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Indirect fitness consequences of mate choice in sticklebacks: offspring of brighter males grow slowly but resist parasitic infections

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'Good genes' models of sexual selection suggest that elaborate male sexual ornaments have evolved as reliable signals of male quality because only males of high genetic viability are able to develop and maintain them. Females benefit from choosing such individuals if quality is heritable. A key prediction is that the offspring of males with elaborate mating displays will perform better than those of less elaborate males, but it has proved difficult to demonstrate such an effect independently of the effects of differences in parental investment. We tested for 'good genes' linked to male ornamentation in the three-spined stickleback *Gasterosteus aculeatus* using *in vitro* fertilization to generate maternal half-siblings, which were raised without parental care. Maternal half-siblings sired by brightly coloured males grew less quickly than half-siblings sired by dull males but were more resistant to a controlled disease challenge. Among the offspring that became infected, those with brighter fathers had higher white blood cell counts. This suggests that highly ornamented males confer disease resistance on their offspring. The association with reduced growth suggests a mechanism for the maintenance of heritable variation in both disease resistance and male sexual coloration.

Keywords: sexual selection; *Gasterosteus aculeatus*; stickleback; parasites; ornamentation; growth

1. INTRODUCTION

Many studies have shown that parasitic infections can have a detrimental effect upon male sexual ornamentation, in that well-ornamented males are less heavily infected (Buchanan *et al*. 1999; Figuerola *et al*. 1999; Merila *et al*. 1999) and are preferentially chosen as mates (Borgia & Collis 1989; Pruett-Jones *et al*. 1990; Zuk *et al*. 1990; Petrie *et al*. 1991). Possible direct benefits of such preferences include reduced risk of sexually transmitted disease, reduced risk of vertical transmission of microparasites to offspring and, where relevant, improved parental care (but see Stott & Poulin 1996).

It is also suggested that females may make indirect fitness gains from choosing parasite-free males if, by doing so, they select genes for traits related to improved offspring performance, such as increased resistance to parasites (Andersson 1994). In a range of species, a weak but significant correlation has been found between offspring survival and male ornamentation (Møller & Alatalo 1999). Several experimental studies also indicate that highly ornamented males may pass on inherited beneficial traits to their offspring (e.g. Reynolds & Gross 1992; Petrie 1994).

However, unambiguous detection of the effects of 'good genes' has remained a problem for a number of reasons (Siva-Jothy & Skarstein 1998). It is often very difficult to control for maternal effects: large females producing large eggs may pair naturally with attractive males, potentially creating a non-causal association between male ornamentation and offspring performance (e.g. Kraak & Bakker 1998). In addition, parents may vary post-fertilization investment, including parental care, depending on the ornamentation of their mate, so even if females are paired at random with respect to male ornamentation, male trait and offspring performance may be related through non-genetic effects.

One way of circumventing these problems is to use a species with external fertilization, the advantage being that simple *in vitro* techniques can be used to generate maternal half-siblings, sired by different males, derived from a single female's simultaneously developed oocytes (Barber & Arnott 2000). We adopted this approach to examine the performance of three-spined stickleback (*Gasterosteus aculeatus*) fry sired by fathers that differed in their degree of carotenoid-based sexual ornamentation.

The three-spined stickleback has become an important model organism for studying the evolution of male ornamentation (Braithwaite & Odling-Smee 1999). Males develop carotenoid-based pigmentation in the skin of their lower throat during the breeding season (Czeczuga 1980; Wedekind *et al*. 1998; Barber *et al*. 2000). This coloration is condition dependent and impaired by some parasitic infections (Milinski & Bakker 1990). Females prefer males with more intense red sexual coloration (Milinski & Bakker 1990; Braithwaite & Barber 2000). Our aim in this study was to examine possible indirect (i.e. genetic) fitness consequences of this well-documented sexual preference for bright male ornamentation, whilst controlling for both maternal effects and parental-care effects. Our approach was to measure several indexes of performance, including survival, growth and resistance to parasitic infection, in groups of maternal half-siblings sired by bright and dull male sticklebacks.

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2. MATERIAL AND METHODS

(a) *Fish collection and husbandry*

We collected three-spined sticklebacks in April 1997 and 1998 from Inverleith Pond, Edinburgh, UK. To supplement the number of breeding males in 1998, a small number of fishes were sampled from a second Edinburgh pond (Craiglockhart). Following transfer to the laboratory, breeding males were placed into individual aquaria (35 cm × 20 cm × 20 cm) and stimulated to build nests by providing nesting material and presenting breeding females (in a jar) twice daily. One day after nest completion (i.e. the first time the male 'crept through' it; Van Iersel 1953) males were screened for intensity of red coloration (see § 2(b)). Females were housed in communal holding tanks (100 cm × 40 cm × 30 cm) and fed daily, to excess, with bloodworms (*Chironomus* sp.).

(b) *Quantifying breeding coloration*

Individual males were placed in a black foam-lined water-filled cell (10 cm × 2 cm × 2 cm) with a high-quality glass front window through which the lateral and ventral surfaces of the fishes were photographed (Frischknecht 1993). An SLR camera (model T90; Canon, Tokyo, Japan) (shutter speed 1/90 s; aperture f11) with a macro lens (90 mm, ϕ55, 1 : 2.5) and colour transparency film (Ektachrome 200ASA; Kodak, Rochester NY, USA) was used. Illumination was from two simultaneously activated flash units (model 283; Vivitar, Swindon, UK) angled at 45° to the box. Images were scanned into a PC and imported into the photographic publishing package PhotoShop 4.0 (Adobe Systems, Inc., San Jose, CA, USA) for colour analysis (Villafuerte & Negro 1998; Barber *et al*. 2000). Areas of nuptial coloration were selected using the 'magic wand' tool and analysed for colour on a pixel-by-pixel basis. Mean brightness for each of three colour channels (red, green and blue: $\bar{r}$, $\bar{g}$, and $\bar{b}$, respectively) was recorded. Redness intensity (i) was calculated for the lateral and ventral images of each fish, using equation (1) (Frischknecht 1993):

$$i = \frac{\bar{r}}{\bar{r} + \bar{g} + \bar{b}}. \quad (1)$$

Since lateral and ventral intensity scores were highly correlated ($r = 0.79$, $n = 42$, $p < 0.001$), a single index of redness intensity (I) was derived for each fish from a weighted average of the lateral (multiplied by two to take into account the two sides of the head) and ventral scores.

(c) *Split clutch* in vitro *fertilization*

Maternal half-sibling embyros were generated using sperm from pairs of sexually receptive males (same population source as the mother) matched for body size, using an *in vitro* fertilization (IVF) protocol adapted from Hagen (1967) (for details of IVF, see Barber & Arnott (2000)). The male with the higher redness score within each pair was termed the 'bright' male and the other was termed the 'dull' male. Gravid females that had recently given 'head up' responses to another (non-experimental) courting male were selected from the holding tanks and egg clutches stripped into moistened watch glasses. Each full-clutch was divided into two approximately equally sized half-clutches, which were placed into separate fresh watch glasses. One half-clutch was then fertilized with sperm from the bright male of a father pair and the other was fertilized with sperm from the dull male. The order in which the bright and dull sires were used was randomized. Sperm was extracted by teasing apart the testes of freshly sacrificed males with fine needles. Sperm and eggs were mixed by gentle agitation of the watch glass, left for 15 min and transferred to 1-l aerating incubators (Barber & Arnott 2000).

(d) *Egg care and fry rearing*

Fertilized half-clutches were examined daily. Eggs that were unfertilized, non-developing or showed any early signs of fungal infection were removed from the clutch using fine forceps. The proportion of eggs hatching from each half-clutch was recorded. On hatching, fry were transferred to 1-l containers and, after 21 days, to 20-l aquaria. Fry were fed initially on Liquifry no. 1 Egglayers^{TM} fry food (Interpet Ltd, Dorking, UK), before progressing onto freshly hatched brine shrimp (*Artemia*) nauplii and finally chopped bloodworms (*Chironomus* sp.). Water quality was maintained by the use of biofilters and by frequent changes with matured water. Temperature was maintained at 14 ± 1 °C throughout the study. Post-hatch survival within each half-brood was monitored on a daily basis.

(e) *Growth measurement* (*1997*)

A census of the lengths of fry in individual half-broods was made 62 days after hatching. Because of the fragility of the small fry, a photometric technique was used. Half-broods were transferred to shallow Petri dishes and photographed from directly above against a 1 mm grid. Photographs were digitized and fry lengths measured using the public-domain NIH-Image program (US National Institute of Health: http://rsb.info.nih.gov/nih-image/) on an Apple Macintosh^{TM} computer. Growth rate of half-broods (measured as the mean length-at-age of fry in the half-brood) was strongly dependent on rearing density. To control for this, rearing density was quantified as the cumulative number of fry present in the rearing tank on each day from day 0 to day 62 (cumulative competitor density, CCD). Mean length-at-age was significantly correlated with CCD (length = −0.0517 CCD + 28.6, $F_{1,31} = 91.90$, $p < 0.0005$), so growth rates of fry within groups were expressed as residuals from this relationship (density-corrected length-at-age, rLength). Since temperature was held constant throughout the study period, no adjustment was needed for temperature-dependent variation in growth rates.

(f) *Experimental infections* (*1998*)

After an initial batch-rearing period of approximately two months, half-siblings from nine full-broods (four to 18 fishes per brood, 78 fry altogether) were transferred to individual 1-l perforated containers. These were maintained in a series of 10-l tanks, each of which contained up to six full- and half-sibling fish containers that shared a common water supply. Fishes were fed to satiation daily on frozen bloodworm. Experimental infections were carried out (UK Home Office Licence number 60/2025) at approximately three months after hatching, when many fry in our study populations naturally acquire the infection (Tierney *et al*. 1996). The fishes were starved for 48 h, transferred to individual 300 ml plastic cups and fed a single copepod containing a known dosage of infective (i.e. cercoma-bearing; Smyth 1969) larvae of the cestode parasite *Schistocephalus solidus*. Infective parasite stages were reared using a protocol adapted from Smyth (1962). Parasite eggs, acquired by *in vitro* maturation of adult *S. solidus* worms, were hatched and fed to a culture of laboratory-reared copepods (*Cyclops strenuus abyssorum*; Sciento, Manchester, UK), which are intermediate hosts of the parasite. Infected copepods were maintained for 30 days at 15 °C, then

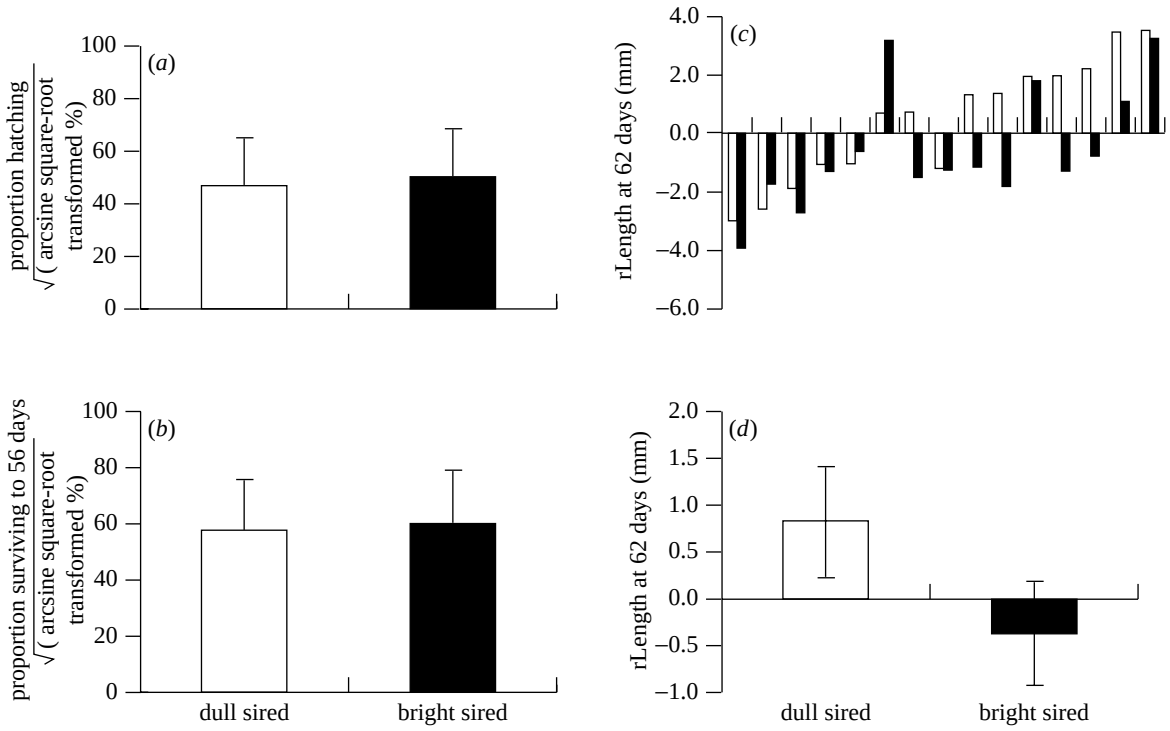

Figure 1. Performance in half-sibling sticklebacks sired by bright and dull males. (*a*) Hatching success (mean ± s.d.). (*b*) Survival to 56 days after hatching (mean ± s.d.). (*c*) Density corrected length-at-age (rLength, see § 2(e) for details) of dull- (open bars) and bright- (closed bars) sired split broods (maternal half-sibling pairs together) at 62 days after hatching. Maternal pairs are ranked along the *x*-axis by the growth performance of the dull-sired fry. (*d*) Mean (± s.d.) rLength of dull- and bright-sired half-sibling groups at 62 days after hatching.

scored under a microscope to determine infection levels. Numbers of infective stages per copepod varied between one and three parasites but the exposure level was standardized between half-siblings. Once a copepod had been consumed, the fish was replaced in its 1-l container. Fishes were sacrificed humanely after a further 67 days, weighed, measured and examined internally to assign gender and check for the presence of *S. solidus* plerocercoids.

(g) *White blood cell counts (1998)*

At the time of sacrificing fishes that underwent the experimental infection, a thin blood smear was taken. Smears were Giemsa stained (Pienaar 1962) and examined at × 40 magnification. Combined white blood cell count (thrombocytes, leucocytes, granulocytes and macrophages) was expressed as a percentage of total cell count (i.e. including erythrocytes).

(h) *Statistical analysis*

Proportional data were square-root arcsine transformed prior to statistical analysis (Zar 1996). To test for maternal effects on hatching success, offspring survival, growth and percentage of infected offspring we used Pearson correlation analysis on data from paired bright- and dull-sired maternal half-siblings. Where maternal effects were found, as indicated by a significant correlation, paired *t*-tests were used to identify consistent differences in the performances of bright- and dull-sired maternal half-siblings.

A logistic regression (SPSS, Inc. 1998) was used to test the effects of paternal redness, brood (i.e. common mother) and parasite exposure level (i.e. number of parasites in the copepod eaten) on the proportion of offspring that became infected. All parameters and their interactions were initially included in the model. Terms were progressively dropped if their removal had a non-significant effect on the model ($p > 0.05$), assuming the change in scaled deviance followed a χ^2-distribution. Linear regression was used to test the relationship between white blood cell counts and paternal redness. Analysis of covariance (ANCOVA) was used to investigate the effects of parasite exposure level on white blood cell counts.

3. RESULTS

(a) *Hatching success (1997)*

Fifteen maternal egg clutches produced viable offspring in both bright- and dull-sired half-clutches and 53 ± 28% (mean ± s.d., $n = 30$ half-clutches) of these eggs hatched. There was a significant positive correlation between hatching success in half-clutches from the same mother ($r = 0.861$, $p < 0.001$), suggesting a maternal effect on this index of performance. Within paired half-clutches, however, hatching success was not consistently linked with sire brightness (paired *t*-test, $n = 15$ pairs, $t = 1.01$, $p = 0.33$; figure 1*a*).

(b) *Post-hatching survival (1997)*

Positive correlations were found between the proportion of bright- and dull-sired half-siblings surviving to four and eight weeks post-hatching ($r = 0.884$, $p < 0.001$ and $r = 0.776$, $p < 0.001$, respectively), indicating a strong maternal effect on post-hatch survival. Dull-sired fry had similar survival rates to their related bright-sired half-siblings at 28 days (paired *t*-test, $n = 15$ pairs, $t = -0.04$, $p = 0.97$) and 56 days ($t = 0.52$, $p = 0.61$; figure 1*b*).

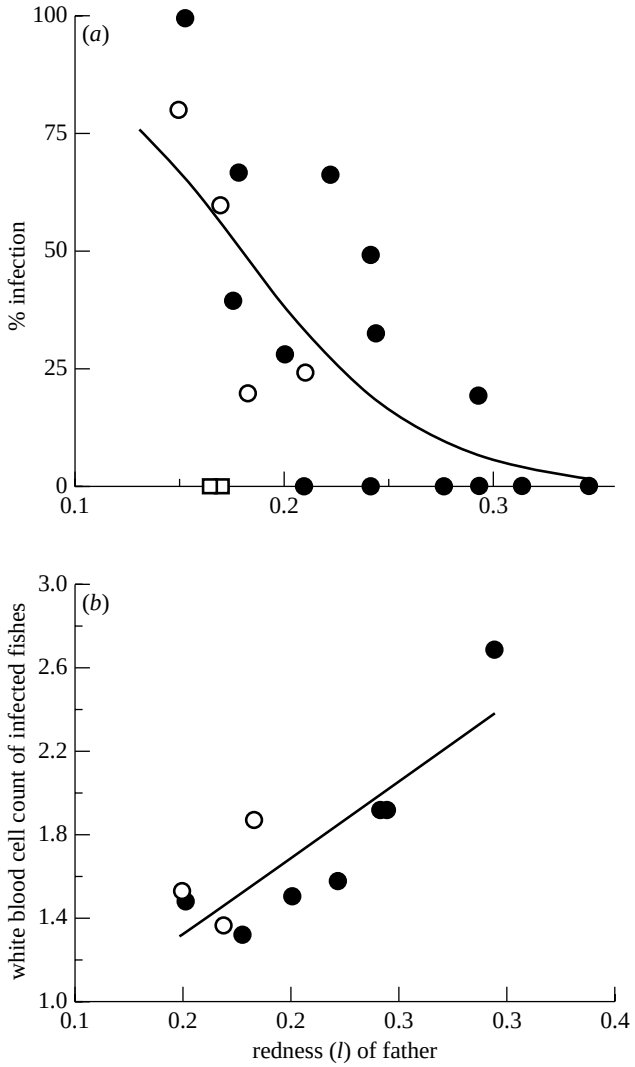

Figure 2. Responses of half-sibling sticklebacks to experimental infection with the parasite *Schistocephalus solidus*. (*a*) Negative relationship between the percentage of half-brood that became infected and paternal-redness score. The fitted line is derived from the logistical regression model $y = e^z/(1 + e^z)$, where $z = e^{(4.08-22.59l)}$. (*b*) In fishes that became infected, there is a positive relationship ($p = 0.002$) between white blood cell count (mean of fishes infected in half-brood) and paternal-redness score. Filled circles, Inverleith pond fishes; empty circles, Craiglockhart fishes; squares, brood from Inverleith that caused the significant brood effect, dropped from further analysis.

(c) *Growth* (*1997*)

Strong maternal effects on density-corrected fry length at 62 days post-hatching were indicated by a strong positive correlation between length-at-age of bright- and dull-sired half-siblings ($r = 0.685$, $p = 0.005$). However, amongst maternal half-siblings, fry fathered by brightly coloured males were smaller than those sired by duller males (paired *t*-test, $n = 15$ pairs, $t = -2.62$, $p = 0.02$; figure 1*c*,*d*).

(d) *Parasitic resistance* (*1998*)

A logistical regression was used to test the likelihood of infection with respect to paternal redness, brood (i.e. mother) and parasite-exposure level (i.e. number of *S. solidus* in the eaten copepod). In an initial analysis, in which data from all nine broods (78 fishes) were entered, significant terms retained within the model were redness ($\chi^2 = 9.26$, d.f. $= 1$, $p < 0.01$) and brood ($\chi^2 = 16.60$, d.f. $= 7$, $p < 0.05$), whilst all other terms were removed from the model ($p > 0.15$).

To examine the brood effect further, re-runs of the logistical regression were performed, selectively removing a single brood on each run. This indicated that the brood effect was brought about by one Inverleith brood of eight fishes (none of which became infected). In a regression with the remaining eight broods (70 fishes), the only significant term retained in the model was paternal redness ($\chi^2 = 15.33$, d.f. $= 1$, $p < 0.001$; for excluded terms, $p > 0.32$). In these eight broods, there was a negative relationship between paternal redness and the proportion of his offspring that became infected (fitted line in figure 2*a*).

White blood cell counts were taken from 18 out of the 23 fishes that became infected and 49 out of the 55 fishes that did not become infected. In uninfected fishes there was no significant relationship between paternal redness and the mean white blood cell count of his offspring (linear regression, $F_{1,15} = 1.77$, $p = 0.203$). The mean white blood cell count of all uninfected fishes was 1.55 (s.d. $= 0.37$). In fishes that developed infections, the mean white blood cell count among fishes from each split brood increased significantly with increasing paternal redness ($F_{1,8} = 19.73$, $p = 0.002$; figure 2*b*). The number of ingested parasite larvae had no significant effect upon this relationship (ANCOVA, comparison of slopes, $F_{6,1} = 0.01$, $p = 1.0$; elevations, $F_{7,1} = 2.53$, $p = 0.156$).

4. DISCUSSION

Since offspring of brightly coloured stickleback males are more resistant to infection by *S. solidus* than are those of dull sires, red coloration may act as an honest indicator of genes for parasite resistance in this species. *S. solidus* is prevalent in our study areas and has significant negative consequences for stickleback hosts (Tierney *et al.* 1996), so preferential mating by females with red males may promote offspring fitness. A positive relationship between sire ornamentation and offspring survival has been reported in many species (Møller & Alatalo 1999), a trade-off between inherited differences in immune status and ornamentation has been documented (Verhulst *et al.* 1999) and enhanced survival of brightly ornamented males following an epidemic has been reported (Nolan *et al.* 1998). However, few studies have directly explored the relationship between sire ornamentation and disease resistance. Kurtz & Sauer (1999) found a non-significant tendency for the offspring of male scorpion flies (*Panorpa vulgaris*) with well-developed sexual 'ornamentation' (secretion of saliva used during nuptial feeding) to have a slightly higher index of immunocompetence. One possible explanation for a link between ornamentation and disease resistance in the present context is that carotenoid-based pigments are limiting in nature (Grether *et al.* 1999) and are also required for the development of a fully effective immune system (Saino *et al.* 1999). Carotenoid-based displays therefore provide females with reliable information on disease resistance in potential mates (e.g. Skarstein & Folstad 1996).

Although sire ornamentation was positively related to parasite resistance in the present study, it was also associated with a negative effect on growth rates. This is in contrast to positive associations between sire ornamentation and offspring growth rate that have been demonstrated experimentally in peacocks (Petrie 1994) and guppy fish (Reynolds & Gross 1992). We have no information on the causes of the negative paternal effects on growth in sticklebacks but our results suggest that increased disease resistance and/or the development of red coloration may involve a cost in terms of reduced early growth rate (see discussion in Arendt (1997)). Our results also suggest a mechanism that could explain why heritable variation in male sexual coloration persists in spite of strong sexual selection, and why female sticklebacks do not always choose bright males as mates (e.g. Bakker *et al*. 1999). Early growth is important for small fishes such as sticklebacks, since it removes them from vulnerability to certain types of predators and also creates a larger adult body size, which may aid overwinter survival and result in higher fecundity (Wootton 1984). Depending on food availability and prevalence of parasites, rapid growth of fry, rather than resistance to infection, may be at a premium in some populations, or in some years, in which case females might benefit by choosing a duller male and producing faster-growing offspring.

This study has also demonstrated clear maternal effects on fry hatching success, survival and growth in sticklebacks. Such effects have been reported for many species of fish (e.g. Benoit & Pepin 1999; Heath *et al*. 1999), and may arise from differences in egg size and nutrient content (Einum & Fleming 1999) and/or in transfer of maternal hormones (McCormick 1999). A key role for maternal effects as a mechanism for generating adaptive phenotypic variation is suggested by Mousseau & Fox (1998) and demonstrated by Rolff (1999).

In conclusion, by using artificially fertilized split broods and rearing fry without any parental care, we have shown that the offspring of brightly coloured male sticklebacks grow more slowly than those of duller males but are less vulnerable to infection by a cestode parasite. Our results provide a clear positive test of one of the key predictions of 'good genes' models for the evolution of sexual ornamentation. In addition, the apparent trade-off between parasite resistance and early growth rate suggests a mechanism for the maintenance of heritable variation in both disease resistance and male sexual coloration.

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