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LETTER

Sexually extravagant males age more rapidly

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Abstract

Evolutionary theories of ageing posit that increased reproductive investment occurs at the expense of physiological declines in later life. Males typically invest heavily in costly sexual ornaments and behaviour, but evidence that the expression of these traits can cause senescence is lacking. Long-lived houbara bustards (*Chlamydotis undulata*) engage in extravagant sexual displays to attract mates and here we show that males investing most in these displays experience a rapid senescent deterioration of spermatogenic function at a younger age. This effect is sufficiently large that the expected links between male 'showiness' and fertility reverse in later life, despite 'showy' males continuing to display at near maximal levels. We show that our results cannot be explained by the selective disappearance of competitive phenotypes and that they are instead consistent with an early vs. late life trade-off in male reproductive competence, highlighting the potential significance of sexual selection in explaining rates of ageing.

Keywords

Ageing rate, fertility, houbara bustard, ornaments, senescence, sexual display, sexual selection, sperm competition, spermatozoa, trade-off.

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INTRODUCTION

Senescence is the deterioration in physiological function that occurs with advancing age (Rose 1994). The reason why an organism should senesce has been an evolutionary puzzle, as natural selection would be expected to 'weed-out' the genes responsible for these age-related declines. Key theories that have been forwarded to explain the evolution of senescence highlight the inherent 'riskiness' of life, meaning that genes or phenotypes that would be expressed late in an organism's life will rarely get to be expressed (Medawar 1952). As a consequence, genes or phenotypes that are beneficial early in life can be selected for even though they have negative effects later i.e. even if they cause senescence (Williams 1957; Kirkwood 1977). The extent to which organisms invest in reproduction has often been forwarded as an example of how early investment may be positively selected even if it causes age-related declines in fitness later (Williams 1957; Kirkwood 1977; Kirkwood & Rose 1991). However, there is a little supporting evidence on long-lived species and that which is available is limited to observations that trade-offs can occur between early and late reproduction in females (Charmantier *et al.* 2006; Nussey *et al.* 2006).

A lack of evidence for these hypotheses of senescence in males is surprising, as it is males that tend to be the shorter-lived of the two sexes, at least in species with significant male sexual competition (Clarke & Mittwoch 1995; Liker & Szekely 2005). This is thought to be due to the greater 'pay-offs' available to males through elevating their reproductive investment, potentially being able to sire the offspring of many females, but also due to the higher risks associated with male reproductive behaviour and competition (Vinogradov 1998; Bonduriansky *et al.* 2008). A further contrast with females is that greater reproductive investment by males is usually expressed in the production of costly sexual traits, such as ornamental plumage, elaborate displays and complex vocalisations (Andersson 1994), and these are characteristics that are exhibited in abundance by male houbara bustards of North Africa.

In the wild, male houbara bustards attract mates using repeated cycles of a complex courtship display (Gaucher *et al.* 1996). After an initial period of pre-display strutting, males erect an ornamental 'shield' of long white feathers in front of them as they begin to run at high speed, often circling a rock or bush. This display culminates in a flash of both black and white ornamental feathers and is often accompanied by several subsonic 'booming' calls (Gaucher *et al.* 1996). Males exhibit variations of this display behaviour for around 18 h a day (Y. Hingrat, unpublished data), which seems likely to be the main cause of a 10% loss in body mass that occurs over the 6 months period that males perform their sexual displays (Saint Jalme *et al.* 1996). Despite this surfeit of male effort, females of this species appear to mate promiscuously, as 60% of clutches contain multiple sires (Lesobre *et al.* 2010). Thus, in addition to outcompeting rivals in elaborate behavioural displays, males also need to produce large ejaculates to be competitive in the sperm competition that may follow copulation (Parker 1970; Dziuk 1996).

We test hypotheses linking male reproductive investment to their senescence using observations on the sexual displays and ejaculates of male houbara bustards residing within a conservation programme in eastern Morocco, where 10 years of longitudinal records on these characteristics have been collected from more than 1700 males ranging from 1 to 24 years of age. In total, pertinent data on 158 799 individual ejaculates were used in mixed model analyses (see Figure S1 for further details). Assays of the quality of these ejaculates are ideally suited for our purpose as spermatogenesis is thought to be particularly vulnerable to senescence (Pizzari *et al.* 2008) and a compromised ability to produce viable sperm would carry readily apparent fitness costs (Parker 1970; Dziuk 1996). By statistically controlling for within year seasonal influences on ejaculate production and analysing changes in spermatogenesis and male behaviour as they age across years, we test the hypothesis that excessive male investment in reproduction occurs at a cost of subsequent age-related declines in physiological function. Specifically, we predict that males investing more in energetically costly sexual displays will initially also produce the

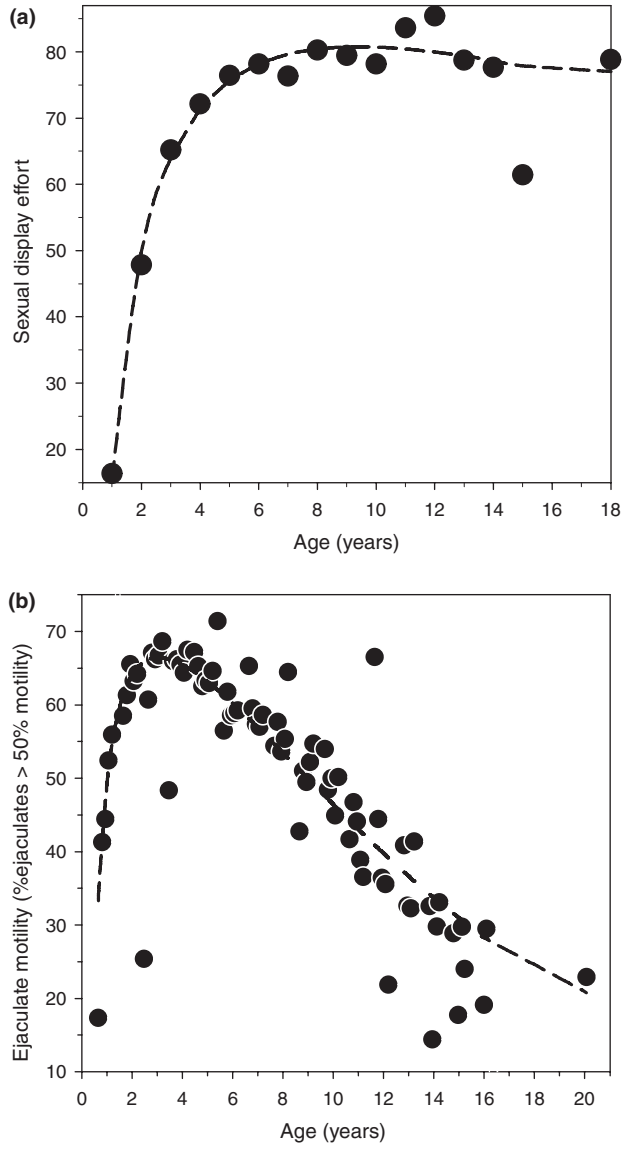

Figure 1 Male display effort and ejaculate quality through life. (a) Male display rate increases to a plateau at 6 years of age, thereafter showing little evidence of a senescent decline. (b) Ejaculate motility increases up to 4 years of age, and then exhibits a senescent decline (analysis in Table 2). All fitted lines are formed from model predictions. For illustrative purposes, these data are grouped at 2 months intervals. Data beyond 16 years of age in both plots are grouped owing to small sample size.

highest quality ejaculates, but that these same males will pay the cost later in life in the form of a more rapid senescent decline in spermatogenic function, as revealed by the production of sperm with morphological abnormalities and ejaculates with low motility.

MATERIALS AND METHODS

Housing conditions

All males considered here were of known age having originated from either eggs that had been collected in the wild or from eggs that had been produced by the captive breeding programme. Males were housed in three different locations in Eastern Morocco (Almis, Missour and Enjil), but they were reared and held under similar conditions. Males that were used as sperm donors were caged individually (cage size: 2 × 4 m), had *ad libitum* access to food and water, and were in visual and auditory contact with other males.

Behavioural data collection

The complex sexual displays of males that are observed in the wild are well-preserved in captivity, although males are limited to running within the confines of their cage [see Gaucher *et al.* (1996) for details on the behaviour of captive houbara males]. Differences in the time males invested in this display behaviour were quantified by recording whether or not males had been engaged in sexual display at the outset of each day (*c.* 8 am). This was recorded by a single observer who walked the caging area before the team of semen collectors began their work (see below). The observer noted the presence or absence of display for each male before approaching their cage, thus preventing any observer effects on male behaviour. The number of days on which males were observed displaying was then summed for each year to form an annual index of male 'sexual display effort'. Thus, a record of each male's 'sexual display effort' was available for each year of life as they aged.

Ejaculate collection and assessment

Ejaculates were routinely collected from pre-determined males by a large (>10 people) team of experienced collectors according to the requirements of the artificial insemination programme. A detailed description of the collection and assessment methodology can be found elsewhere (Saint Jalme *et al.* 1994). Briefly, a dummy female was used to elicit male copulation, with the ejaculate subsequently collected on a Petri dish that was placed under the copulating male prior to ejaculation. Samples were immediately transferred from this Petri dish into an Eppendorff tube using an aspirator, before the sample was taken for analysis by a different team of assistants in the laboratory located directly adjacent to where the donor males were housed. Males were then usually allowed at least one day's recovery between collections (in 97% of cases).

Ejaculates were routinely assessed for motility under a light microscope (×100) and were subjectively scored as being either greater or less than 50% motile. Estimates of the numbers of sperm contained within each ejaculate were obtained using a spectrophotometer (after appropriate dilution using Lake 7.1 diluent, Lake & Ravie 1984), with the optical density read against a validated scale for this species. A large subset of males had a more detailed evaluation of ejaculate characteristics that included assessment of sperm morphology. Following standard methods (Lindsay *et al.* 1999), diluted semen was eosin-nigrosin stained and a minimum of 100 sperm underwent morphological assessment using a light microscope (×1000). Ejaculates typically contained sperm with aberrant morphology, which were characterised predominately by sperm which had a swollen membrane or extended nuclei, a double flagella or double heads.

Using these data collection methodologies, large numbers of each male's ejaculates could be rapidly and reliably collected and assessed within and across multiple years of their lives and could then be paired with records of their sexual display effort in the same year.

Statistical analysis

Data availability

Data were analysed using Generalized and Linear mixed models [(G)LMM] implemented in Genstat (8th edition), which are powerful tools to examine within individual pattern of ageing using longitudinal data (see Nussey *et al.* 2006, 2008). Here, we make full use of the longitudinal assessments of male ejaculate quality by incorporating all of the repeated measures data available to us from both within (mean = 28.5, range = 1–90) and between years (mean = 3.15, range = 1–10); see Figure S1 for details of specific frequencies. In total, 10 years of data were available for analysis derived from a maximum of 1792 males that ranged from 1 to 24 years of age, the number that were included in each analysis was dependent on the availability of different forms of data and is shown in respective table legends.

Statistical control

In our analyses of ejaculate quality, male identity, the origin of eggs (wild or captive bred), the breeding site (Almis, Missour or Enjil) and the year of ejaculate collection (from 2000 to 2009),were statistically controlled for as categorical random effects (Genstat 5 Committee 1993). Differences in ejaculate quality that are expected due to yearly seasonal changes in spermatogenesis (e.g. Penfold *et al.* 2000) were controlled by fitting 'day of year' as a cubic fixed term in models to allow for its cyclical nature. Ejaculate quality may also be influenced by collection frequency, which can result in a depletion or ageing of sperm reserves (e.g. Fan *et al.* 2004). Thus, the 'number of ejaculates collected to date' in a given year and the 'days since last ejaculation', were also considered in models to statistically control for depletion or ageing effects if present.

Life trajectories and critical tests

The explanatory terms that were included in models to test predictions were the age of males on the day of ejaculate collection and the sexual display effort of that male in the same year (see above). Age was considered as a quadratic function to allow for the curvilinear association with reproductive parameters that have been observed in other studies of reproductive senescence (e.g. Charmantier *et al.* 2006; Nussey *et al.* 2006) and which was readily observed in the raw data prior to in depth analysis.

The interaction between male age and sexual display effort in determining reproductive parameters (and their senescence) was assessed for significance in models, as these terms would ascertain how the ejaculate quality of males changed over each year of their lives in relation to the amount of effort they invested in sexual displays. The different life trajectories of males could then be calculated and described within the text using the model parameters reported in the corresponding table and its legend (Genstat 5 Committee 1993). To provide explicit tests of any changes in the relationship between ejaculate quality and display rates in ageing males, we subsequently fitted male age as a categorical term in these same models (two level grouping). The groupings chosen were informed by the large changes in ejaculate quality that have been observed in ageing males and were designed to show whether relationships between display rate and sperm quality in males up to and including their peaks ages of ejaculate production (0–6 years) are statistically different to those that are found in senescing males (> 6 years); the significance of these interaction terms is reported in associated figure legends. Parameter estimates of each of the two separate slopes (before and after 6 years) are presented at an appropriate point in the text and have been bootstrapped (for 1000 cycles) to provide robust 99% confidence intervals (CI) and associated *P* values.

During the analyses, examination of model fit for male age and ejaculate number indicated that there was a systematic departure from the data, this was corrected through $\log_e$-transformation of these terms. For our primary analyses, the final models that are reported had non-significant ($P > 0.05$) terms removed (McCullagh & Nelder 1983). In figures, the fitted lines in plots are derived from the model predictions associated with them and the data plotted are corrected for all remaining fixed and random terms (including individual identity) that were fitted in the model.

RESULTS

Patterns of senescence

LMM analyses showed that, during their mating season, males increase the time they spend displaying to females with each year of life, before approaching a plateau of *c.* 80 display days yearly (range 1–188) at around 4–6 years of age (Fig. 1a; LMM: $n_{years} = 5698$, $n_{males} = 1792$, intercept = 15.44, $\log_e$Age estimate = 58.370, SE = 2.262, Wald statistic = 666.20, $P < 0.001$; $\log_e\text{Age}^2$ estimate = −13.040, SE = 1.048, Wald statistic = 154.84, $P < 0.001$). Males that begin with a strategy of displaying for longer periods of the year maintain this as a fixed strategy, as male sexual display effort during their first 6 years was similar, relatively speaking, to their display effort through later life (from 7 to 24 years, repeatability of the average sexual display effort for each male across these age classes = 0.37, $F_{295,296} = 2.17$, Lessells & Boag 1987). Thus, individual houbara males display at relatively consistent levels across different years with little evidence of a senescent decline as they age.

We found that the ejaculates produced by males during their mating season had marked variation in the number of sperm they contained (median: 19.77 million, range: 0–761 million) and ejaculates frequently had heavily impaired motility, comprising of less than 50% motile sperm (38% of all ejaculates collected). We analysed how these key reproductive parameters changed through a male's life using generalized and linear mixed models and found clear evidence of spermatogenic senescence in males of this species. Both the numbers of sperm produced by males and the motility of their ejaculates show an initial increase in early life, before peaking at 4 years of age on average and showing clear evidence of senescent declines once they are beyond 6 year of age (Age + Age^2 terms in Tables 1 and 2; Fig. 1b). These models indicate that in the years following these peak ages, the numbers of sperm ejaculated by males dropped by over 60%, well below the level at which fertility using artificial insemination is negatively affected (Saint Jalme *et al.* 1994), while the probability of producing an ejaculate with severely impaired motility more than doubled to over 0.7. These results highlight the extent to which the average male's ejaculate quality deteriorates with advancing age in this species.

Reproductive effort and senescence

To evaluate if male reproductive effort was reflected in the quality of their ejaculates, but might later also increase the rate at which they senesced, we used display rate as a proxy for reproductive effort and

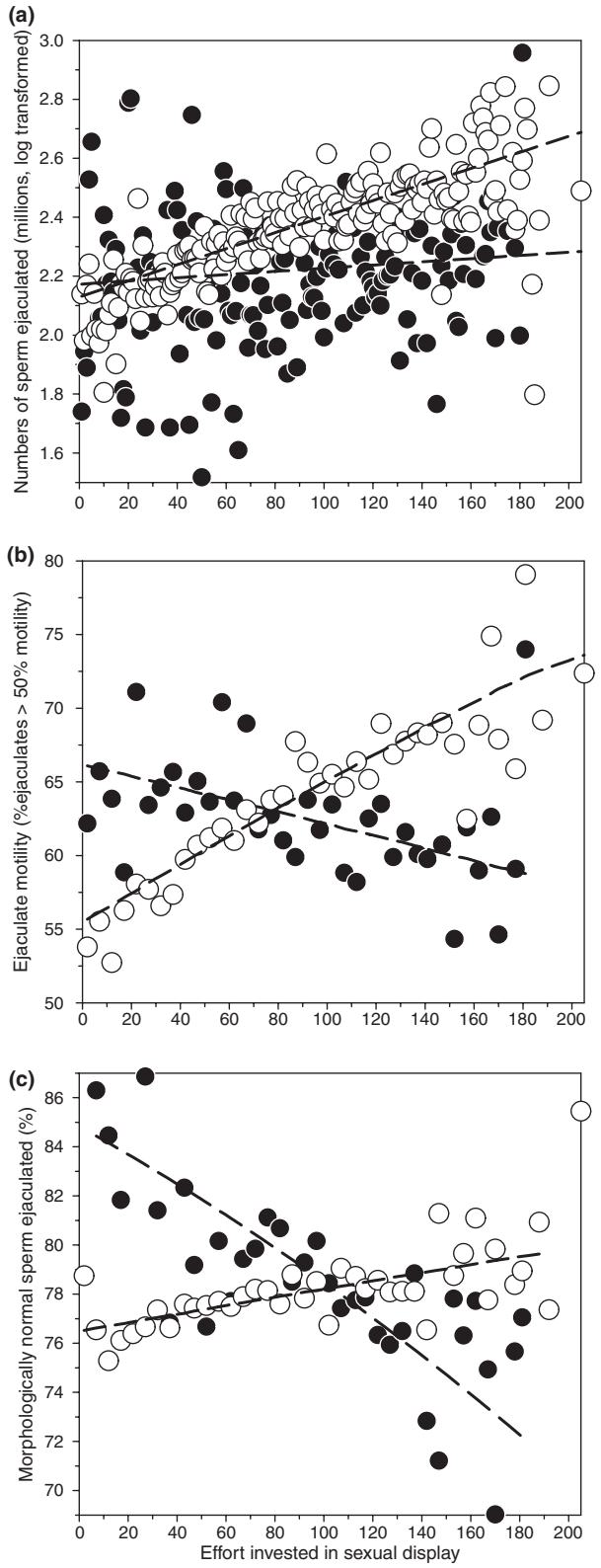

Figure 2 Evidence of early senescence in males that invest in sexual displays. The plots illustrate how sexual displays are associated with (a) the numbers of sperm ejaculated by males, (b) the overall motility of ejaculates, and (c) the percentage of morphologically normal sperm in ejaculates from males up to (white points) and after (black points) 6 years of age. All plots are from the models reported in Tables 1–3, but with age fitted as an explanatory factor (grouping: 1–6 years and 7–24 years of age). In each case, the interaction terms were highly significant (Wald statistics > 60, d.f. = 1, $P < 0.001$). Fitted lines are based on the bootstrapped model estimates reported within the main text. For illustrative purposes, data are grouped at every increment in the display index for plot (a), and every five increments for plots (b) and (c), thus the number of data points are not indicative of the sample size; please refer to Tables 1–3 for these and other specific details of the analysis.

assessed its relationship with the observed senescence in ejaculate quality that occurs over the lives of males. Consistent with expectations, our results show that males displaying at higher levels appear to also produce higher quality ejaculates in the first 6 years of their lives (display: age interactions in Tables 1 and 2; Fig. 2a,b), resulting in a significant positive relationship between display effort and both the number of sperm in ejaculates and the probability their ejaculates would contain a larger percentage of motile spermatozoa (bootstrap estimates of the correlation between sexual display effort and ejaculate parameters up to 6 years of age : sperm numbers, estimate = 0.00271, SE = 0.000129, 99% CI = 0.002365–0.003049, $P < 0.001$; ejaculate motility, estimate = 0.00404, SE = 0.000373, 99% CI = 0.003126–0.004937, $P < 0.001$; Fig. 2a,b). Furthermore, detailed morphological evaluation of a subset of ejaculates indicated that there was also display-related differences in the quality of sperm (Table 3), with males who display at higher rates producing ejaculates containing a small but significant increase in the percentage of morphologically normal sperm during this same period of their lives (bootstrap estimate of the correlation for males up to 6 years of age = 0.000960. SE = 0.000283, 99% CI = 0.000265–0.001774, $P < 0.001$; Fig. 2c). Thus, within the first 6 years of their lives, males that invest more effort into sexual displays also have higher sperm production rates with fewer instances of poor motility and aberrant morphology.

To test our primary prediction, that increased investment in reproduction would cause accelerated senescence, we further examined these relationships through later stages of male's lives. As predicted, we found that spermatogenic processes in males that displayed at higher rates appeared to 'age' more rapidly (display: age interactions, Tables 1–3). Our models showed that the advantage in terms of sperm numbers ejaculated by males that displayed at the highest levels deteriorated by around 85% in later life (bootstrap estimate of the relationship between sexual display effort and sperm numbers beyond 6 years of age: 0.000538, SE = 0.000193, 99% CI = 0.000050 to 0.001033, $P = 0.002$; Fig. 2a), while the relationships between display rates and ejaculate quality reversed (bootstrap estimates: sperm motility, estimate = −0.001757, SE = 0.000555, 99% CI = −0.003223 to −0.000213, $P < 0.001$; sperm morphology, estimate = −0.00409, SE = 0.00131, 99% CI = −0.00708 to −0.00112, $P < 0.001$; Fig. 2b,c). Thus, males displaying at the highest levels appeared to undergo a more rapid reproductive decline, which led to them producing the poorest quality ejaculates as they aged.

Although the analyses presented thus far examine measurements of male reproductive effort and spermatogenesis that were recorded within the same year, it is implicit within our results that display levels in males in their physiological prime (4 years of age in terms of sperm production) will also be indicative of their later reproductive decline. We examined this explicitly by fixing the 'sexual display effort' of males in our analyses to the level they expressed when they were at this physiological prime; we note that this approach also precludes the possibility that only co-variation between display and ejaculation

Table 1 Linear mixed model analysis of ejaculate size with age. The aim of the analyses was to examine the potential influence of male reproductive effort (using a sexual display index as a proxy) on the rate of senescent decline in the number of spermatozoa produced by males in their ejaculates. Sperm counts for each ejaculate were obtained using validated spectrophotometric methods and were $\log_e$ transformed prior to analysis to meet assumptions of normality. A linear mixed model was implemented; constant = −31.93. In total, the analysis includes assessments of 109 475 ejaculates collected from 1715 males over 10 years. See Methods for further details

Term	d.f.	Effect	SE	Wald statistics (χ^2)	*P*-value
Day of year	1	0.0440	0.000940	2196.55	< 0.001
Day of year^2	1	−0.000392	0.00000947	1709.18	< 0.001
Day of year^3	1	0.000000965	0.0000000306	991.64	< 0.001
$\log_e$Ejaculate number	1	0.2413	0.00646	1395.08	< 0.001
$\log_e$Age	1	8.823	0.2472	1273.46	< 0.001
$\log_e$Age^2	1	−0.601	0.0451	1071.76	< 0.001
Display effort	1	0.0971	0.00983	97.70	< 0.001
$\log_e$Age: display effort	1	−0.0249	0.00274	82.83	< 0.001
$\log_e$Age^2:display effort	1	0.00160	0.000190	70.87	< 0.001

Table 2 Generalized linear mixed model analysis of ejaculate motility with age. The aim of the analyses was to examine the potential influence of male reproductive effort (using a sexual display index as a proxy) on the rate of senescent decline in ejaculate motility. A binary variable was used which indicated whether each ejaculate contained more or less than 50% motile sperm when viewed under a microscope (scored as 1 or 0 respectively). A logistic GLMM was implemented using a logit link function and an estimated dispersion parameter. The constant = 57.52. In total, the analysis includes assessments of 158 799 ejaculates collected from 1770 males over 10 years. See Methods for further details

Term	d.f.	Effect	SE	Wald statistics (χ^2)	*P*-value
Day of year	1	0.0241	0.0120	408.53	< 0.001
Day of year^2	1	0.000151	0.00000474	1015.8	< 0.001
$\log_e$Ejaculate Number	1	0.523	0.194	728.35	< 0.001
$\log_e$Age	1	15.32	0.734	435.62	< 0.001
$\log_e$Age^2	1	−1.042	0.0539	374.57	< 0.001
Display effort	1	0.3291	0.0311	111.79	< 0.001
$\log_e$Age: display effort	1	−0.08508	0.00866	96.52	< 0.001
$\log_e$Age^2:display effort	1	0.00547	0.000601	82.68	< 0.001

Table 3 Generalized linear mixed model analysis of sperm quality with age. The aim of the analyses was to examine the potential influence of male reproductive effort (using a sexual display index as a proxy) on the rate of senescent decline in the number of morphologically normal spermatozoa within ejaculates. Binomial proportions were used as the response variable that indicated the number of morphologically normal sperm that were present in counts of *c.* 100 sperm from each ejaculate that had been stained with eosin nigrosin and viewed under a microscope. A logistic GLMM was implemented using a logit link function and an estimated dispersion parameter. The constant = 4.725. All other terms tested in the model were excluded with $P > 0.7$. In total, the analysis includes assessments of 5876 ejaculates collected from 1080 males over 9 years. See Methods for further details

Term	d.f.	Effect	SE	Wald statistics (χ^2)	*P*-value
Day of year	1	0.00151	0.000255	35.2	< 0.001
$\log_e$Age	1	−1.247	0.634	3.87	0.049
$\log_e$Age^2	1	0.104	0.0451	5.32	0.021
Display effort	1	−0.0886	0.0280	10.02	0.002
$\log_e$Age: display effort	1	0.0264	0.00782	11.41	< 0.001
$\log_e$Age^2:display effort	1	−0.00196	0.000545	12.88	< 0.001

production during maturation (or senescence) drives the observed relationships. Consistent with our previous analysis and interpretation, these results show that males displaying at higher levels during their physiological prime lost their ability to invest in much larger ejaculates (see Table S1; Fig. 3a), became more likely to produce ejaculates with severely impaired motility (Fig. 3b), and appeared to suffer from a greater and increasing number of errors during spermatogenesis (Fig. 3c). That is, they underwent earlier reproductive senescence.

Accounting for selective (dis)appearance

Age-specific changes in population parameters can occur because of the 'selective (dis)appearance' of individual phenotypes at specific life stages through a number of mechanisms (e.g. delayed maturation or early mortality of 'poor quality' individuals, see van de Pol & Verhulst 2006). Since these changes that occur at the population level have some potential to confound the within-individual analyses we present here, we further examined our data and models to preclude them as a possible explanation for our findings. To do so, we first restricted our dataset by excluding all individuals that joined the breeding programme late (e.g. adults transfers), and any individuals that left the dataset at any stage (e.g. due to mortality), and then reran our models. In all cases, the descriptions, tests, and levels of significance reported for model terms remained robust when examined in these restricted datasets (see Table S2). Second, we applied statistical control for selective (dis)appearance events in our models reported previously (Table 1–3), by fitting 'age at first ejaculation' and 'age at last ejaculation' as covariates in each of these models on the original unrestricted dataset (see Nussey *et al.* 2006; van de Pol & Verhulst 2006). Again, our results remained qualitatively unchanged (see Table S3). Together, these analyses indicate that our results showing earlier reproductive senescence of males displaying at higher rates are not driven by selective (dis)appearance events.

DISCUSSION

Our results provide clear evidence of reproductive senescence in male houbara bustards, with three important components of ejaculate viability showing rapid declines after their quality peaks at around 4 years of age. Crucially for the aims of the present study, it is the males that invest most effort into extravagant sexual display that experience this spermatogenic 'burn-out' at an earlier age. Our analyses showed that males that devoted more time and energy to sexual displays at younger ages continued to do so through later life, but they appeared to lose their ability to invest in greater sperm production, as evidenced by the declining numbers of sperm contained in their ejaculates. This 85% reduction in sperm production occurs alongside an increasing number of fine scale errors during spermatogensis that result in the production of sperm with

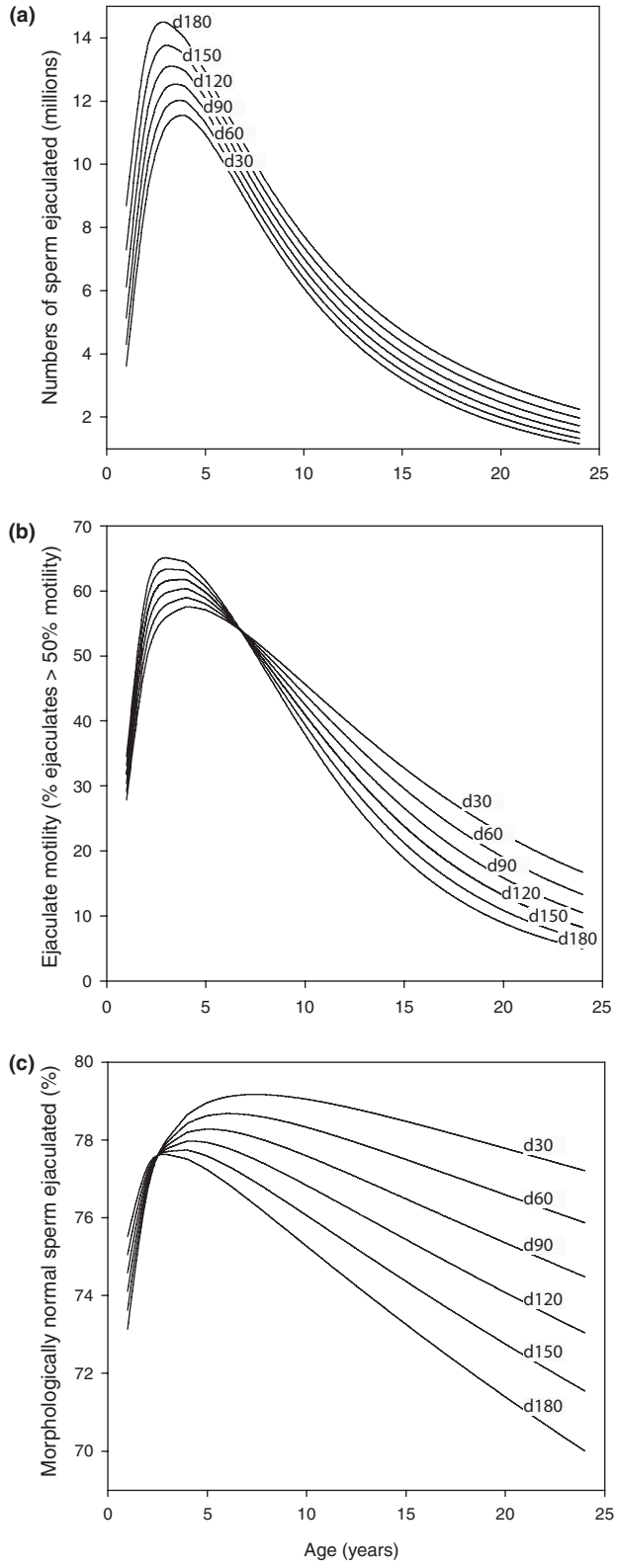

Figure 3 Ejaculate quality in ageing males according to their sexual display. The plots show model derived estimates of how the 'sexual display effort' of males at their physiological prime (4 years of age) is associated with differing age trajectories of ejaculate quality, as indicated by (a) the number of sperm ejaculated, (b) the overall motility of ejaculates and (c) the percentage of morphologically normal sperm ejaculated. Levels of sexual display vary from 30 to 180 days a year (denoted by 'd30' to 'd180' respectively).

morphological abnormalities such as double heads or flagella. It also coincides with what are presumably larger scale perturbations that result in the production of ejaculates with severely impaired fertility, owing to more than 50% of the sperm they contain being immotile.

Recently, interest has begun to intensify on the potential influence of male reproductive senescence on their ability to perform in sexual competition, with studies suggesting it may have a broad influence ranging from a reduced endurance during the mating season (Nussey *et al.* 2009), ability to attain dominance (Dean *et al.* 2010), and to develop sexually attractive ornaments (Balbontin *et al.* 2011). It has also been argued that male's post-copulatory sexual performance will be especially vulnerable to age-related declines, because high rates of spermatogenesis leave germ lines particularly susceptible to mutation accumulation with age, meaning older males produce sperm that may be both less fertile and competitive than their younger rivals (Pizzari *et al.* 2008). This theoretical possibility has received some support with sperm having been found to have deteriorated in a number of aspects of their performance in older males (Hale *et al.* 2008; Møller *et al.* 2009; Dean *et al.* 2010) However, other studies have been more equivocal (e.g. Gasparini *et al.* 2009), and we are unaware of any study that has found deterioration in the sperm of older males while accounting for the equally relevant possibility that the observed effect occurs instead at the population level i.e. that sexually vigorous and highly fertile males die sooner leaving only poorer quality males to survive to old age ('selective (dis)appearance', see van de Pol & Verhulst 2006; Dean *et al.* 2010). In our study, we show that male houbara undergo rapid declines in fundamental measures of ejaculate quality as they age, well beyond the level at which artificial insemination success is severely impaired (Saint Jalme *et al.* 1994), and that these effects are robust when removing the possible influence of any individual's appearance or disappearance from our analyses. Our study thus points to a hitherto hidden cost of ageing for males, which also has the potential to have direct fertility consequences for females.

Our central finding, that it is the males that invest most effort into reproducing that experience the earliest senescent deterioration of their spermatogenic machinery, provides the first phenotypic support in males for 'disposable soma' and 'antagonistic pleiotropy' hypotheses on the evolution of senescence (Williams 1957; Kirkwood 1977), and suggest a major additional factor likely to shape age-related sexual strategies and preferences of both sexes. While genetic support has yet to be uncovered in males, studies have found analogous effects at the phenotypic and genetic level in females. At the phenotypic level, female red deer that are more fecund in their early years have been shown to suffer an earlier subsequent deterioration in the timing and quality of the offspring they produce (Nussey *et al.* 2006). At the genetic level, negative genetic correlations have been shown to exist between early and late reproduction in female swans (Charmantier *et al.* 2006). It should be noted that other studies have failed to find similar relationships, however, possibly due to a confounding relationship between breeding success across life and female quality (e.g. in fur seals, Beauplet *et al.* 2006).

A further key finding of our study was that the early ageing of 'showy' males is severe enough to lead to the uncoupling of expected relationships between elaborate male display and the quality of sperm within ejaculates (Trivers 1972; Charge *et al.* 2010). Past their physiological prime, males investing most in sexual display produced the poorest quality ejaculates with greater numbers of morphologically abnormal sperm. These findings clearly have implications for theories on the evolution of female mate preferences that link male displays of

sexual vigour to their fertility (Trivers 1972). Indeed, if spermatogenic senescence is mirrored by the senescence of other physiological systems and ultimately male physical condition, as seems plausible, then male 'showiness' would be unreliable as an indicator of current competitiveness or competence (Andersson 1994), in this and potentially other systems (e.g. antlers in red deer, Nussey *et al.* 2009). Future studies should further address the reliability of male ornamental traits and displays as indicators of viability in both ageing males and their sperm, and assess the extent to which early actuarial senescence of sexually extravagant males might enforce the honesty of their sexual signalling while they are alive. In this respect, we note that adult houbara have a 90% annual survival rate in the wild (Y. Hingrat, unpublished data), suggesting that they will survive well beyond that age at which sperm senescence will become relevant to them; assuming senescence occurs at similar or faster rates to those occurring in the captive birds we consider here. If this is the case, then assessing the extent to which age-related variation in ejaculate quality influences sperm competition success and female promiscuity will be a key future objective.

Finally, our results support ideas that link differences in the intensity of sexual selection between species and so their likely investment in costly sexual traits, to variation in rates of senescence and potential longevity, at least for males (Promislow *et al.* 1992; Clutton-Brock & Isvaran 2007; Bonduriansky *et al.* 2008). Thus, they suggest that sexual selection may be an important evolutionary driver of ageing rates in nature.

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AUTHOR CONTRIBUTIONS

BTP, MSJ, YH, FL & GS conceived the study; YH & FL supervised and participated in data collection; BTP designed and performed the analysis with GS; BTP wrote the first draft of manuscript, and all authors contributed substantially to revisions.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Figure S1 The frequency of ejaculate assays within and across years.
Table S1 Mixed model analyses of reproductive senescence relative to the display effort of males at their 'physiological prime'.
Table S2 Mixed model analyses of reproductive senescence with a restricted dataset that excludes individuals that could drive selective (dis)appearance events.
Table S3 Mixed model analysis of reproductive senescence with statistical control for selective (dis)appearance of individuals.

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