaval.ca/louisbernatchez/>) and allowed us to establish number of offspring and number of partners for each adult over the study period.

Measure of genetic diversity

We used IR (Amos et al. 2001) as a measure of individual genetic diversity. IR is based on the relatedness measure of Queller and Goodnight (1989), which is usually applied to comparisons between pairs of individuals. In this case, however, the method is applied to data in which, at each locus, two alleles rather than two pairs of alleles are compared such that:

$$\frac{(2H - \sum f_i)}{(2n - \sum f_i)}$$

where H is the number of loci that are homozygous, n is the number of loci and f_i is the frequency of the i th allele contained in the genotype. Allele frequency estimates for the alleles carried by the individual concerned are not adjusted (Queller and Goodnight 1989; see also Amos et al. 2001). When calculated over several loci, the resulting values are approximately normally distributed and centered on zero for individuals born to unrelated parents, with negative values suggesting relatively outbred individuals and high positive values being suggestive of inbreeding (see Amos et al. 2001). IR was calculated using an Excel macro written in Visual Basic provided by William Amos (Department of Zoology, Cambridge University, UK).

Statistical analyses

Internal relatedness was calculated for each individual and then averaged over all offspring assigned to a given adult for a resulting average offspring within-individual genetic diversity. Mean offspring IR (within-individual offspring genetic diversity, henceforth mean IR_o), adult own IR (within-adult genetic diversity, henceforth IR_a), adult size and number of partners were included as predictors of reproductive success in a general linear model with a Poisson error distribution using Genstat (GenStat V7.1, VSN 2003; this analysis was weighted by the mean proportion of offspring assigned to each individual to restrict the potential confounding effect of sampling a limited number of offspring with very high or very low IR for a given individual). Finally, for both males and females, we compared observed within-adult IR at the population level with observed average within-partners IR (henceforth mean IR_a), using one-sample t -test. We thus established if individuals of each sex mated preferentially with relatively more outbred individuals (mean IR_a significantly lower than the population mean). All analyses reported in results were also carried out using standardized heterozygosity (SH), but as IR and SH were highly correlated (all $r^2=0.96$, all $P<0.001$) all results were similar and therefore only results from IR analyses are presented here.

Results

Parentage analyses allowed us to assign a single parental pair to 91% of offspring sampled (936/1,029) (details in Garant et al. 2001, 2003). Females had on average 8.9 partners (0–19) and 29 offspring assigned (0–72). Males had a mean of 7.3 mating partners (0–17) and 23 offspring assigned (0–77). The average IR_a of females was -0.086 (-0.272 to 0.372) and the average IR_a of males was -0.007 (-0.251 to 0.558). Mean IR_o of females was 0.009 (-0.079 to 0.280) and that of males was -0.004 (-0.211 to 0.208). This difference was not significant; $t_{68}=0.70$, $P=0.489$.

Results of linear regression analyses showed a significant negative relationship between the number of partners and the mean IR_o for females ($b=-0.005\pm 0.002$, $t_{29}=2.10$, $P=0.044$), but not for males ($b=0.001\pm 0.003$,

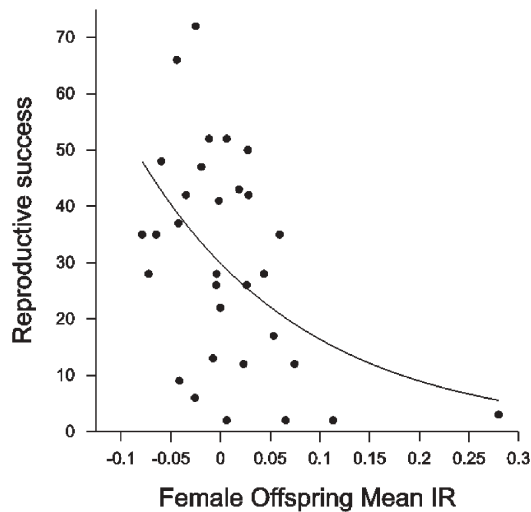


Fig. 1 Relationship between average mean internal relatedness (IR) of offspring (mean IR_o) and reproductive success of each female, defined as the number of offspring assigned overall, and fitted from a general linear model ($b=-6.01\pm 2.28$, $t=-2.64$, $P=0.013$) in female Atlantic salmon. The relationship is significant even after excluding the individual with the highest progeny IR ($b=-5.58\pm 2.59$, $t=-2.15$, $P=0.040$)

Table 1 Results of the general linear model assessing the effects of potential determinants of individual reproductive success for A female and B male Atlantic salmon. Number of partners (nb partners), within-individual offspring genetic diversity (mean IR_o), adult body size (fork length) and within-adult genetic diversity (IR_a) were included in the model as predictor variables. Factors shown in **bold** significantly affected reproductive success

	Estimate	SE	<i>t</i>	<i>P</i>
A Female				
Constant	1.584	0.863	1.84	0.078
nb Partners	0.0897	0.0198	4.53	<0.001
Offspring mean IR	-5.34	1.97	-2.71	0.012
Adult length	0.0014	0.0012	0.95	0.353
Adult parent IR	-0.099	0.495	-0.20	0.842
B Male				
Constant	1.979	0.329	6.01	<0.001
nb Partners	0.1658	0.0194	8.56	<0.001
Offspring mean IR	-1.42	1.11	-1.27	0.211
Adult length	-0.0004	0.0006	-0.77	0.448
Adult parent IR	-0.400	0.484	-0.83	0.414

$t_{37}=0.26$, $P=0.799$). The general linear model, which included potential predictors of reproductive success, showed that for females, the number of partners (positive effect) and mean IR_o (negative effect; see also Fig. 1) were the only significant predictors of individual reproductive success (Table 1A). These two effects were largely independent as a partial correlation analysis including mean IR_o , number of partners and reproductive success revealed that the partial relationship between mean IR_o and reproductive success ($pr=-0.296$, $P=0.015$) and number of partners and reproductive success ($pr=0.758$, $P<0.001$) was strong and significant, whereas the partial correlation between mean IR_o and the number of partners was weak and non-significant ($pr=-0.002$,

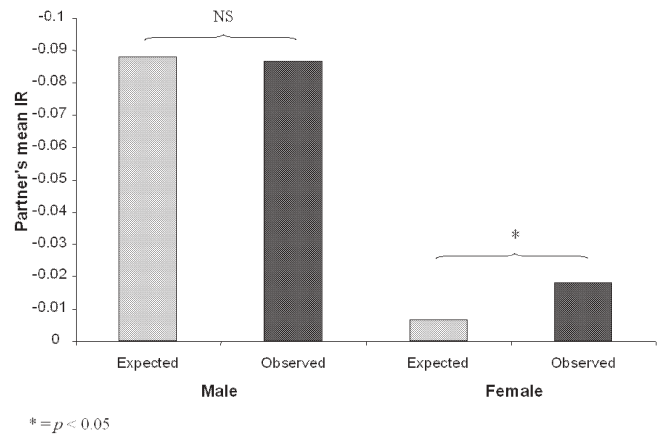


Fig. 2 Expected (population mean) and observed (mean of all partners) mean IR for male and female (mean IR_a) Atlantic salmon mating partners. Difference is significant for females ($P=0.035$) but not for males ($P=0.827$). Note that the y-axis is reversed

$P=0.665$). For males, only the number of partners showed a significant positive effect (Table 1B). There was no effect of either within-adult genetic diversity (IR_a) or size in both sexes (see Table 1A, B).

Finally, our results showed a preference for females to mate with males that were significantly more outbred than the male population mean IR_a (female partner's mean $IR_a = -0.0182$, one sample t -test=2.11, $P=0.035$; Fig. 2). No such pattern was observed in males (male partner's mean $IR_a = -0.0867$, one sample t -test=0.22, $P=0.827$; Fig. 2).

Discussion

Our results demonstrate significant effects of both multiple paternity and individual offspring genetic diversity on reproductive success of females in a population of wild Atlantic salmon. We also found that females with a higher number of males fertilizing their eggs had more outbred progeny on average (higher mean IR_o), but that both of these effects separately resulted in greater reproductive success, expressed in terms of surviving offspring. The genetic diversity–fitness relationship we observed may be attributed to a lower survival of inbred individuals at early life stages, related to their poorer viability, which would then result in individuals producing more outbred individuals having a higher number of offspring recruiting on average. These results thus show that females benefit from having offspring with a high within-individual genetic diversity (IR_o).

Our findings also indicate that this genetic diversity–fitness association is more important for females as this pattern was not significant in males. The relationship observed between reproductive success and both the number of partners and the average degree of within-individual offspring diversity (mean IR_o) indicates that multiple paternity results in important direct (by increasing number of mates) and indirect (by potentially increasing individual offspring genetic diversity) genetic

benefits for females. This observation contradicts traditional views of sexual selection theories (Bateman 1948; Trivers 1972), which predict that females would gain very little (in terms of offspring produced) by mating with multiple males. However, recent studies have demonstrated that females may gain different advantages from such behavior (see for example Tregenza and Wedell 2002). Thus, traditional theoretical views of cost–benefit trade-offs for males and females might not be valid in cases where multiple mating occurs in both sexes. For example, taking into account the inequality between sexes in the costs of multiple mating might be a more appropriate approach to explain the difference we observed here. In polygynous systems, inbreeding should be more costly for females than males, as inbred matings benefit males by adding a few offspring to their reproductive output without forfeiting other mating opportunities whereas for females, this may result in drastic reductions in their lifetime reproductive output (see Lehmann and Perrin 2003). As such, the payoff for multiple mating should be greater in females, leading us to propose that females may be more discriminating in selecting outbred mates than males, a possibility also supported by our results (see Fig. 2). As suggested by Kokko et al. (2003), the direct cost of multiple mating, roughly the inverse of the female's fecundity, is a U-shaped function of the effort that females expend screening males. Low screening effort leads to maladaptively frequent mating, whereas too high an effort leads to infertility, or is, by itself, energetically costly (Kokko et al. 2003; see also Lehmann and Perrin 2003). The fact that females might be discriminating among mates has been previously demonstrated by studies showing female preferences for mates with complementary genotypes in order to generate more diversified progeny (Landry et al. 2001; Aeschlimann et al. 2003). Moreover, recent evidence suggests that females have greater offspring viability after selectively seeking multiple paternity in both mammals and birds (see Hohoff et al. 2003; Foerster et al. 2003). The present study represents, to our knowledge, the first evidence of such a phenomenon in fishes where external fertilization is thought to reduce the potential for female choice and provide ample opportunities for multiple fertilizations of a given batch of eggs.

There are suggestions in the literature that the maximization of offspring diversity is not necessarily the best way of achieving the highest fitness. Instead, optimal outbreeding theory predicts that individuals should select mates that are neither too close nor too distant with respect to their own genotype to avoid a depression in offspring fitness (see Pusey Wolf 1996). Indeed, the maximization of offspring diversity can potentially lead to outbreeding depression, which would disrupt or prevent the development of local adaptations that could ultimately impact the fitness of Atlantic salmon (see McGinnity et al. 2003 for an example of the impacts of the loss of local adaptations). As such, we have also tested if a model of optimal within-individual diversity would better explain the resulting reproductive success observed by applying a

quadratic function (optimal function) instead of the model presented in Fig. 1. We found that the quadratic function was not significant in either sex (results not shown), which suggests that our former model of maximization of diversity in females performs better at explaining the pattern of reproductive success we found. This suggests that maximization rather than optimization of outbreeding has occurred in the present population. Yet, further experimentation and modeling are required to clearly establish the conditions under which our observations would stand.

It could be argued that the results reported here are specific to small or bottlenecked populations. However, the population from which our sample was randomly drawn has a much larger number of individuals (annual run of ~1,000 adults for the entire river). Nevertheless, it has been shown that within-river population structure is significant in salmon (Garant et al. 2000) and, as such, salmon form smaller subpopulations where the opportunity for inbreeding increases. In fact, the probability that all offspring of a female could be the result of a sib-mating decreases with the number of mating events, and the same should be true for males. However, we observed that the number of partners was the same for both males and females and that the association between the mean internal genetic diversity of progeny and the number of partners was not significant in males. One further possibility is that because females were on average more outbred than males in the study population, even unselective males obtained mates that were more outbred than the partners of discriminating females (Fig. 2). It could also be argued that an atypical aspect of the study was that mature male parr (small males that reach sexual maturity in freshwater as early as the second summer of life), which are often significant genetic contributors during the spawning season in Atlantic salmon (see Garant et al. 2002), were absent from our study site. However, the incidence of parr maturity within a given population is usually highly variable in the wild and ranges from 0 to 100% (Aubin-Horth and Dodson 2004). Our population might therefore lie at one extreme of a natural situation, but is still representative of situations encountered in wild rivers. Therefore, the phenomenon observed here is not an artifact and could be widespread in Atlantic salmon.

The importance of this result for conservation genetics is obvious. Atlantic salmon is declining dramatically over most of its range (Kellog 1999) and consequently, numerous supportive breeding programs are attempting to increase numbers of individuals through artificial reproduction. Supportive breeding schemes usually use one mate per female and force reproduction between individuals under controlled conditions. Our results suggest that such procedures might impair the subsequent survival of offspring produced. Knowledge regarding the reproductive aspects of the species' behavioral ecology in such programs should therefore be beneficial to their long-term achievement. There is also a need for more studies investigating the benefits of individual genetic diversity to be conducted under natural conditions, as laboratory ex-

periments might mask the potential negative effects that inbreeding could have in the wild (Joron and Brakefield 2003).

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