Are secondary sex traits, parasites and immunity related to variation in primary sex traits in the Arctic charr?

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Primary and secondary sex traits are influenced by the same sex hormones, and the expression of secondary sex traits may consequently signal males' capacity for sperm production. Sperm quality may also be influenced by immune activity, as sperm are non-self to the male. Parasite infections alter immune activity and may thus reduce ejaculate quality. In the Arctic charr (*Salvelinus alpinus*) the red abdominal colour is considered an ornament that signals important information in mate choice. We captured and individually caged sexually mature male Arctic charr during the spawning period. Afterwards we estimated abdominal colour, parasite infections, gonad mass and several spermatological and immunological variables. Intensity of abdominal colour was positively correlated to testes mass, milt mass and sperm cell numbers produced. Additionally, males with low parasite intensities had high testes mass and produced milt with high sperm density, indicating a trade-off between parasite resistance and development of primary sex traits. Our measures of immunity were, however, not related to primary sex traits. We conclude that females evaluating male abdominal coloration may obtain information about differences between males in fertilization potential and parasite resistance.

Keywords: Arctic charr; sperm production; secondary sex traits; parasite infections; immunocompetence; female choice

1. INTRODUCTION

Benefits from female mate choice may either be direct, such as increased fertilization rate (Nakatsuru & Kramer 1982), or indirect, such as heritable parasite resistance, resulting in parasite-resistant offspring (Hamilton & Zuk 1982). Recently developed theory suggests, however, that females selecting males with elaborate secondary sex traits may obtain both high fertilization probability and good genes for their offspring (Folstad & Skarstein 1997). This idea rests on two important observations. First, sperm cells are non-self to the male (Beer & Billingham 1976), and are exposed to immune attack within the testes (Shulman 1995). Parasite-resistant males may therefore be at an advantage as they may be able to lower immune

activity during spermatogenesis, and thus produce ejaculates of higher quality compared with non-resistant males (Folstad & Skarstein 1997; Hillgarth *et al.* 1997). Support for this prediction has been reported in humans (*Homo sapiens*) where a general reduction in non-gonadal bacterial infections in men with high densities of leukocytes in semen results in reduced densities of leukocytes and improved ejaculate quality (Skau & Folstad 2003). Second, there seems to be a link between a male's ability to undergo immunosuppression and the development of secondary sex traits, as androgens, which promote both expression of primary and secondary sex traits, are also immunosuppressive (Folstad & Karter 1992). Thus, a female selecting males based on the expression of secondary sex traits may receive both high-quality ejaculates, ensuring fertilization of her eggs, and parasite resistance genes for her offspring.

We tested some predictions of the above theory in the Arctic charr (*Salvelinus alpinus*), which develop a red spawning coloration, signalling both parasite intensity and immune activity (Skarstein & Folstad 1996; Liljedal *et al.* 1999). We predicted the following: (i) a positive correlation between primary and secondary sex traits; (ii) a negative correlation between primary sex traits and parasite intensities; and (iii) a negative correlation between immune activity and primary sex traits.

2. MATERIAL AND METHODS

During 19–30 September 1998 we caught 36 sexually mature male Arctic charr (mean body length $\pm$ s.d. = 23.3 ± 2.3 cm; mean body mass $\pm$ s.d. = 159.1 ± 50.4 g) on a spawning ground in Fjellfrøsvatn, northern Norway. The fish were stripped for all available milt and placed in individual cages for 6 days to prohibit spawning activity during ejaculate production (see also Gage *et al.* 1995). After 6 days we collected the produced milt and estimated sperm density, total milt mass and sperm number (see Liljedal & Folstad 2003). We also collected blood samples from the caudal vessel for estimation of lymphocyte and granulocyte densities (see Liljedal *et al.* 1999). Thereafter the fish were killed, photographed and frozen. We estimated intensity of spawning coloration by using the photographs to produce numerical estimates for three colour properties; hue, saturation and brightness (see Skarstein & Folstad 1996).

During dissection eight groups of parasites were identified and counted; the digeneans *Phyllidostomum umblae*, *Diplostomum* spp. and *Crepidostomum* spp.; the cestodes *Diphyllbothrium* spp., *Eubothrium salvelini* and *Cyathocephalus truncatus*; the nematode *Cystidicola farionis*; and the crustacean *Salmincola* sp. We weighed the spleen and testes to the nearest 0.001 g, and counted the number of growth zones on the otoliths to determine fish age.

Using factor analysis, we constructed three principal components (PCs) from the parasitological variables. These PCs describe 67.2% of the variation in the parasitological variables (PC1, 33.9%; PC2, 20.4%; and PC3, 12.9%). We also constructed a PC representing the average abdominal colour for each fish. The PC describes 77.4% of the variation in abdominal colour variables.

Only one of the predictor variables (i.e. abdominal colour PC, the three parasite PCs and the immune variables) was tested against one of the primary sex traits (i.e. milt mass, sperm density, sperm numbers and testis mass) in each multiple regression model. In each model fish length and capture time were entered as covariates (capture time refers to the number of nights after the first fish was captured). Sample size is 36 in all tests.

Further details of methods are available in electronic Appendix A available on The Royal Society's Publications Web site.

3. RESULTS

(a) Secondary sex traits versus primary sex traits

Abdominal colour was not significantly related to sperm density (partial $r = 0.21$, $p = 0.2$), but was positively related to milt mass ($r = 0.53$, $p < 0.001$, figure 1a). Abdominal colour did, together with capture time, also explain variance in sperm numbers (abdominal colour:

Figure 1. The relation between abdominal colour PC and (a) log milt mass, (b) residual log sperm numbers, and (c) residual testis mass in sexually mature Arctic charr. The abdominal colour PC is a PC composed of the three colour variables: hue, saturation and brightness. Residual sperm numbers are the residuals from the linear regression between sperm numbers and capture time, whereas residual testis mass is the residuals from the linear regression between testis mass and fish length.

partial $r = 0.56$, $p < 0.001$, figure 1b; capture time: partial $r = -0.62$, $p < 0.001$). Moreover, abdominal colour was positively related to testis mass (partial $r = 0.44$, $p < 0.01$, figure 1c), although fish length (partial $r = 0.57$, $p < 0.001$) and capture time (partial $r = -0.36$, $p < 0.04$) also explained variance in testes mass.

(b) Parasite infections versus primary sex traits

Parasite PC1 and capture time were both negatively related to sperm density (parasite PC1: partial $r = -0.36$, $p < 0.04$, figure 2a; capture time: partial $r = -0.71$, $p < 0.001$). Parasite PC2 and PC3 did not, however, explain a significant portion of the variance in sperm density (parasite PC2: partial $r = 0.05$, $p = 0.77$; parasite PC3: partial $r = 0.06$, $p = 0.75$). Parasite PC2 was negatively related to testis mass, when including fish length in the model (parasite PC2: partial $r = -0.41$, $p < 0.02$, figure

Figure 2. The relation between parasite PC1 and (a) residual sperm density, and (b) the relation between parasite PC2 and residual testis mass in sexually mature Arctic charr. The parasites PC1 and PC2 are two of three PCs composed of eight parasite groups. Residual sperm density is the residuals from the linear regression between sperm density and capture time, whereas residual testis mass is the residuals from the linear regression between testis mass and fish length.

2b; fish length: partial $r = 0.76$, $p < 0.001$). PC3 and PC1 were not significantly related to testis mass (parasite PC3: partial $r = -0.28$, $p = 0.10$; parasite PC1: partial $r = -0.18$, $p = 0.32$). None of the three parasite PCs were significantly related to milt mass or sperm numbers (partial r -range: -0.19 – 0.04 , p -range: 0.26 – 0.8).

(c) Immunity versus primary sex traits

We did not find any significant associations between primary sex traits and the immune variables measured (i.e. granulocytes, lymphocytes and spleen mass; partial r -range: -0.24 – 0.10 , p -range: 0.16 – 0.99). It is, however, noteworthy that all but one of the 12 associations between primary sex traits and the immune variables were negative (for further details see electronic Appendix A).

4. DISCUSSION

Testis mass, milt mass and sperm numbers were all positively associated with abdominal coloration in the sexually mature male Arctic charr. Relations between ornamental development and primary sex traits are also documented in other species. For example, in greenfinches (*Carduelis chloris*) there is a positive correlation between feather brightness and testis size (Merilä & Sheldon 1999), and sperm quality is positively related with display rate in

other species (Mjelstad 1991; Matthews *et al.* 1997). Such observations are as expected given that high androgen levels promote both expression of secondary sex traits and sperm production (Folstad & Skarstein 1997; Hillgarth *et al.* 1997). Our results show that highly ornamented males possess larger testes and produce more sperm for a given time compared with less ornamented males. Thus, expression of spawning ornamentation seems to signal males' capacity for sperm production. However, red male Arctic charr caught on the spawning ground seem to have lower sperm density than pale males (Liljedal *et al.* 1999). This apparent contradiction may result from red males having higher mating frequencies relative to pale males. Thus, attractive males may under normal mating activity experience sperm depletion, even if they have a high rate of sperm production relative to other males (Engen & Folstad 1999). Alternatively, as suggested by a recent study, pale subordinate males may increase their sperm density to meet a higher level of sperm competition than in dominant males (Liljedal & Folstad 2003).

Testicular investment and sperm density were negatively related to parasite intensity. This supports earlier results in Arctic charr (Liljedal *et al.* 1999), and similar results are also reported for the red jungle fowl (*Gallus gallus* (Zuk *et al.* 1990)) and for oldfield mice (*Peromyscus polionotus* (Meagher & Dudek 2002)). A negative effect of parasites on testicular development and sperm production may be caused by parasites draining resources from the host. Alternatively, infected males may canalize resources to increased immune activity, resulting in reduced resource availability for testes development and sperm production. Additionally, raised immune activity may itself be harmful for sperm production. The immune system perceives sperm cells as non-self and immune responses interfere with sperm cells inside the testes (Folstad & Skarstein 1997). Parasite infections triggering immune reactions may thus create differences in fertilization capacity between males (Folstad & Skarstein 1997). A negative association between ejaculate quality and both the levels of circulating granulocytes and spleen mass has previously been documented in free-ranging male Arctic charr (Liljedal *et al.* 1999), and a negative relation between immune activity and testis size is also recorded in bats (*Chalinolobus morio* and *Nyctophilus geoffroyi* (Hosken & O'Shea 2001)). We did not, however, find any significant relations between the immune variables and primary sex traits in our study, although 11 of 12 correlations were negative. Thus, the present study does not provide support for the assumption that raised immune activity would negatively affect sperm production.

However, in summary our results suggest that males' sperm production is limited by their current intensities of parasitic infections, and that female charr can obtain information on males' sperm production by evaluating the

expression of their secondary sex traits. Given some level of heritability, females using this information in mate choice may produce parasite-resistant offspring, and additionally, sons with high fertilization potential.

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