

Does testis size track expected mating success in yellow dung flies?

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Summary

1. We quantitatively compare the fitness function linking male mating success in the field to body size and the allometric relationship linking testis length (estimating sperm production) to body size, both empirically derived, for the yellow dung fly *Scathophaga stercoraria* (Diptera: Scathophagidae).

2. We find that both relationships are similarly non-linear (quadratic) and increasing. The most parsimonious interpretation of this congruence is that the evolution of testis size (and hence sperm numbers) evolutionarily ‘tracks’ the number of copulations a male of a given size can expect, resulting in the particular non-linear testis size-body size allometry evident.

3. We offer a general, two-tiered statistical approach for this kind of quantitative comparison.

Key-words: Akaike’s information criterion (AIC), allometry, *Scatophaga*, sperm competition, sexual selection.

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Introduction

Comparative studies suggest that when sperm competition is greater, males invest more in testes to increase sperm numbers (e.g. Møller 1988, 1989; Gage 1994; Hosken 1997; Wedell 1997; Stockley *et al.* 1997). Corresponding evidence within species is scant and restricted to species with alternative mating strategies (Gage, Stockley & Parker 1995; Tomkins & Simmons 2002). Moreover, species with higher mating rates are also expected to have relatively larger testes and produce more sperm (e.g. Short 1979), as are particular phenotypes of a given species which expect to gain more mates. Therefore, large testes may indicate high (expected) mating success and/or high sperm competition risk. In the literature, testis size is consequently a commonly used estimator of sperm numbers, the degree of polyandry, and ultimately the strength of post-copulatory sexual selection (e.g. Hosken & Ward 2001). In this study we ask whether testis size tracks the expected mating success in the field for a particular species, the yellow dung fly *Scathophaga stercoraria* (Diptera: Scathophagidae; sometimes *Scatophaga*).

Mating success of a given male can depend on a variety of morphological, behavioural and physiological traits, which as a result are subject to sexual selection (Andersson 1994). One prominent trait affecting

mating success in many species is body size, larger males typically acquiring more mates (Andersson 1994). This is also the case in yellow dung flies (Borgia 1981; Jann, Blanckenhorn & Ward 2000; Kraushaar & Blanckenhorn 2002). One might therefore expect that large males have evolved relatively large testes. The strength of phenotypic selection on a given trait can be quantitatively estimated using standardized methods (Arnold & Wade 1984; Schluter 1988; Brodie, Moore & Janzen 1995). In general, a fitness component (e.g. mating success) is regressed on the trait of interest (e.g. body size). These fitness (component) functions describe the relationship between the trait and fitness, which can be complex and non-linear (Schluter 1988; Brodie *et al.* 1995). Analogously, static allometry is defined as the relationship of a given trait to body size within a species (Gould 1966; Cheverud 1982). Again, this relationship is often positive but can be equally complex and non-linear (e.g. sigmoid: Stern & Emlen 1999). For example, larger males generally have larger testes, although this varies considerably among species (Møller 1988, 1989; Pitnick 1996; Wedell 1997). Even though historically they were derived for different purposes and using different methods, fitness functions and allometries are thus quite analogous, and consequently can be analysed similarly and compared. What links them in this particular case is the dependence on body size, the intermediary variable.

We here compare two empirically derived phenomenological relationships, that between mating success and body size and that between testis size and body size. We show that they are similar, and thus conclude

that testis size tracks the expected mating success in the yellow dung fly. In so doing, we introduce a general statistical method for quantitative comparisons of fitness functions and/or allometric relationships in any species.

Materials and methods

STUDY SPECIES

The yellow dung fly is a classic model species to investigate sexual selection and sperm competition (Parker 1970; Simmons 2001). Males are larger than females, and the intensity of pre-copulatory sexual selection on male size varies both within and among populations but is, on average, strong and positive (Jann *et al.* 2000; Kraushaar & Blanckenhorn 2002). At this level, sexual selection is largely mediated by male–male competition, although subtle forms of pre- and post-copulatory female choice also occur (Borgia 1981; Reuter, Ward & Blanckenhorn 1998; Ward 2000). Sperm competition is intense in this species but in the wild approximately the same for all males, as they on average displace about 80% of stored sperm and female storage organs cannot expand to hold more (Parker 1970; Simmons 2001). So males rarely compete against more than two competitors at any appreciable level. Male body size also affects ejaculatory duct dimensions and hence copula duration and potentially also fertilization success (Parker & Simmons 2000; Simmons 2001; p. 351ff). Furthermore, testis size evolves in response to elevated levels of polyandry (Hosken & Ward 2001) and changes allometrically with body size (Stockley & Seal 2001; Hellriegel & Blanckenhorn 2002).

STATISTICAL METHODS

We use data from two studies published in other contexts. Jann *et al.* (2000) assessed male mating success (mated or not) as a function of body size, estimated by hind tibia length (HTL), in the field over two seasons in one yellow dung fly population, for a total of $n = 7424$ males. From this data set we estimated the fitness function linking male mating success to body size specifically for this study. Hellriegel & Blanckenhorn (2002) investigated the allometry of testis length and body size, again estimated by HTL, of $n = 138$ males reared at various temperatures and food levels in the laboratory to produce a full range of body sizes (see their Fig. 2a for the raw data; cf. Stockley & Seal 2001).

The relationship between a fitness component and a trait can take any form, and can be visualized by non-parametric regression (Schluter 1988; Brodie *et al.* 1995). It may also follow a particular (*a priori*) parameterized mathematical function. Typically, linear models are used to demonstrate directional selection and quadratic models to demonstrate stabilizing, disruptive or asymptotic selection (Brodie *et al.* 1995). The proper technique to estimate the best parameterized

model for a given relationship between Y and X is model selection based on Akaike's information criterion, AIC (Burnham & Anderson 1998). This technique quantifies the fit of all models considered, picked *a priori* and/or after inspection, relative to the best-fit model, controlling for the number of parameters, K , in the various models. Note, in contrast, that the R^2 -value of regular least-squares regression necessarily increases with the number of parameters (i.e. variables). However, while model selection is well suited for estimation of functional relationships, it does not accommodate significance testing and entirely refrains from setting arbitrary significance thresholds (such as P -values).

We therefore used a two-tiered approach, combining two established pre-existing statistical methodologies. We first used model selection techniques on the raw data to estimate the best fitting model describing the relationships between mating success and HTL on the one hand, and between testis length and HTL on the other. The simplest and most important question to ask was whether the relationships are linear or non-linear. In our particular case we therefore chose only two models, linear and quadratic, as higher order polynomials could be excluded by inspection. We are aware that other non-linear (e.g. logistic) functions might fit our data equally well (or even better; Burnham & Anderson 1998), but the quadratic is the simplest and most intuitive for our purpose here and is typically used when computing selection differentials (Arnold & Wade 1984). If the data are best described by the same mathematical function one can then use significance (t -)tests to quantitatively compare them. The X -axis was the same in both data sets (HTL), but the Y -axes were different. To quantitatively compare the parameters of the fitted functions, the Y therefore had to be standardized. Two standardizations of data are commonly used, both applied in selection differentials (Arnold & Wade 1984). Dividing each data point by the mean of the entire population standardizes the data about a mean = 1, but does not standardize the variance. Alternatively, z -scores, whereby the population mean is subtracted from each data point and this difference divided by the population standard deviation (SD), standardize the data to a mean = 0 and a SD = 1. As mating success is a binary and testis length a continuous variable, we had to use the latter standardization.

Results

Model selection results indicate that of the two simplest models that could fit the data, the quadratic model best fitted both data sets (lowest AIC; Table 1). The evidence ratio column indicates that for the testis length data the quadratic model (with $K = 3$ parameters) describes the fit about twice as well as the linear ($K = 2$), while for the mating success data it is about 500 times better (Table 1). We therefore conclude that the relationship of both variables with HTL is best

Table 1. AIC model selection statistics for the two data sets (see Burnham & Anderson 1998 for details)

Model	LS R^2	Log likelihood	K	AIC	Delta AIC	AIC weight	Evidence ratio
Mating success data ($n = 7424$)							
Linear	–	–4448.3	2	8900.7	12.46	0.002	0.002
Quadratic	–	–4441.0	3	8888.3	0.00	0.998	1
Testes data ($n = 138$)							
Linear	0.332	45.1	2	–86.1	1.41	0.331	0.495
Quadratic	0.349	46.8	3	–87.5	0.00	0.669	1

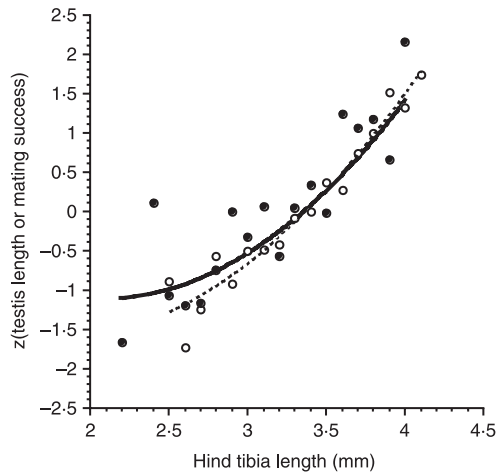


Fig. 1. Regression of z -scored testis length (filled circles and solid line) or mating success (open circles and broken line) on body size (hind tibia length: HTL) for the yellow dung fly. For clarity, one mean value per 0.1 mm size category is depicted ($n = 1–18$ males for testis length, and $n = 35–1135$ males for mating success per size category). The quadratic regression line is based on the raw data ($n = 138$) for testis length ($z(\text{testis length}) = 0.677 \times \text{HTL}^2 - 2.802 \times \text{HTL} + 1.777$) and on weighted means for mating success ($z(\text{proportion mated}) = 0.605 \times \text{HTL}^2 - 2.082 \times \text{HTL} + 0.142$).

described by an increasing non-linear (quadratic) function. For testis length the log likelihood, on which the AIC is based, was calculated from the R^2 values of a regular least squares analysis using SPSS, as can be conveniently done (Burnham & Anderson 1998; pp. 17 and 48). The binary mating success data were analysed using logistic regression with the software program MARK (White & Burnham 1999).

Because the best fitting functions here were the same, we could directly compare them. Figure 1 shows that the quadratic fits of testis length and mating success (both z -scored) on HTL look remarkably similar: $z(\text{testis length}) = 0.677 \times \text{HTL}^2$ (SE: 0.138) $- 2.802 \times \text{HTL}$ (SE: 0.858) $+ 1.777$ (SE: 1.307); and $z(\text{proportion mated}) = 0.605 \times \text{HTL}^2$ (SE: 0.067) $- 2.082 \times \text{HTL}$ (SE: 0.081) $+ 0.142$ (SE: 0.132). Comparing both the quadratic and linear components using t -tests yields non-significant differences ($t < 1.2$; $P > 0.2$).

Discussion

Our analysis indicates that the fitness relationship between mating success and hind tibia length, and the allometric relationship between testis length and hind tibia length are similarly non-linear and increasing. This means that males acquire increasingly more mates (i.e. pre-copulatory sexual selection is directional and accelerating; Brodie *et al.* 1995) and also have increasingly larger testes as they get larger, and/or mating success and testis size reach an asymptote at some low but non-zero value (these two interpretations cannot be discriminated). The most parsimonious interpretation of this congruence of these relationships is that testis size, and hence sperm production, is adjusted to the number of copulations a male of a given size can expect. That is, the evolution of testis size apparently ‘tracks’ mating success. This is an evolutionary (as opposed to phenotypic or behavioural) response to sexual selection, as testis length (when completely filled with sperm) and body size are heritable and fixed at emergence, i.e. before sexual reproduction begins (Hosken & Ward 2001; Blanckenhorn 2002; Hellriegel & Blanckenhorn 2002).

The testis length data stem from a laboratory study where a range of body sizes was produced by manipulating temperature and larval food levels (Hellriegel & Blanckenhorn 2002), whereas the pairing success data set stem from the field (Jann *et al.* 2000). It is well known that investment in sperm reflects strategic decisions on the part of a male that are subject to life history trade-offs, in addition to being influenced by various environmental factors (Simmons 2001, e.g. Parker & Simmons 1994, 2000; Stockley & Seal 2001). So the body size/testis size relationship produced under controlled laboratory conditions may not be the same as that expressed in the field. We nonetheless obtained a close match between the two data sets. This probably relates to the fact that our pairing success data are averages of over 7000 flies taken over 2 years and multiple environmental and competitive conditions. Both relationships as well as our approach thus appear to be quite robust.

Our comparison was performed at the phenomenological level. That is, the data sets analysed contain no information about the behavioural mechanisms underlying sexual selection or the physiological mechanisms underlying testis development. This approach contrasts with the behavioural experiments (Simmons 2001) or comparative studies across taxa (e.g. Møller 1988, 1989; Gage 1994; Hosken 1997) that are more typical in the fields of sperm competition and sexual selection. Nevertheless, our results agree well with what we know about yellow dung fly sexual selection and behavioural ecology. Pre-copulatory sexual selection on male size is largely mediated by male–male competition and strong in *S. stercoraria* (Jann *et al.* 2000), and body size appears to respond to selection in the wild (Kraushaar & Blanckenhorn 2002). In contrast, post-copulatory sexual selection on male size appears weak. This is primarily

because sperm displacement yields diminishing returns, so it does not pay males to keep copulating after some time. Larger males can transfer a given (optimal) sperm number faster and have better prospects to obtain more mates (Parker & Simmons 1994, 2000). As a result, small males copulate for longer, and fertilization success is typically not affected by male size (Parker & Simmons 1994). Nonetheless, when Hosken & Ward (2001) experimentally altered polyandry levels, they found evolutionary responses in testis but not body size. Yellow dung fly males are larger than females and thus can force copulations, although females attempt to sneak up towards particular large or dominant males (Borgia 1981; Reuter *et al.* 1998). Even though females can store enough sperm for at least three clutches (Parker 1970), they consequently mate at least once every time they lay a clutch of eggs. However, female mating frequency in nature is unknown, as in many species. Despite strong male control of mating, females of any size should still prefer to mate with larger males because size is heritable and conveys strong fitness benefits (Jann *et al.* 2000; Blanckenhorn 2002) but incurs no visible costs beyond those of mating itself (Blanckenhorn *et al.* 2002; Hosken, Uhía & Ward 2002). Evolution of cryptic (i.e. post-copulatory) female sperm selection mechanisms allowing preferential use of large males' sperm is also possible, but such effects are likely to be weak in comparison and remain to be demonstrated (though cryptic female choice for other male characters has been shown: Ward 2000). In conclusion, comparative and microevolutionary studies suggest sperm competition strongly selects on testis size in this and other species. Our study shows that testis size evolution in yellow dung flies is additionally driven by the number of mates a male of a given size can expect, presumably resulting in the particular non-linear testis size-body size allometry evident in Fig. 1 (cf. Hellriegel & Blanckenhorn 2002).

Finally and importantly, our study offers a two-tiered, general statistical approach to estimate and compare fitness functions and/or allometric relationships, particularly when they are non-linear, as is often the case. The novelty lies in combining two distinct established statistical approaches in a new context of comparing two different types of empirical relationships (allometries and fitness functions). The need for such comparisons arises not only in (sexual) selection studies, but also in many other realms of organismic biology such as physiology or life history theory. First the best-fit model is determined using model selection (Burnham & Anderson 1998). Thereafter, the functions can be quantitatively compared using significance testing, but only if the relationships are described by the same mathematical function. Otherwise the relationships are likely different, although often a particular relationship can be described by several, mathematically quite different functions. It is therefore of utmost importance to focus on the particular question asked when choosing the functions considered (Burnham & Anderson 1998).

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