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Evolutionary biology

Context-dependent expression of sperm quality in the fruitfly

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In most species, females mate multiply within a reproductive cycle, invoking post-copulatory selection on ejaculatory components. Much research has focused on disentangling the key traits important in deciding the outcomes of sperm competition and investigating patterns of covariance among these traits. Less attention has focused on the degree to which such patterns might be context-dependent. Here, we examine whether the expression of sperm viability—a widely used measure of sperm quality—and patterns of covariance between this trait and male reproductive morphologies, change across distinct age classes and across naturally occurring genotypes, when expressed in both heterozygotic (extreme outbred) and homozygotic (extreme inbred) states in the fruitfly *Drosophila melanogaster*. Older males, and heterozygous males, generally exhibited higher sperm viability. The male age effect seems at least partly explained by a positive association between sperm numbers and viability. First, old males possessed more stored sperm than young males, and second, sperm numbers and viability were also positively associated within each age class. Furthermore, we found a positive association between sperm viability and testis size, but only among heterozygous, old males. These results suggest that sperm quality is a labile trait, with expression levels that are context-dependent and shaped by multiple, potentially interacting, factors.

1. Introduction

Females of many species mate with multiple partners. This generates sexual selection on the male ejaculate and ensures that male post-copulatory adaptations will be profound contributors to male reproductive success [1]. Increases in phenotypic expression across numerous ejaculate and sperm traits have been linked to increased male fertility under reproductive competition. These traits include those that augment sperm production capacity (sperm numbers) [1], sperm quality [2,3] and those that increase competitiveness of the seminal fluid itself [1].

In recent years, sperm viability (the proportion of live, fertilization competent sperm within an ejaculate) has been widely used as a measure of sperm quality [4], and this metric seems well justified within the insects. It has been shown to be a key predictor of male post-copulatory success in crickets [5], and representative sperm viability values across insect species have been positively tied to the level of post-copulatory sexual selection experienced by these species [6]. The intrinsic factors affecting the expression of sperm viability, however, remain generally unclear, and limited information exists as to how this trait is involved in genetic [7–10] or allocation trade-offs with other life-history traits [11–13], which could ultimately impact on a male's realized reproductive outcomes. In particular, little is known as to whether likely targets of post-copulatory sexual selection, specifically intra-ejaculate traits, will routinely trade-off against each other. That is, do males face a trade-off (and potentially alternative post-copulatory reproductive strategies), whereby investment into large numbers of sperm occurs at the expense of sperm quality, and *vice versa* [2]?

Here, we report the results of an experiment, in which we screened for interacting intrinsic (male age) and genetic effects (distinct globally sourced genotypes expressed in both homozygous and heterozygous states) on the expression of

sperm viability, in the fruitfly *Drosophila melanogaster*. As part of the design, we screened for phenotypic covariance involving ejaculate (stored sperm numbers and sperm length) and reproductive traits (testis, seminal vesicle and accessory gland size), and their potential interactions with age and genetic effects, on sperm viability.

2. Material and methods

Full methods are provided in the electronic supplementary materials. The experiment harnessed five near-isogenic strains of *D. melanogaster*—four derived from globally diverse wild populations (DAH, PUER, ZIM and MAD, denoted 'wild-type') and a fifth (w^{1118}) from the Canton-S laboratory population.

The experiment was conducted in four temporal blocks. Environmental sources of variation were controlled, including maintaining egg numbers at a set density in vials that produced the focal flies. In the parental generation, virgin males of each wild-type strain were crossed either to virgin females of the same strain (in eight pairs per replicate) to create a standard homozygous focal male genotype per strain, or to virgin females from w^{1118} (eight pairs per replicate) to create a standard heterozygous male genotype per strain. In each block, we generated three replicate crosses per wild-type strain, in each of the homozygous and heterozygous states (see electronic supplementary material, table S1). Thus, we could measure the contribution of each of four distinct genotypes in the context of both a diploid (i.e. two copies of each autosomal gene derived from the focal genotype—genome-wide homozygosity) and haploid (i.e. one copy of each autosomal gene derived from the focal genotype—genome-wide heterozygosity) states (see the electronic supplementary material, for motivation).

Ten days after these parental crosses, virgin males were collected and stored in groups of eight, with each group assigned to an age treatment. Males assigned to the young class were held in vials for three days prior to dissection; males in the old class for 23 days. We measured sperm viability (Molecular Probes, Eugene, OR, USA), testis and accessory gland areas, seminal vesicle area and representative sperm head length from each dissected male.

Data were analysed using generalized linear models with binomial errors and logit link, corrected for overdispersion in R v. 2.11.1 [14]. The response variable was our measure of sperm quality (live sperm and dead sperm). The expression of several of the reproductive traits (total sperm, testis, accessory gland and seminal vesicle areas, but not sperm length) varied markedly across the two male age classes (see electronic supplementary material, figure S1), and it was not possible to include these variables and age in the same analysis. Therefore, we fitted two sets of models; first a model in which age (3, 24 days), genetic strain (DAH, PUER, ZIM and MAD) and genetic status (homozygote state and heterozygote state) were fitted as fixed factors and sperm length as a covariate; then age-specific models (one for young males and one for old), in which the reproductive morphological traits were fitted as covariates. In each analysis, full models were progressively simplified, removing non-significant parameters one at a time and comparing the models with *F*-tests.

Table 1. Final models of the effects of reproductive traits, genetic strain, genetic status and age, on sperm viability. SS, sum of squares.

source	SS	d.f.	*F*	*p*
(*a*) age-general model				
genetic status	2000	1	5.974	0.016
genetic strain	485	3	0.483	0.695
age	2286	1	6.826	0.010
sperm length	519	1	1.551	0.215
residuals	50 562	151		
(*b*) young males model				
genetic status	2.9	1	0.0232	0.880
genetic strain	602	3	1.5815	0.205
accessory gland area	76	1	0.5991	0.442
testis area	30.6	1	0.2411	0.625
sperm length	18.4	1	0.1447	0.705
seminal vesicle	463.3	1	3.6514	0.061
total sperm	1364.1	1	10.7508	0.002
residuals	6598.2	52		
(*c*) old males model				
genetic status	2471.1	1	5.9059	0.018
genetic strain	1219.0	3	0.9711	0.411
accessory gland area	1287.9	1	3.0782	0.083
testis area	1191.5	1	2.8476	0.096
sperm length	454.7	1	1.0867	0.301
seminal vesicle	236.8	1	0.5660	0.454
total sperm	2376.0	1	5.6786	0.020
genetic status × testicle area	2359.8	1	5.6399	0.020
residuals	30 962.5	74		

3. Results

There were no significant differences in the expression of sperm viability across the four genetic strains (table 1*a–c*). The full model revealed that old males had higher sperm viability than young males (figure 1*a*). This pattern is, however, probably attributable to old males storing larger numbers of sperm than young males (see electronic supplementary material, figure S1), because there was a clear association within each age-class for sperm viability to increase with increasing sperm numbers (table 1*b*,*c* and figure 1*b*,*c*).

Although males whose genotypes were expressed in a heterozygous state generally exhibited higher sperm viability than their homozygous counterparts (table 1), this relationship was strongly age-dependent and, furthermore, contingent on testis size (table 1). Specifically, testis size was positively associated with sperm viability, but only among old males whose genotypes were expressed in a heterozygous state (table 1*c* and figure 1*d*).

4. Discussion

The expression of sperm viability, and its association to the reproductive morphologies investigated in our study, were dependent both on the genetic status (i.e. whether the male's genotype was expressed in a homozygous or heterozygous state) and the age of the focal males. We detected a context-dependent positive association between testis size and sperm viability, which was only apparent in the cohort of old males

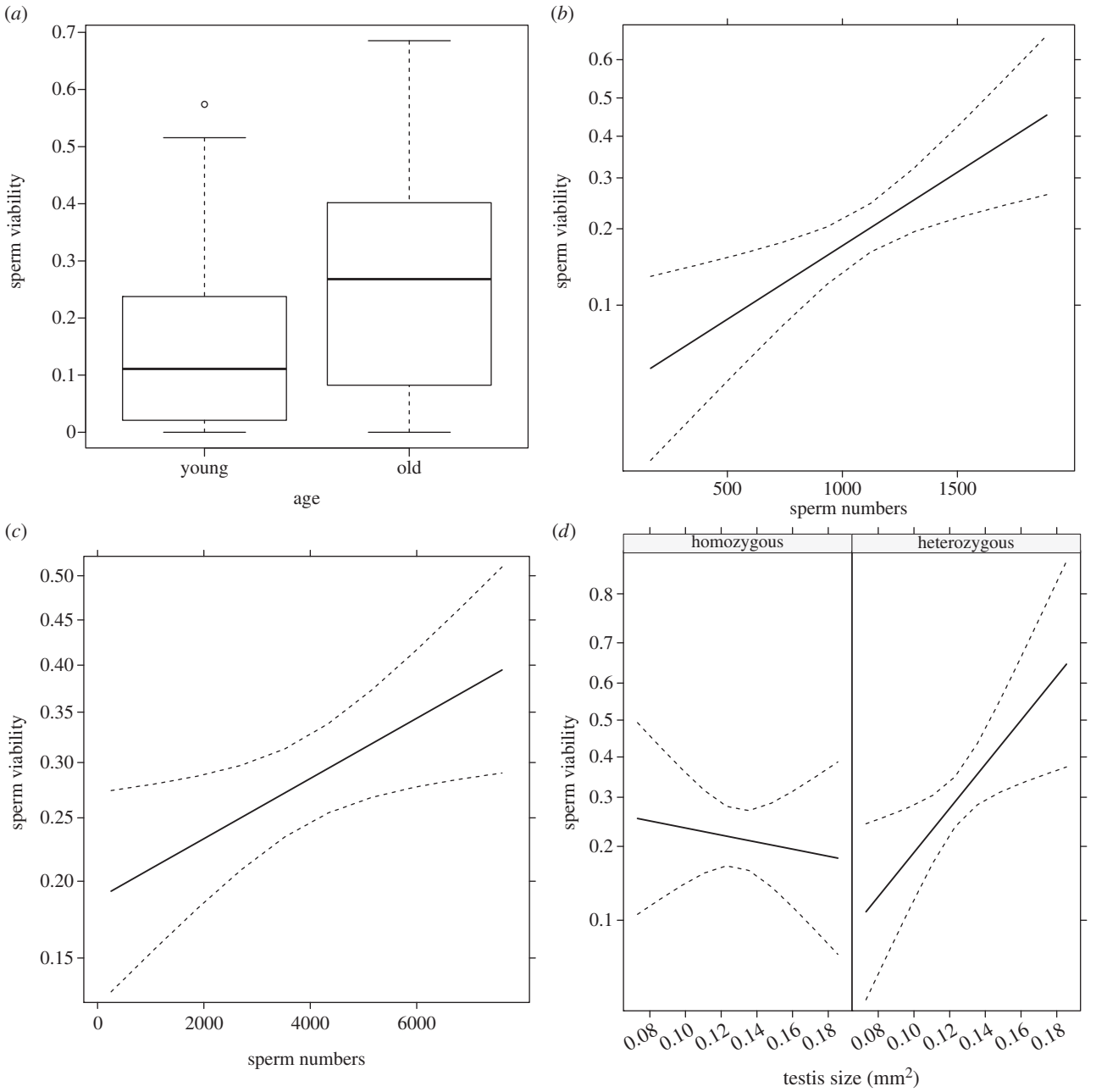

Figure 1. (*a*) Boxplots of proportion sperm viability across young and old males; (*b*,*c*) linear regression line and 95% confidence envelopes for the association between sperm numbers extracted from the dissected seminal vesicle and sperm viability in (*b*) young and (*c*) old males; (*d*) plot of interaction between testis size and genetic status on sperm viability in old males. Dotted lines show 95% confidence envelopes.

whose genotypes were heterozygously expressed. Although our study did not set out to test inbreeding effects, the effects we found are consistent with the idea that the association between testis size and sperm quality is eroded by inbreeding, with old males exhibiting greater sensitivity to such effects (see the electronic supplementary material for broader discussion of this effect). Testing this would, however, require new experiments under a range of inbreeding coefficients typical of those found in natural populations.

The age effects revealed in this study are worth critical enquiry. Males of both young and old cohorts were kept as virgins until their dissection, and thus old males were storing many more sperm—as reflected in their larger seminal vesicles and greater sperm counts per vesicle (see electronic supplementary material, figure S1). Old males also possessed larger accessory glands, responsible for producing the seminal proteins, but smaller testes (see electronic supplementary material, figure S1), which suggests that their sperm production capacity had decreased with age. Although old males were storing sperm that was likely to be generally older than those of their young counterparts, the viability of these old sperm was around 75% higher than the sperm of young males (see electronic supplementary material, figure S1). This observation suggests that intra-ejaculate sperm numbers could well be the critical determinant of sperm viability, rather than male age *per se*, with increases in sperm numbers acting in some currently unknown way to protect the viability of sperm [15]. This suggestion is strengthened greatly by the finding of positive associations between sperm numbers and viability within each age class. A broader implication of this finding is that it suggests that males who mate less regularly relative to their competitors will display superior fertility under sperm competition once engaging in copulation. Ultimately, definitively disentangling which of male age or sperm numbers is the primary driver of the sperm viability phenotype would require a design in which the males of each age class are

mated multiply and at different mating rates. That said, the relationship described above, between testis size and sperm viability in old and heterozygous males did indeed account for confounding effects of sperm numbers—and this result thus highlights dedicated male age effects on the expression of sperm viability.

It has been suggested that intra-ejaculate traits will be subjected to allocation trade-offs [2]. While some studies support this idea, showing trade-offs between sperm size and quantity/quality [13,16,17], evidence for trade-offs between sperm quantity and quality have remained elusive, with growing support for the suggestion that expression of these two intra-ejaculate traits is usually either unlinked [7] or otherwise positively associated [15,18], as we report here. In our study, this positive association was robust across distinct age classes and genotypes regardless of whether they were expressed homozygously or heterozygously. Thus, the current weight of evidence indicates that post-copulatory sexual selection does not regularly drive trade-offs between sperm numbers and sperm quality [2,7,15,18]. Yet, evidence exists that production of high-quality sperm nonetheless does routinely come at a cost to males, by invoking trade-offs between investment in sperm quality and in other life-history phases [11,13].

In sum, our results suggest that sperm viability—a widely used measure of sperm quality and likely key determinant of male fertility outcomes—is a dynamic trait, whose phenotypic expression is context-dependent and potentially shaped by numerous interacting factors and morphological associations.

Data accessibility. Raw data are available in figshare: http://dx.doi.org/10.6084/m9.figshare.805223 [19].

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