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A novel test of the phenotype-linked fertility hypothesis reveals independent components of fertility

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The phenotype-linked fertility hypothesis predicts that male sexual ornaments signal fertilizing efficiency and that the coevolution of male ornaments and female preference for such ornaments is driven by female pursuit of fertility benefits. In addition, directional testicular asymmetry frequently observed in birds has been suggested to reflect fertilizing efficiency and to covary with ornament expression. However, the idea of a phenotypic relationship between male ornaments and fertilizing efficiency is often tested in populations where environmental effects mask the underlying genetic associations between ornaments and fertilizing efficiency implied by this idea. Here, we adopt a novel design, which increases genetic diversity through the crossing of two divergent populations while controlling for environmental effects, to test: (i) the phenotypic relationship between male ornaments and both, gonadal (testicular mass) and gametic (sperm quality) components of fertilizing efficiency; and (ii) the extent to which these components are phenotypically integrated in the fowl, *Gallus gallus*. We show that consistent with theory, the testosterone-dependent expression of a male ornament, the comb, predicted testicular mass. However, despite their functional inter-dependence, testicular mass and sperm quality were not phenotypically integrated. Consistent with this result, males of one parental population invested more in testicular and comb mass, whereas males of the other parental population had higher sperm quality. We found no evidence that directional testicular asymmetry covaried with ornament expression. These results shed new light on the evolutionary relationship between male fertilizing efficiency and ornaments. Although testosterone-dependent ornaments may covary with testicular mass and thus reflect sperm production rate, the lack of phenotypic integration between gonadal and gametic traits reveals that the expression of an ornament is unlikely to reflect the overall fertilizing efficiency of a male.

Keywords: female preference; *Gallus*; sperm quality genes; phenotypic integration; sexual ornaments; testicular asymmetry

1. INTRODUCTION

> Professor Mantegazza is inclined to believe ('Lettera a Carlo Darwin', *Archivio per l'Anthropologia*, 1871, p. 306) that the bright colours, common in so many male animals, are due to the presence and retention by them of the spermatic fluid; but this can hardly be the case...
> (Darwin 1882, p. 224)

This quote shows that a link between the sexual ornaments of a male and the quality of his semen had been already hypothesized when Darwin formulated his theory of sexual selection. The idea that the display of sexual ornaments signals male fertilizing efficiency was explicitly proposed by Trivers (1972; see also Williams 1978), and generated much interest when it became clear that widespread sexual promiscuity renders male fertilizing efficiency an important component of fitness and a target of sexual selection (Birkhead & Pizzari 2002). The phenotype-linked fertility hypothesis (Sheldon 1994) predicts that male fertilizing efficiency is reflected by the phenotypic expression of male ornaments, allowing female birds to reduce the risk of infertility by selecting relatively fertile copulation partners. Similarly, Møller & Birkhead (1994) argued that plumage ornamentation in male birds has evolved as a response to female selection of extra-pair partners. Finally, Møller (1994) added a corollary to the phenotype-linked hypothesis by suggesting that the degree of testicular asymmetry, often observed in birds (Friedmann 1927; Lake 1981), reflects male fertilizing efficiency and covaries with the expression of sexual ornaments. More recently, several studies have suggested different physiological mechanisms through which ornament expression may covary with fertilizing efficiency (Folstad & Skarstein 1997; Hillgarth *et al.* 1997; Blount *et al.* 2001).

The underlying idea of the phenotype-linked fertility hypothesis is that female preference for male ornaments has been directly selected owing to fertility benefits to females and indirectly owing to superior fertilizing efficiency in sons and adaptive partner choice in daughters (Pizzari & Birkhead 2002). This idea implies that the covariance between male ornaments and fertilizing efficiency is condition dependent (Sheldon 1994). In

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addition, for male ornaments to evolve in response to female pursuit of fertility benefits (Møller & Birkhead 1994), additive genetic variation must underlie the phenotypic relationship between male ornaments and fertility. Testing the phenotype-linked fertility hypothesis is crucial to understanding the evolution of male ornaments and female preference. For example, by obtaining high-quality inseminations, females may minimize the number of costly copulations (Pizzari 2002). However, although it has received considerable experimental attention, the phenotype-linked fertility hypothesis has often been tested in natural populations (but see Birkhead *et al.* 1995; Matthews *et al.* 1997), with ambiguous results (reviewed in Pizzari & Birkhead 2002; Birkhead & Pizzari 2002). This is not surprising considering that in natural populations environmental effects mask genetic effects, because:

(i) environmental variables such as male age, condition and copulation history are difficult to measure and control; and
(ii) natural and sexual selection are expected to reduce additive genetic variance in ornaments and fertilizing efficiency (although the extent to which this happens remains debatable).

Here, we adopt a novel experimental approach, increasing genetic variation and reducing environmental variation, to test whether the expression of male ornaments reflects male fertilizing efficiency in the fowl, *Gallus gallus*.

The mating system of the red jungle fowl, *Gallus gallus* ssp., and that of feral populations of its domesticated descendant, the domestic fowl, *G. g. domesticus*, is characterized by high levels of sexual promiscuity exposing males to intense sexual selection both pre- and post-insemination (McBride *et al.* 1969; Pizzari *et al.* 2002). Consistent with this is the fact that male fowl display multiple ornaments (Zuk *et al.* 1990) and relatively high investment in gonadal mass (Pizzari 1999). In the fowl, male fertilizing efficiency is determined largely by a gonadal and a gametic factor:

(i) the number of sperm inseminated by a male into a female, which depends on the rate of sperm production and, ultimately, testicular mass (Burrows & Titus 1939; Parker *et al.* 1942; Amann 1970; Lake 1971; Martin & Dziuk 1977; De Reviers & Williams 1984); and
(ii) the quality of inseminated ejaculates (Froman *et al.* 2002), which depends on the metabolic performance and ATP content of spermatozoa (Etches 1996; Cummins 1998; Froman *et al.* 1999).

Both (i) and (ii) are important determinants of the fertilizing efficiency of an insemination (Froman *et al.* 2002). The extent to which ornaments predict variation in overall male fertilizing efficiency in the fowl depends on the relative importance of (i) and (ii), and on whether (i) and (ii) are phenotypically integrated.

After domestication, some fowl populations have undergone an intense process of artificial selection for egg production in which the number of males inseminating females has been minimized, often with the help of artificial insemination (Etches 1996), thus relaxing (or even preventing) sexual selection. In addition, consistent artificial selection for egg production is likely to accumulate alleles allowing females to sustain higher reproductive investment. Because the functions of the two sexes are typically divergent and yet many genes are bisexually expressed (Rice 1984; Chippindale *et al.* 2001), artificial selection for egg production may favour female-beneficial genetic combinations that, when expressed in males, have potentially deleterious effects on ornament expression and/or fertilizing efficiency.

We studied males of the second (F_2) generation of the inter-cross between a red jungle fowl population, where male investment in ornaments and fertilizing efficiency is favoured by sexual selection, and a domestic strain artificially selected for egg production. Segregation of wild and domestic alleles at the F_2 generation of the crossing reveals the genetic variation underlying both male ornaments and fertilizing efficiency (Andersson 2001). In addition, we minimized environmental variation by standardizing the age at which males were studied and environmental conditions to which males had been exposed from incubation.

Our aim in this study was to test the following:

(i) the relationship between male ornamentation and gonadal and gametic components of fertilizing efficiency;
(ii) the relationship between gonadal and gametic components of fertilizing efficiency; and
(iii) the hypothesis that testicular asymmetry covaries with ornament expression (Møller 1994).

2. MATERIAL AND METHODS

(a) *Study population*

We used the F_2 generation of an inter-cross between a red jungle fowl population housed at the research station of Stockholm University (Sweden), and a Scandinavian domestic strain of White Leghorn (SLU13), which has a long history of artificial selection for egg mass (Liljedahl *et al.* 1979), and maintained at the Swedish University of Agricultural Sciences (Skara; see Carlborg *et al.* (2003) for details). Birds from each batch were hatched and raised together on floor in indoor pens (3 m × 3 m) in mixed sex groups of about 40 animals under standardized housing conditions with *ad libitum* food and water. We studied 44 males from the F_2 batch hatched on December 1999 ($n = 247$ at hatching).

(b) *Phenotypic measurements*

Forty-four males were kept isolated from females two months before the study to ensure sexual rest and the replenishment of their sperm reserves. In June 2002 males were housed in single-sex outdoor pens at Tovetorp Research Station, Stockholm University, for 3 days when one sperm sample was manually collected (Burrows & Quinn 1937) from 38 of them. Each semen sample was diluted in Gold's Eagle Growth medium to a concentration of 2×10^6 sperm ml^{-1}. Thirty microlitres of this solution were mounted on a microscope slide and video-recorded with a CCD KP-M1E/K Hitachi Denshi Ltd camera (Japan) connected to a BH-2 Olympus microscope (Japan) with dark field optics at a magnification of ×20. We quantified sperm quality by measuring the performance of individual motile sperm by

Table 1. Dissection of phenotypic covariance in (*a*) mean testicular mass and sperm quality, (*b*) VSL and (*c*) VAP.

covariate	d.f.	*F*	*p*
(*a*) mean testicular mass and sperm quality			
comb mass	1,34	7.16	0.01
spur length	1,34	0.01	0.92
body mass	1,34	2.91	0.097
(*b*) VSL			
comb mass	1,34	0.76	0.39
spur length	1,34	0.93	0.34
body mass	1,34	0.89	0.35
(*c*) VAP			
comb mass	1,34	1.12	0.30
spur length	1,34	1.61	0.21
body mass	1,34	1.59	0.22
adjusted R^2	0.29		

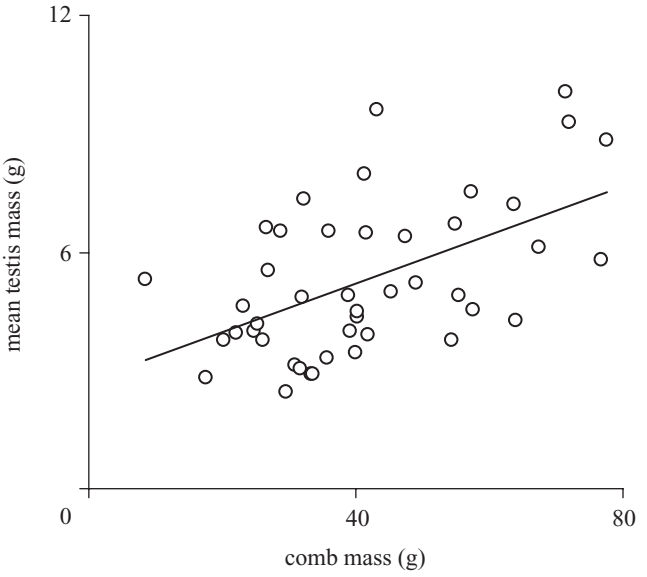

Figure 1. Phenotypic relationship between male comb and mean testicular mass (see table 1*a*).

using computer-assisted sperm analysis (Hobson Sperm Tracker) in two ways as follows:

(i) average path velocity (VAP, μm s^{-1}); and
(ii) straight line velocity (VSL, μm s^{-1}).

VAP was calculated by dividing the smoothed distance of each sperm track by the time taken to cover that distance. VSL was calculated by dividing the straight line distance between the start and endpoint of the track, by the time taken to cover that distance. For each male, 100 sperm were individually tracked within 5 minutes of recording, and mean VAP and VSL were obtained. VAP and VSL are positively correlated with fertility in several taxa (Liu *et al*. 1991; Moore & Akhondi 1996; Hirano *et al*. 2001; Tash *et al*. 2001; Al-Qarawi *et al*. 2002; Kupriyanova & Havenhand 2002; see also Birkhead *et al*. 1995) including the fowl (Wishart & Palmer 1986), and with sperm mobility (Donoghue *et al*. 1998; Froman & Feltmann 2000; King *et al*. 2000), another important predictor of fertilizing efficiency in Galliformes (see Froman *et al*. 1997; Donoghue *et al*. 1998; Birkhead *et al*. 1999; Froman *et al*. 2002; see also Donoghue 1999).

The day after sperm collection males were killed, weighed to the nearest 10 g, and dissected. Both testicles and the comb were removed and weighed to the 0.01 g, in all 44 males. The length of both spurs was measured as the chord connecting the base and the tip of the spur for the 38 males for which sperm quality was measured. We chose male comb size because female red jungle fowl prefer to copulate with large-combed males (Zuk *et al*. 1990; Ligon & Zwartjes 1995). In addition, male comb size may reflect male condition (Zuk *et al*. 1995; Verhulst *et al*. 1999) and male social dominance (Ottinger 1983; Sullivan 1991; Parker *et al*. 2002), another trait which female fowl favour in their copulation partners (Pizzari & Birkhead 2000; Johnsen *et al*. 2001; Pizzari 2001). Spur length was chosen because it may reflect male fighting ability and influence female choice in some Galliformes (see Davison 1985; Von Schantz *et al*. 1989; Mateos & Carranza 1996; Badyaev *et al*. 1998), although the functional significance of male spurs remains unresolved in the fowl (see Zuk *et al*. 1990).

This experimental design assumes different levels of investment in male ornaments and fertilizing efficiency in the two parental populations. We tested this by examining the somatic investment in both mean testicular mass and comb mass in adult (*ca*. 1 year old) males of both parental populations (white leghorn and red jungle fowl). In addition, we analysed sperm quality (VAP) in white leghorn and red jungle fowl males. Parental males were kept at the same site under the same environmental conditions, with the exception of the white leghorn males which we used to analyse sperm quality and which were kept in single cages at the Swedish University of Agricultural Sciences (Uppsala).

(c) *Analysis*

First, we tested whether comb mass and mean spur length ((left + right) × 0.5) explained a significant proportion of the variance in mean testicular mass ((left + right) × 0.5) and in sperm quality (VSL, VAP) by using an analysis of covariance (ANCOVA). To control for allometric effects we also entered body mass as a covariate. All variables were checked for normality. The distribution of testicular mass was normalized by log transformation. In addition, we tested differences in relative mean testicular mass and relative comb mass (i.e. standardized over body mass) between parental populations through Mann–Whitney tests, and differences in VAP through a one-way ANOVA with population as a fixed effect.

Second, we tested the relationship between mean testicular mass and sperm quality (VSL, VAP) using ANCOVA.

Finally, we tested the hypothesis of Møller (1994) that the typically smaller right testis enlarges to compensate for any condition-dependent deficiency in the left testis. This hypothesis predicts:

(i) a negative relationship between relative testicular ((left − right)/(left + right) × 0.5) asymmetry and right testis mass; and
(ii) a positive relationship between directional (left–right) asymmetry and ornament expression.

We tested (i) using Spearman rank correlation, and (ii) through a multiple stepwise regression with directional testicular asymmetry as the dependent variable and comb mass, mean spur length and body mass as the independent predictors.

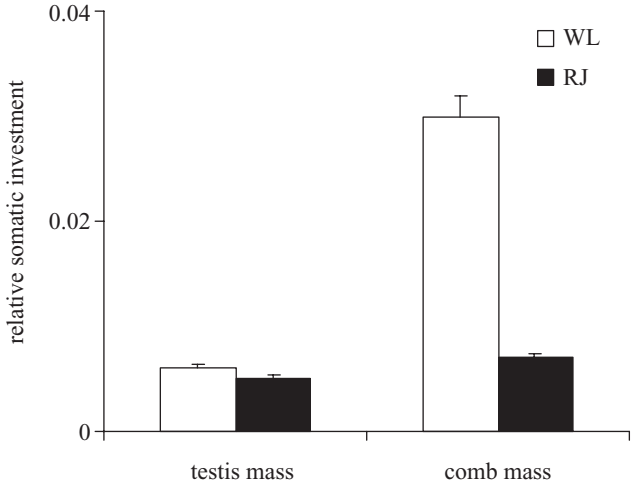

Figure 2. Somatic investment in mean testicular mass (mean testis mass per body mass) and comb mass (comb mass per body mass) in adult male white leghorn (WL) and red jungle fowl (RJ); (Mann–Whitney: relative testis mass, $U = 5.00$, $p = 0.04$, $n_{WL} = n_{RJ} = 6$; relative comb mass, $U = 0.00$, $p = 0.00$, $n_{WL} = n_{RJ} = 5$).

Table 2. Phenotypic covariance between in mean testicular mass and sperm quality: (*a*) VSL and (*b*) VAP.

covariate	d.f.	*F*	*p*
(*a*) VSL			
mean testis mass	1,35	0.43	0.52
body mass	1,35	0.00	0.99
(*b*) VAP			
mean testis mass	1,35	0.31	0.58
body mass	1,35	0.03	0.86
adjusted R^2	0.04		

3. RESULTS

Consistent with the phenotype-linked fertility hypothesis, male comb mass was a significant predictor of mean testicular mass (table 1; figure 1). In addition, consistent with the idea that the observed covariance in testicular mass and comb mass in the F_2 hybrids was mainly genetic, we found a significant difference in gonadal and comb investment between the parental populations. Males of the domestic strain invested significantly more in both, testicular and comb mass (figure 2). Mean spur length, on the other hand, did not covary significantly with mean testicular mass (table 1). Similarly, variation in sperm quality (VSL, VAP) was not explained by the expression of either male sexual ornaments, or by body mass (table 1).

Mean testicular mass and sperm quality did not covary (table 2; figure 3*a*,*b*), indicating that these fertility traits were not phenotypically integrated. Consistent with the idea that in the fowl testicular mass and sperm quality are genetically determined but not genetically integrated with each other, we found that males of the parental white leghorn population produced sperm of significantly lower quality than those produced by males of the red jungle fowl parental population, with the F_2 hybrids producing sperm of intermediate quality (figure 4).

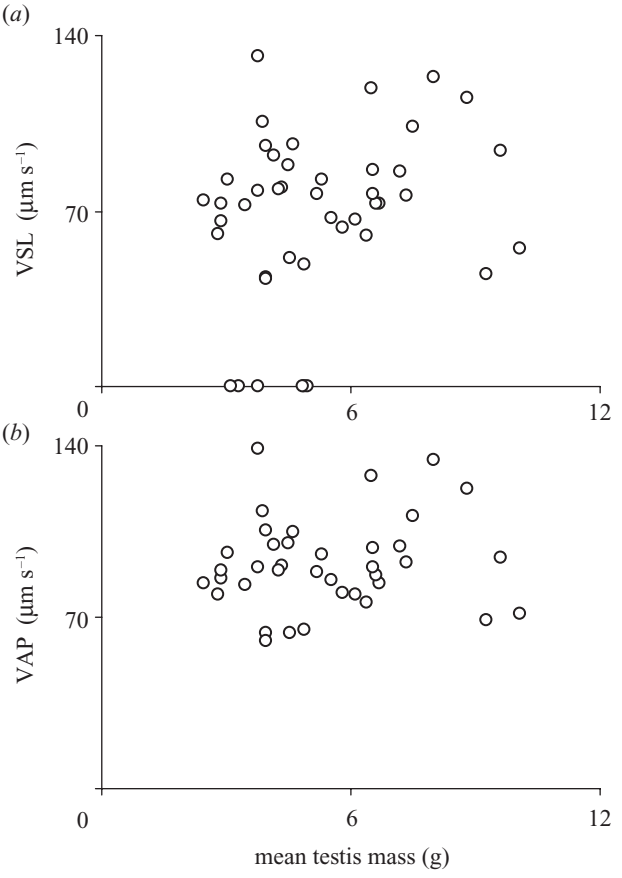

Figure 3. Phenotypic relationship between mean testicular mass and two measures of sperm quality (*a*) VSL and (*b*) VAP.

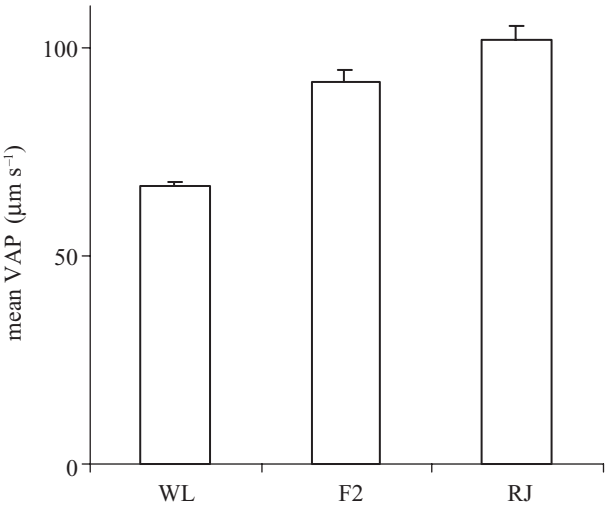

Figure 4. Mean sperm quality, measured as VAP in adult male white leghorn (WL), F_2 hybrids (F2), and red jungle fowl (RJ). There was an overall population effect ($F_{2,148} = 70.09$, $p < 0.0001$, $n_{WL} = 74$, $n_{F2} = 38$, $n_{RJ} = 37$), mainly because of the sperm quality of WL males being significantly lower than that of both, F2 and RJ males (mean difference ± s.e.m., 35.18 ± 3.20, *post hoc* tests: Tukey Honestly Significant Difference, THSD, $p < 0.0001$, Dunnett's T3, $p < 0.0001$). Similarly, there was a tendency for sperm quality to be higher in RJ than in F2 (10.07 ± 3.68, THSD, $p = 0.017$, Dunnett's T3, $p = 0.084$).

We found no support for the hypothesis of Møller (1994). Although the left testis was on average larger, this difference was not significant (mean ± s.e.m., left 5.32 ± 0.34; right 5.22 ± 0.27 g, Wilcoxon test: $Z = -0.035$, $p = 0.97$, $n = 44$), indicating no overall directional asymmetry. Contrary to the prediction that the right testis develops to compensate for the left, we found no correlation between relative testicular asymmetry and right testis mass ($r^s = -0.09$ $p = 0.54$, $n = 44$). Furthermore, we found no evidence consistent with the prediction that directional testicular asymmetry covaries with ornament expression, neither comb nor mean spur length explained variance in directional testicular asymmetry (none of the independent variables had a significant effect on variance in directional asymmetry and was included in the model, $n = 44$). Finally, there was no correlation between directional testicular asymmetry and mean testis mass ($r^s = 0.15$, $p = 0.35$, $n = 44$), suggesting that directional testicular asymmetry is unlikely to reflect the rate of sperm production.

4. DISCUSSION

The present study shows that, consistent with the phenotype-linked fertility hypothesis, the expression of a sexual ornament, the comb, reflects testicular mass and thus the rate of sperm production in male fowl. However, the gametic component of fertilizing efficiency, sperm quality, was not predicted by ornament expression and was independent of testicular mass.

These results suggest that male fertilizing efficiency consists of two independent components in the fowl. One, gonadal investment, is predicted by the phenotypic expression of a sexual ornament. The link between testicular and comb mass is likely to be steroid mediated (Astiningsih & Rogers 1996). In birds and other vertebrates, spermatogenesis occurs in the seminiferous tubules of the testis, where spermatocytes develop into haploid spermatozoa through two meiotic divisions (Johnson 1991; Etches 1996). Sertoli cells, in the adluminal region of the tubules, provide the microenvironment for spermatogenesis by ensuring high levels of testicular steroid hormones through the release of androgen-binding protein (Kirby & Froman 2000). Testicular ability to produce sperm is associated with the proliferation of Sertoli cells at the onset of sexual maturity in the male fowl (Etches 1996), and because the number of Sertoli cells is proportional to testicular size, larger testes produce more sperm (Burrows & Titus 1939; Amann 1970; Martin & Dziuk 1977; De Reviers & Williams 1984).

Testosterone is produced by the Leydig cells, which in the fowl are in the interstitial tissue of the testis (Kirby & Froman 2000). In mammals, Leydig cells contribute to testicular mass, and the number of Leydig cells is positively correlated with sperm production (Johnson 1991). This also appears to be the case in birds where testicular mass is positively correlated with testosterone production both seasonally (Young *et al.* 2001) and through ontogeny (Schwabl & Farner 1989; Cecil & Bakst 1991; Deviche *et al.* 2000). More importantly, castration in male fowl interrupts the negative feedback loop that regulates levels of luteinizing hormone through testosterone inhibition of the gonadotrophin-releasing hormone (Scanes *et al.* 1984; Etches 1996), demonstrating the causal link between testes and testosterone production. Testosterone is the primary androgen in adult male fowl (Bahr & Johnson 1991; Etches 1996) with a crucial influence on male sexual and competitive behaviour (Allee *et al.* 1939; Ottinger 1983; Bahr & Johnson 1991) and on comb size (Zuk *et al.* 1995; Fennell *et al.* 1996; Parker *et al.* 2002). Comb size may thus reflect male social status (Parker *et al.* 2002) and steroid-mediated immune responses (Zuk *et al.* 1995; Fennell *et al.* 1996; Verhulst *et al.* 1999). However, the relationship between comb and testicular mass in male fowl has been controversial. For example, Von Schantz *et al.* (1995) found that artificial selection for increased male comb size in a domestic fowl strain originally selected for egg production resulted in a weak negative correlation between male comb size and both testicular mass and spur length. Alternatively, McGary *et al.* (2002) found that male comb size was positively correlated with relative testicular mass in one of two domestic broiler strains (i.e. selected for meat production). However, different artificial selection regimes have resulted in divergent mechanisms of testosterone modulation of comb expression in different strains (Dorfman & Dorfman 1948; Astiningsih & Rogers 1996), which are difficult to interpret at an evolutionary level. A more promising approach is to compare artificially selected strains with natural populations (Astiningsih & Rogers 1996). By adopting this design, the present study provides evidence consistent with the idea that genetic combinations coding for relatively high gonadal investment are also associated with superior investment in comb expression.

A second component of fertilizing efficiency, the motile quality of ejaculated spermatozoa, appears to be independent from testicular mass. Sperm quality depends ultimately on the metabolic performance of mature spermatozoa, which is determined by mitochondrial ATP synthetic ability (Wishart & Palmer 1986; Cummins 1998; Froman *et al.* 1999, 2002). Hence, sperm quality may be less dependent on the testicular steroid milieu during spermatogenesis and thus less likely to covary with comb mass. The fact that testicular mass and sperm quality are not phenotypically integrated, despite their functional interdependence (Froman *et al.* 2002), may therefore be explained by the fact that sperm quality is particularly vulnerable to the performance of maternally transmitted mitochondrial genes (Cummins 1998; Pizzari & Birkhead 2002), which may prevent correlational directional selection on testicular mass and sperm quality (Froman *et al.* 2002; Pizzari & Birkhead 2002). Earlier work suggests that variance in sperm quality in the fowl is determined by both mitochondrial and nuclear genes (Froman *et al.* 2002). Our breeding design reduced the effect of mitochondrial genes on sperm quality because only white leghorn females contributed to the F_1 generation (Carlborg *et al.* 2003), resulting in all the F_2 males sharing leghorn mitochondrial genes. The result that the F_2 males produced sperm of intermediate quality strongly suggests that nuclear genes are also importantly involved in determining variation in sperm quality in the fowl. This result is also consistent with the idea that the relaxation of sexual selection through domestication and artificial selection for female reproductive traits (e.g. egg production) has resulted in the accumulation of genetic variants with

detrimental effects on some male fertility traits. The mechanisms through which mitochondrial and nuclear genes interact to determine sperm quality remains to be investigated (Cummins 1998). Similarly, mitochondrial genes that affect sperm quality may also mediate a phenotypic relationship between some ornaments and sperm quality. However, our experimental design is likely to have reduced mitochondrial genetic variation in the F_2 birds, and thus the possibility of detecting potential relationships between ornaments and sperm quality mediated by mitochondrial genes. The different housing conditions in which the males of the two parental lines for which sperm quality was measured (see § 2) may also contribute to explain the observed line difference. However, this seems unlikely because:

(i) a line difference was also observed in testicular and comb mass, which were measured in male leghorn and red jungle fowl kept in similar conditions; and
(ii) F_2 males had higher sperm quality than male red jungle fowl although both were housed in similar conditions.

We found no support for the idea that the degree of testicular asymmetry covaries with ornament expression (Møller 1994). First, we found no evidence of directional testicular asymmetry, as found in previous studies of red jungle fowl (Kimball *et al.* 1997) and wild passerines (Merilä & Sheldon 1999). Second, the lack of covariance between directional testicular asymmetry and male ornaments is consistent with findings in a captive population of red jungle fowl (Kimball *et al.* 1997) and in other species of wild birds (Birkhead *et al.* 1997, 1998). Furthermore, both testes are known to be functional in the fowl (Johnson 1976; Etches 1996), and both the left and right *vasa deferentia* eject semen (T. Pizzari, P. Jensen and C. K. Cornwallis, personal observations; Etches 1996, fig. 8.8D, p. 220) during manual collection of semen samples. This indicates that, at least during manually induced ejaculation, both testes contribute to the ejaculate. This observation seems to contradict the assumption that only the left testis typically contributes to an ejaculate, upon which the hypothesis of Møller (1994) of functional testicular asymmetry rests. A non-functional explanation suggests that directional testicular asymmetry in male birds is a genetic correlate of the more pronounced female ovarian asymmetry (Stanley & Witschi 1940). Clearly, more work is needed to understand the functional significance of avian testicular asymmetry.

In conclusion, testicular mass and sperm production may be more likely to be predicted by steroid-dependent phenotypic traits than sperm quality. This may contribute to explain female preference for steroid-dependent male ornaments in the fowl (Zuk *et al.* 1990) and other Galliformes (Hagelin & Ligon 2002). However, the extent to which female preference for large-combed males has been selected by direct and indirect fertility benefits, and by other effects associated with high testosterone levels (social status) remains unclear. In addition, the number of sperm inseminated by a male into a female is determined by multiple contingent social cues in many taxa (Wedell *et al.* 2002) including the fowl (Pizzari *et al.* 2003), suggesting that ejaculate size is not entirely predicted by the expression of male ornaments.

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