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# Melanin-based coloration of sneaker male Atlantic salmon is linked to viability and emergence timing of their offspring

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The 'good genes' hypothesis of sexual selection predicts that male ornaments are favoured by female mate choice because male ornament reveals genetic quality. In species with different male reproductive tactics, variation in genetic quality among 'sneaking' males has rarely been investigated, as usually 'sneakers' are thought not to be chosen by females. Here we focused on the alternative reproductive tactic in Atlantic salmon (*Salmo salar* Linnaeus, 1758) to test whether the skin colour of sneakers may reveal the performance traits of their offspring. A fully factorial breeding design was realized between 20 sneakers and two females using *in vitro* fertilization. We quantified the red and dark colorations of males and measured the survival of their progeny under semi-natural conditions. In addition, the size of offspring and their emergence timing from the gravel nest were monitored in the laboratory. We found that darker males sired more viable offspring, whereas red coloration was negatively correlated with offspring survival. Nevertheless, darker and redder male pigmentations were linked to a delay in offspring emergence. These results demonstrate that colours can reveal individual genetic quality in an alternative male reproductive tactic, with male melanin-based coloration being linked to both beneficial and detrimental effects for the offspring. Our results imply that sneaker ornaments may potentially play a role in both intra- and intersexual selection.  *Biological Journal of the Linnean Society*, 2014, **111**, 126–135.

ADDITIONAL KEYWORDS: alternative reproductive tactic – carotenoid coloration – good genes – male–male competition – melanin coloration – *Salmo salar*.

## INTRODUCTION

The 'good genes' hypothesis of sexual selection predicts that males of high genetic quality (i.e. providing indirect genetic benefits to females) are favoured by female choice (Andersson, 1994). Males can use colours as secondary sexual characters to signal their quality and attract mates. Colours are considered to be costly signals to produce and maintain, so that only high-quality males can afford them to display their quality (Griffith, Parker & Olson, 2006). For instance, three-spined stickleback males (*Gasterosteus aculeatus* Linnaeus, 1758) use their conspicuous red coloration to attract females (Milinski & Bakker, 1990), and redder males are dominant in male–male interactions (Bakker & Sevenster, 1983). Male–male competition seems to increase the honesty of the signal, as subordinate males decrease their degree of coloration during interactions with dominant individuals to signal their status and avoid fights (Candolin, 2000). Thus, mate

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choice and male–male competition could both promote colours as indicators of high-quality males.

In the animal kingdom, red and dark colorations are often involved in intra- and intersexual selection (Griffith *et al*., 2006). Red or orange coloration results from carotenoid pigments acquired through the diet. These pigments are involved in primary functions like antioxidant activity or the immune system (Peters, 2007). Therefore, red ornamentation has been described as an honest signal of the physiological and trophic condition of an individual (Olson & Owens, 1998; Griffith *et al*., 2006). As dominance and physiological condition are often correlated, Griffith *et al*. (2006) suggested that red coloration could predict the outcome of male–male competition (e.g. Wolfenbarger, 1999). It has also been shown that male coloration could be linked to sperm velocity. For instance, in the artic charr [*Salvelinus alpinus* (Linnaeus, 1758)] redder males produce faster sperm (Janhunen *et al*., 2009). The dark (or black) coloration is based on another pigment, melanin, which is metabolized through the melanocortin system. Ducrest, Keller & Roulin (2008) argued that genes involved in the melanocortin system also influence the expression of several physiological and behavioural traits. This would explain why darker males are often more aggressive and sexually active, for instance (e.g. West & Packer, 2002; Horth, 2003), as well as more resistant to stress (e.g. Senar *et al*., 2000; Kittilsen *et al*., 2009). Carotenoid- and melanin-based colorations involve different acquisition pathways, but they may both play a role in intrasexual selection, with darker and redder males being more often aggressive and dominant (Griffith *et al*., 2006).

One possible evolutionary outcome of male–male competition is the development of alternative male reproductive tactics (Gross, 1996; Snover, Watters & Mangel, 2006; Takegaki, Svensson & Kvarnemo, 2012). Besides males that invest in monopolizing access to females (hereafter called fighters), some males may alternatively exploit the investment of fighters to obtain access to female gametes (hereafter called sneakers) (Gross, 1996; Taborsky, Oliveira & Brockmann, 2008). Alternative reproductive tactics are very common in fishes, in which females are generally considered to preferentially reproduce with fighters rather than with sneakers (see Taborsky, 2008); however, because mating with multiple males may notably increase the fertilization rate of eggs (e.g. Smith & Reichard, 2005) and confer genetic benefits to females (e.g. Evans & Magurran, 2000), the presence of sneakers may be expected to improve female fitness in certain species. For instance, female Bitterling fish [*Rhodeus sericeus* (Pallas, 1776)] spawn more frequently in close proximity to sneakers and lay more eggs in the presence of high-quality sneakers, thereby improving the fertilization of the eggs (Smith & Reichard, 2005). Although alternative reproductive tactics are widespread in fish mating systems, relatively little is known about how precopulatory sexual selection operates in sneaker males. Notably, the link between ornament and genetic quality has never been investigated in sneakers, although it could reveal a potential role of sneaker ornament in competition among sneakers and female mate choice (Reichard, Le Comber & Smith, 2007).

In Atlantic salmon (*Salmo salar* Linnaeus, 1758), fighters (also called territorial males) and sneakers (also called precocious males) often coexist. Fighters are large anadromous males that spend 1–5 years at sea (depending on the population) before returning to freshwater for spawning. Sneakers are small, 1- to 4-year-old males that mature in freshwater without seaward migration. They display various patterns of dark and red coloration but, to our knowledge, the possible role of these colours in sexual selection has never been investigated. Anadromous males fight intensely for access to spawning territories or to females, and they establish a dominance hierarchy (Fleming & Reynolds, 2004). Conversely, sneakers remain hidden, to avoid attacks from large males, and they get close to the female only during spawning to furtively fertilize ova (Fleming & Reynolds, 2004; Verspoor, Stradmeyer & Nielsen, 2007). A strong competition also occurs among sneakers that can increase their reproductive success by defending the best position close to the female when ova are released (Myers & Hutchings, 1987; Mjølnerød *et al*., 1998; Esteve, 2005; Grimardias *et al*., 2010a). Once fertilized, eggs are buried by the female into the gravel where embryonic development lasts for several months. The mortality of salmonid embryos can be extremely high during this period because of hypoxia and pathogens (Bardonnet & Baglinière, 2000; Pompini, Clark & Wedekind, 2013). When the yolk sac is depleted, juveniles emerge from the gravel, begin to feed on invertebrate prey, and become territorial. Emergence timing and size at emergence are major life-history traits in salmonid fishes, as large and early-emerged fry are believed to acquire and defend the best feeding positions, and undergo lower predation rates (Brännäs, 1995; Einum & Fleming, 2000).

Empirical tests of good genes models of sexual selection should avoid confounding effects like differential female investment in offspring of attractive males or direct benefits provided by males (Uller, Eklöf & Andersson, 2005). In *S. salar* there is no evidence of parental care and fertilization is external, thereby allowing experiments based on *in vitro* fertilization. Male genetic quality should ideally be assessed through total fitness, inferred from lifetime

reproductive success (Hunt *et al.*, 2004), but this is extremely challenging to realize in many organisms. Here we evaluate the genetic quality of *S. salar* sneakers through several traits of their offspring (see below). In particular, we investigated whether sneakers vary in genetic quality, and whether this variation could be related to their colours. Dark and red coloration are linked to genetic quality in some other salmonids (e.g. Wedekind *et al.*, 2008; Eilertsen *et al.*, 2009), but to our knowledge such effects have never been tested in an alternative reproductive tactic. To infer the genetic quality of males we conducted a full factorial breeding design between 20 sneakers and two females, and measured embryo survival under semi-natural conditions, as well as juvenile emergence timing and body size in the laboratory.

## MATERIAL AND METHODS

### Fish origin and experimental design

Two females that had spent two years at sea and 20 sneaker males were caught in December 2008 by electric fishing in the Oir River, Normandy, France. Males and females were then kept in two separate tanks supplied with river water. All fish were anaesthetized with benzocaine, measured to the nearest 0.1 cm (standard length), and scales were sampled for age estimation by scalimetry. Pictures of each male were taken (see below for details) and gametes of all individuals were individually collected. All fish were then released back into the river. Fertilizations were made with a full factorial design: each male was crossed with each female, producing 40 full-sib groups ('North Carolina II' design; Lynch & Walsh, 1998). First, eggs from each female were distributed into 20 Petri dishes (with approximately 200 eggs per dish). Second, a 40-μL milt from each male was micropipetted into each Petri dish, followed by 50 mL of water (Wedekind & Müller, 2004). One hour after fertilization, eggs from each full-sib group were washed and split into two groups for incubation under both laboratory and semi-natural conditions. Water used for fertilizations and washing the eggs was aerated and reconstituted to chemically standardized water according to Organization for Economic Co-operation and Development (OECD) guidelines (OECD, 1992; Jacob *et al.*, 2010).

### Analysis of male coloration

The dark and red coloration of each male was quantified using digital pictures taken under standardized conditions, and we did not notice any skin coloration change resulting from anaesthesia (Fig. A1). Males were placed within a uniform, indirect, and diffuse light box. We took a picture of both sides of each fish using a Canon EOS 30D camera (lens: 17–40 mm, f4) and placed a grey colour patch on each image. Pictures of a colour checker (GretagMacbeth) and uniform grey background control were taken for calibration. The images were analysed using VISILOG 6.6 (Noesis). First, images were normalized by the uniform background picture and the grey colour patch, to correct for any variation in light and potential drift of sensors during the experiment. Second, the colour checker picture was used to transform RGB into XYZ by means of a linear regression model and then a nonlinear XYZ–L*a*b* colour space transformation was performed following the method described by Marty-Mahe *et al.* (2004). The L*a*b* colour space was used because it provides better colour discrimination than the RGB colour space (León *et al.*, 2006).

The relative surface of red or dark skin area was measured on each side of each fish (including the fins). Thresholds for dark colour pixels ($L^* < 35$) and red colour pixels ($a^* > 15$) were visually chosen following the method described by Wedekind *et al.* (2008). For each male's side, we calculated the relative dark and red surfaces (hereafter called darkness and redness) by dividing the number of dark or red pixels by the total number of pixels to control for body size. We averaged values for both sides of the fishes to get individual redness and darkness scores.

### Incubation conditions

After fertilization, 20 eggs of each full-sib group were randomly placed into two fine mesh screen cylindrical capsules (diameter, 15 mm; height, 60 mm; ten eggs per capsule), following Dumas & Marty (2006). Two sets of 40 capsules representing all full-sib groups were separated, then the capsules of each set were randomly pooled in groups of ten and placed into four wire-net baskets (diameter, 150 mm; height, 160 mm), filled with gravel. The resulting two sets of four baskets were introduced into two artificial redds (about 20 m apart) in a small tributary of the Oir River, also used by salmon as a natural spawning ground. Each basket was buried in the gravel at a depth of 15 cm and the water temperature was recorded every 15 min using an electronic thermometer data logger (mean ± SD temperature: 6.5 ± 1.6 °C).

In the laboratory a subset of 20 full-sib groups, corresponding to ten males crossed with both females, was incubated in a recirculated water system at a constant temperature of 10.1 ± 0.1 °C and a dissolved oxygen level of 10.95 mg $L^{-1}$ (95% oxygen saturation). Forty-five eggs from each male × female combination were incubated in three separate containers (50 mm × 50 mm, 15 eggs per container) made with

plastic perforated plates to ensure water circulation. Unfertilized eggs were counted and removed 31 days after fertilization. Embryo mortality and hatching was monitored daily; dead embryos and alevins were removed to prevent any infection.

To estimate the fertilization and early development success, 30 eggs of each full-sib group were incubated in 12-well tissue culture plates (IWAKI) kept in a climate chamber at constant temperature (8.95 ± 0.01 °C). Eggs were placed in individual wells filled with 4.5 mL of chemically standardized water (OECD, 1992). Thirty-five days after fertilization (313 accumulated degree days, ADDs), we calculated the ratio of 'eyed eggs' (i.e. with embryos visible through the egg chorion) to the total number of eggs for each full-sib group. This number reflected the proportion of developed embryos, excluding both unfertilized eggs and embryos that died early. It was used to compute an expected number of developing embryos for each full-sib group to infer unbiased survival rates at a later stage (see below).

### Offspring measurements

To measure embryo survival under semi-natural conditions, egg capsules were removed from the gravel and transferred to the laboratory 66 days after fertilization (430 ADDs). Undeveloped and/or dead embryos were discarded, whereas the others were then incubated in a recirculated water system following the protocol described above. Survival rates were calculated for each full-sib group as the number of embryos alive at the yolk-sac depletion stage (870 ADDs) divided by the expected number of developing embryos.

Sixty-six days after fertilization (670 ADDs), 30 embryos of each of the 20 full-sib groups initially reared in the recirculated water system were transferred into two emergence boxes (15 embryos per box) to study the timing of emergence and to measure size at emergence. These boxes were vertical cylinders (diameter, 90 mm; height, 120 mm) made from plastic pipes, filled with gravel (2–3 cm particle size), and covered with a transparent plastic lid. Boxes were continuously supplied with 10 °C recirculated water entering at the bottom and leaving through a small opening in the lid. Illumination (12-h light/12-h dark) came from overhead neon tubes (Daylight 365; Mazdafluor). A wire-netting trap connected to the outlet of each box was monitored daily to collect emerged juveniles. Each fish was then over-anaesthetized following the guidelines from the 'Ecole Nationale Vétérinaire de Nantes' with a solution of benzocaine (0.050 g $L^{-1}$), and then measured to the nearest 0.1 mm from snout to tail with an electronic Vernier calliper. Overall, we collected three offspring measures: embryo survival under semi-natural conditions; size at emergence; and emergence timing under laboratory conditions.

### Data analysis

We used linear mixed effects models (LMMs) fitted by restricted maximum likelihood (REML) to test the effect of paternal traits on size of alevins at emergence and emergence timing. Embryo survival was modelled by generalized linear mixed effects models (GLMMs) with a binomial error family. All models included the following random terms: male identity; female identity; and their interaction. In the GLMM on survival data under semi-natural conditions, we also entered the 'basket' and redd where the embryos were incubated as additional random effects. In LMMs with size at emergence and emergence timing as response variables, we entered the emergence box where embryos were incubated as an additional random effect. Sire age, length, redness, and darkness were included as fixed effects in the models. Graphical inspections of the data were used to ensure that assumptions of LMMs were met.

We used likelihood ratio tests (LRTs) to test the significance of paternal effects on offspring traits. LRTs are based on the difference in the approximated likelihood following the inclusion of the focal explanatory variable into the partial model comprising all other variables. This procedure, based on the comparison of the goodness of fit between models, is frequently used with LMMs and GLMMs (e.g. Jacob *et al*., 2007; Jaquiéry *et al*., 2010). The scale parameter of the GLMM on survival data was 0.91, indicating a minor level of under-dispersion of the data. Additionally, we computed Spearman's correlation coefficients ($r_s$) between the four male traits, and between the three offspring measurements. We also tested how male darkness and redness correlate with embryo survival and emergence timing by using Spearman's correlations. Analyses were performed with R (R Developpment Core Team, 2009), and we used the lme4 package for LMM and GLMM analyses (Bates & Maechler, 2010).

## RESULTS

The average size (±SD) of males was 124 ± 6 mm; 13 males were aged 1 year, four were aged 0 years, and three were aged 2 years. Male size was positively correlated with male age and negatively correlated with darkness, but we did not detect any other significant correlations among male traits (Table 1).

Very few eggs were unfertilized, as the proportion of developed embryos in tissue culture plates at 313 ADDs averaged (±SD) 98.8 ± 3.7% over the

**Table 1.** Spearman's correlation coefficients between traits in males

| | Male age | Male darkness | Male redness |
|---|---|---|---|
| Male size | **0.80** | **−0.55** | 0.38 |
| Male age | – | −0.36 | 0.37 |
| Male darkness | – | – | −0.18 |

Significant $P$ values are indicated in bold; $N = 20$.

males (range: 81.7–100%). The average survival (±SD) assessed at 670 ADDs for embryos reared in the recirculated water system for the emergence experiment was 96.5 ± 3.1% (range: 91.1–100%). For individuals first incubated in the field and then in the laboratory, the average embryo survival (±SD) assessed at 870 ADDs was 75.1 ± 18.7% (range: 35–100%), and was strongly affected by both female and male effects (Table 2). The effects of age and size of males were not significant, whereas embryo survival was positively related to male darkness and negatively related to male redness (Fig. 1; Table 2). Offspring survival also differed significantly among baskets, and showed significant male × female and male × redd interactions (Table 2). Given this significant male × female interaction, we also performed analyses for each female separately: male darkness was strongly related to increased embryo survival, with no effect of male redness in one female and no significant effect of male darkness, but a negative relationship between male redness and embryo survival was observed for the second female (Table A1). As neither the female × male darkness interaction (LRT, $\chi^2 = 1.507$, d.f. = 1, $P = 0.220$) nor the female × male redness interaction (LRT, $\chi^2 = 0.771$, d.f. = 1, $P = 0.380$) were significant, the effect of male darkness and male redness on embryo survival did not significantly differ between the two females.

On average, emergence occurred 96 days after fertilization (974 ± 3 ADDs). The mean size at emergence (±SD) was 27.7 ± 0.2 mm. Male size was not significantly linked to offspring size or emergence timing (Table 2). Descendants of darker males emerged later and tended to be bigger than those of pale males (Fig. 1; Table 2). Descendants of redder males tended to emerge later than those of less red males (LMM not significant, but significant $r_s$; Fig. 1; Table 2). No significant male × female interaction was observed for offspring size and emergence timing.

Embryo survival and emergence timing tended to be positively correlated ($r_S = 0.55$, $N = 10$, $P = 0.10$), whereas offspring size did not correlate with embryo survival ($r_S < 0.01$, $N = 10$, $P > 0.99$) or with emergence timing ($r_S = 0.14$, $N = 10$, $P = 0.71$).

**Table 2.** Model estimates (±SE), $\chi^2$, and $P$ values of generalized linear mixed models (GLMMs) and linear mixed models (LMMs) testing the effects of parental traits and environmental factors on embryo survival, measured under semi-natural settings, and on size at emergence and emergence timing of juveniles in the laboratory

| | Embryo survival† | | | Size at emergence | | | Emergence timing | | |
|---|---|---|---|---|---|---|---|---|---|
| | Estimate ± SE | $\chi^2$ | $P$ | Estimate ± SE | $\chi^2$ | $P$ | Estimate ± SE | $\chi^2$ | $P$ |
| Male* | – | 25.837 | < 0.001 | – | 0.689 | 0.407 | – | 0.000 | 1.000 |
| Female* | – | 4.446 | 0.035 | – | 0.000 | 1.000 | – | 3.571 | 0.059 |
| Basket* | – | 24.049 | < 0.001 | – | – | – | – | – | – |
| Redd* | – | 0.066 | 0.797 | – | – | – | – | – | – |
| Emergence box* | – | – | – | – | 1.357 | 0.244 | – | 41.611 | < 0.001 |
| Male × female* | – | 8.814 | 0.003 | – | 3.237 | 0.072 | – | 0.000 | 1.000 |
| Male × redd* | – | 13.588 | < 0.001 | – | – | – | – | – | – |
| Female × redd* | – | 0.000 | 1.000 | – | – | – | – | – | – |
| Male size | −0.005 ± 0.019 | 0.083 | 0.773 | 0.006 ± 0.005 | 1.202 | 0.273 | 0.050 ± 0.034 | 2.175 | 0.140 |
| Male age | 0.736 ± 0.804 | 0.811 | 0.368 | – | – | – | – | – | – |
| Male darkness | 0.562 ± 0.198 | 7.660 | 0.006 | 0.132 ± 0.073 | 3.782 | 0.052 | 0.903 ± 0.461 | 3.875 | 0.049 |
| Male redness | −1.336 ± 0.657 | 3.912 | 0.048 | −0.497 ± 0.340 | 2.505 | 0.114 | 3.657 ± 2.139 | 2.985 | 0.084 |

Model estimates for fixed effects were based on full models including all effects. $\chi^2$ and $P$ values refer to likelihood ratio tests (see Material and methods).
*Random effects.
†Binomial distribution.

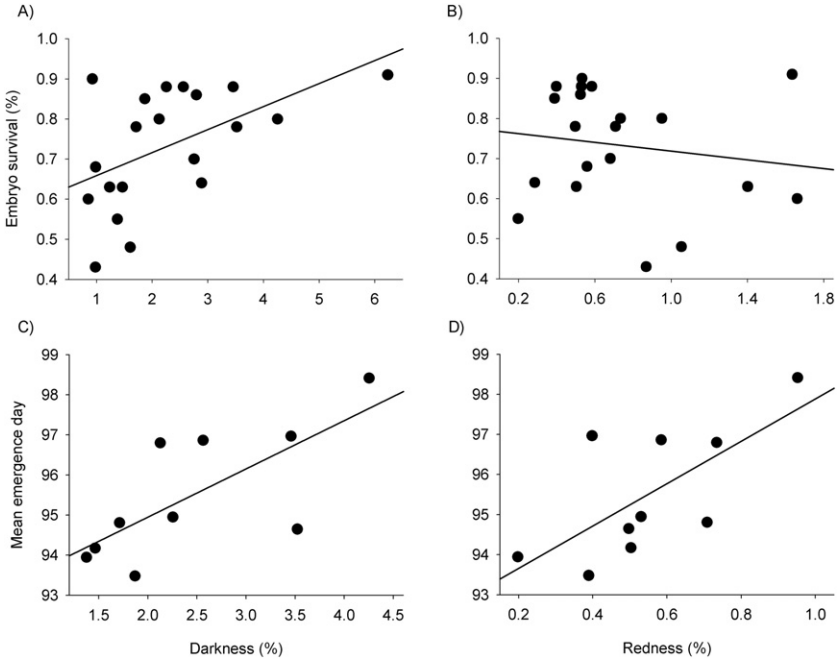


**Figure 1.** Relationships between offspring survival and male darkness (A; $r_s = 0.52$, $N = 20$, $P = 0.018$) and redness (B; $r_s = -0.13$, $N = 20$, $P = 0.586$), and between emergence timing and male darkness (C; $r_s = 0.72$, $N = 10$, $P = 0.024$) and redness (D; $r_s = 0.66$, $N = 10$, $P = 0.044$).

## DISCUSSION

We observed a significant effect of the melanin-based dark coloration of the sneakers on the survival, emergence timing, and, to a lesser extent, size of their offspring. Darker males sired more viable and larger offspring that emerged later than the progeny of lighter males. The carotenoid-based red coloration of males was also marginally linked to the survival rate and timing of emergence, with redder males producing less viable and later-emerging descendants. Effects of male ornament on offspring performance have been reported in other species (e.g. Wedekind *et al*., 2008; Eilertsen *et al*., 2009; Huuskonen, Haakana & Kekäläinen, 2009), which were all based on males from the dominant reproductive tactic. Our results show that sneaker males also vary in genetic quality, with this variation being revealed by melanin-based coloration.

In vertebrates, parental coloration has been shown to correlate with offspring performance, e.g. embryo viability (Wedekind *et al*., 2008), developmental homeostasis (Roulin *et al*., 2003), and immune responsiveness (Roulin *et al*., 2000). In the salmonid species brown trout (*Salmo trutta* Linnaeus, 1758), embryo viability is positively linked with male dark coloration and negatively linked with male redness (Wedekind *et al*., 2008). The study mentioned was performed in a dominant male reproductive tactic, and may be interpreted as a good genes effect, in which female choice could favour the development of honest ornamentation. Interestingly, we found a similar pattern in *S. salar* sneaker males, i.e. embryo survival linked positively with male darkness and negatively with male redness. This suggests that a similar proximate cause may be involved between male coloration and offspring performance in these two closely related species, and across male reproductive tactics. Furthermore, our study revealed that darkness was also correlated with a delay at emergence, which is probably linked to detrimental effects for offspring (Brännäs, 1995; Einum & Fleming, 2000). Male ornamentation, reflecting both fitness costs and benefits, has previously been described between offspring immune defences and viability (Kortet *et al*., 2004) or growth rate (Barber *et al*., 2001). In accordance with Barber *et al*. (2001), we found that highly ornamented males may sire offspring with a slower but higher quality development, as revealed by lower embryo mortality. Additionally, because melanin-based coloration seems to be heritable in salmonids (Wedekind *et al*., 2008; Kittilsen *et al*., 2009), offspring of darker males may have a higher activity of the melanocortin system, compared with offspring of lighter males. Such energy expenditure for the development of dark coloration may decrease the resources available for the development of other traits, hence delaying emergence timing.

Although the pathway through which male coloration correlates with offspring performances is at present unknown in salmonid species, the observed effect leads to perspectives on the potential role of sneaker coloration in both male–male competition and female mate choice. In *S. salar* there is strong competition among sneakers to gain the best position during female spawning, and the dominance among sneakers may involve male coloration and body size. Dark coloration has been associated with subordinate behaviour in juvenile *S. salar* (Thomaz, Beall & Burke, 1997; Garant, Dodson & Bernatchez, 2005), but the role of sneaker coloration on dominance status and reproductive success has never been investigated. In contrast, several studies reported significant effects of male size, with larger sneakers having a higher reproductive success than smaller individuals (Myers & Hutchings, 1987; Thomaz *et al*., 1997; Garant *et al*., 2002). Interestingly, a recent study suggested that this relationship might not hold in habitats where shelters are available for sneakers (Grimardias *et al*., 2010a); however, given the negative correlation we observed between size and darkness of males, it is difficult to make any prediction on the relationship between darkness and dominance in sneakers.

Our study reveals that correlations between male ornamentation and genetic quality can also be detected among sneaker males that are not supposed to be chosen by females. Reichard, Le Comber & Smith (2007) proposed that in certain species, females may in fact benefit from sneaking (but see Taborsky, 2008). For instance, in Bitterling fish (*R. sericeus*), females spawn more frequently close to sneakers and lay more eggs near large sneakers, conferring a higher fertilization rate to females (Smith & Reichard, 2005). In bluegill sunfish (*Lepomis macrochirus* Rafinesque, 1819), sneakers sire offspring with higher growth rates and body sizes than those sired by fighters (Neff, 2004). In *S. salar*, although there is at present no indication that females choose to spawn preferentially in the presence of sneakers, parentage analyses in the wild have revealed that multiple paternity is the rule, with sneakers usually siring many fewer juveniles than fighters, even though the former could sire up to 87% of offspring (Morán *et al*., 1996; Grimardias *et al*., 2010b). There is also some indication that juveniles sired by sneakers may have faster early growth than those sired by anadromous males (Garant *et al*., 2002), and that females with multiple partners have higher reproductive success (Garant *et al*., 2005). Hence, we hypothesize that *S. salar* females may gain indirect genetic benefits from the presence of sneakers in spawning sites. Furthermore, given our results, we speculate that *S. salar* females could select spawning sites where dark sneakers are present. Testing these hypotheses would clearly require additional experiments with larger sample sizes, behavioural assays, and lifetime reproductive fitness analyses.

Finally, we observed a significant male × redd interaction on embryo survival, which suggests a genotype by environment interaction (see Charmantier & Garant, 2005); however, only two redds were used, and a larger sample size would thus be required to investigate such effects in detail. We also detected a significant male × female interaction on embryo survival, which indicates potential genetic compatibility effects among mates (e.g. Rodrìguez-Muñoz & Tregenza, 2009). But this result is difficult to interpret because our experimental design, including many males but only two females, was primarily designed to reveal additive genetic effects. Furthermore, even if separate analyses for each female revealed that male darkness was significantly related to increased embryo survival in only one female, whereas the negative relationship between male redness and embryo survival was only observed in the other female (Table A1), male coloration effects did not significantly differ between the two females. Overall, these results emphasize the need for additional experiments, including a larger number of females to investigate the potential links between genetic compatibility and male coloration in *S. salar*.

In conclusion, our study demonstrates for the first time that male coloration can reveal genetic quality in an alternative reproductive tactic. Darker *S. salar* sneakers sired more viable offspring that nevertheless emerge later. Conversely, redder males seem to produce less viable and later-emerging offspring. Our results suggest that redder males may have a lower genetic quality than darker males. Our finding raises the possibility that, contrary to common expectations, both competition among sneaker males and female mate choice may affect male ornamentation in an alternative reproductive tactic.

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## ARCHIVED DATA

Data deposited in the Dryad repository (Marie-Orleach *et al*., 2013).

## APPENDIX

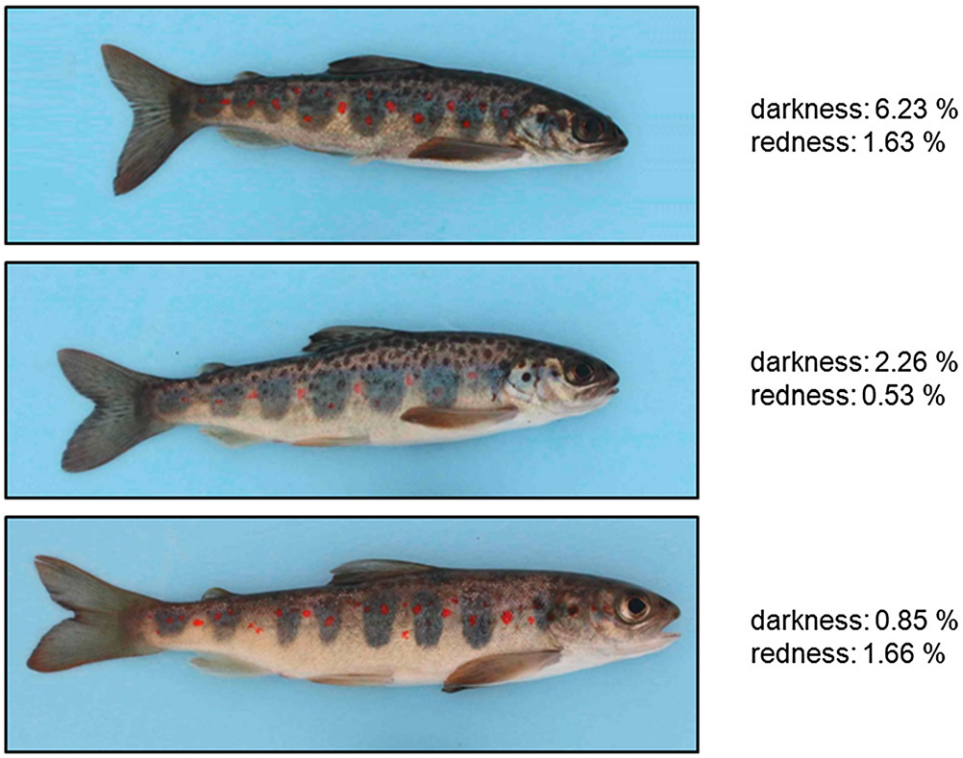


**Figure A1.** Examples of individuals with different levels of darkness and redness.

**Table A1.** Model estimates (±SE), $\chi^2$, and *P* values of generalized linear mixed models (GLMMs), testing for each female separately the effects of male traits and environmental factors on embryo survival measured under semi-natural settings

| | Embryo survival† | | | | | |
|---|---|---|---|---|---|---|
| | Female A | | | Female B | | |
| | Estimate ± SE | $\chi^2$ | *P* | Estimate ± SE | $\chi^2$ | *P* |
| Male* | – | 10.562 | 0.001 | – | 4.248 | 0.039 |
| Basket* | – | 0.000 | 1.000 | – | 5.230 | 0.021 |
| Redd* | – | 7.443 | 0.006 | – | 0.000 | 1.000 |
| Male × Redd* | – | 5.487 | 0.019 | – | 6.236 | 0.013 |
| Male size | 0.043 ± 0.017 | 5.469 | 0.019 | −0.049 ± 0.023 | 4.029 | 0.045 |
| Male age | −1.487 ± 0.722 | 3.824 | 0.051 | 2.705 ± 1.033 | 6.058 | 0.014 |
| Male darkness | 0.698 ± 0.201 | 10.774 | 0.001 | 0.354 ± 0.229 | 2.266 | 0.132 |
| Male redness | 0.126 ± 0.606 | 0.042 | 0.837 | −2.161 ± 0.746 | 7.074 | 0.008 |

Model estimates for fixed effects were based on full models including all effects. $\chi^2$ and *P* values refer to likelihood ratio tests (see Material and methods).
*Random effects.
†Binomial distribution.