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Sperm Viability and Sperm Competition in Insects

F.M. Hunter[1] and T.R. Birkhead
Department of Animal and Plant Sciences
University of Sheffield
Sheffield S10 2TN
United Kingdom

Summary

Sperm quality plays an important role in vertebrates in determining which male has the advantage when two or more males compete to fertilize a female's ova [1, 2]. In insects, however, the importance of sperm quality has never been considered, despite sperm competition being widespread and well studied in this group [3, 4]. We tested the hypothesis that sperm viability, measured as the proportion of live sperm, covaried with the intensity of sperm competition in insects. In a pairwise comparison of seven closely related species pairs, each comprising a monandrous and a polyandrous species (i.e., with and without sperm competition, respectively), we found that in all cases the polyandrous species had a higher proportion of live sperm in their sperm stores. The distribution of the percentage of live sperm showed considerable inter- and intraspecific variation, suggesting that, all else being equal, males will vary in their ability to fertilize ova on the basis of sperm viability alone. Our results suggest that sperm viability is one of a suite of male adaptations to sperm competition in insects.

Results and Discussion

We tested the hypothesis that sperm viability, measured as the proportion of live sperm, is higher in insects with relatively more intense sperm competition.

As predicted, in seven out of seven species pairs, the polyandrous species had a lower proportion of dead sperm (Figure 1); the probability of this occurring by chance is $p = 0.008$ (binomial probability test). Moreover, after accounting for significant differences between species pairs (logistic regression: $F = 45.49$, $df = 6302$, $p < 0.0001$), there was a highly significant difference between polyandrous and monandrous species ($F = 164.90$, $df = 1302$, $p < 0.0001$), with the proportion of dead sperm in monandrous species being on average seven times higher than in polyandrous species (range = 1.34–14.8 times). Moreover, there was a positive correlation between the proportion of dead sperm in the monandrous and polyandrous species of each species pair (Pearson's correlation using arcsine-squareroot transformed data: $r = 0.806$, $df = 5$, $p = 0.026$; Figure 2), indicating that sperm viability has a phylogenetic component, since the variation in sperm viability within species pairs was less than that between pairs, although the correlation was largely due to the high proportion of dead sperm recorded from species pair seven: the cockroaches. The fact that all the points fall above the line of equality in Figure 2 shows again that the monandrous species have a higher proportion of dead sperm. There was considerable variation in sperm viability among males, as illustrated by the range of the proportion of dead sperm for each species (Figure 1).

Our results suggest that males of insect species whose females are polyandrous produce higher quality sperm, in terms of the proportion alive, than monandrous species. This result, although based on a small sample, is consistent with theory—if a male is in competition with other males over a female's ova, it will be in his interests not just to produce many sperm but to maximize the number of those sperm that are viable. Providing there are sufficient sperm of adequate quality for fertilization, there will be no such selective pressure on males in species that are not usually in competition with other males. Sperm viability is likely to be one of a suite of traits favored by sperm competition; other potentially important traits include sperm longevity, motility, swimming speed, and timing of acrosome reaction—as has previously been shown in humans and domesticated mammals and birds [2, 5–11]

Why there should be any dead sperm in the seminal vesicles or testes requires some explanation. The problem is similar to that of why sperm morphology is so variable both within ejaculates from the same individual and among individuals from the same species. The traditional view is that sperm are difficult to manufacture and that deformed or dead sperm are simply production errors [12]. If this is correct, our findings suggest that there is selection on males of polyandrous species either to make fewer production errors or to invest more in maintaining sperm once they are formed. There have been other explanations for variation in sperm morphology which might also encompass dead sperm, notably Baker and Bellis's kamikaze sperm hypothesis [13] (but see [14]).

We found considerable interspecific variation in the proportion of viable sperm. The sperm attributes that are most important to a species are likely to depend on the mechanisms of sperm transfer, storage, and manipulation by the female. Among insects, these mechanisms show considerable variation [4], and it seems likely that viability may be more important for some species than for others. It is also possible that there is a trade-off among quality traits so that some species have maximized swimming speed for example, and others have maximized viability. The trait that is most important will depend on that species' sperm competition mechanism and ecological and behavioral factors, such as timing of insemination relative to ovulation and intensity of sperm competition.

Our results also suggest that considerable intraspecific variation exists in the proportion of dead sperm (Figure 1). To evaluate this properly, one should establish whether individual males show consistent differences in ejaculate viability. Such information is poten-

[1] Correspondence: f.m.hunter@sheffield.ac.uk

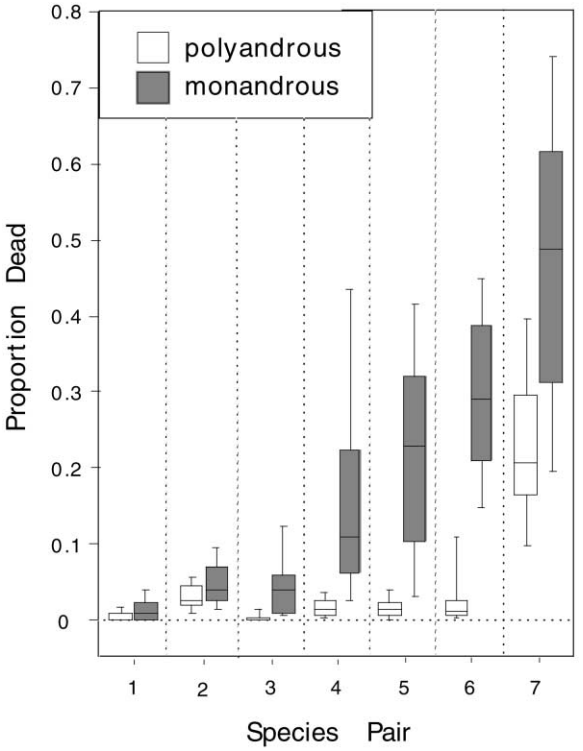

Figure 1. Proportion of Dead Sperm in Polyandrous and Monandrous Species Pairs

1: black larder beetle (*Dermestes ater*, n = 15 males, C), khapra beetle (*Trogoderma granarium*, n = 20, C); 2: honeybee (*Apis mellifera*, n = 30, W), bumble bee (*Bombus terrestris*, n = 22, W); 3: fruitfly (*Drosophila pseudo-obscura*, n = 20, C), fruitfly (*D. subobscura*, n = 20, C); 4: yellow dungfly (*Scatophaga stercoraria*, n = 30, W), housefly (*Musca domestica*, n = 20, C); 5: Wood ant (*Formica truncorum*, n = 20, W), dinosaur ant (*Dinoponera quadriceps*, n = 9, C); 6: seaweed fly (*Coelopa frigida*, n = 32, C), greenbottle (*Lucilia caesar*, n = 32, C); 7: cockroach (*Diploptera punctata*, n = 20, C), cockroach (*Nauphoeta cinerea*, n = 20, C); polyandrous, monandrous, respectively. Species were either collected from the wild (W) or were reared in captivity (C). The 10th, 25th, 50th, 75th, and 90th percentiles are shown for each species.

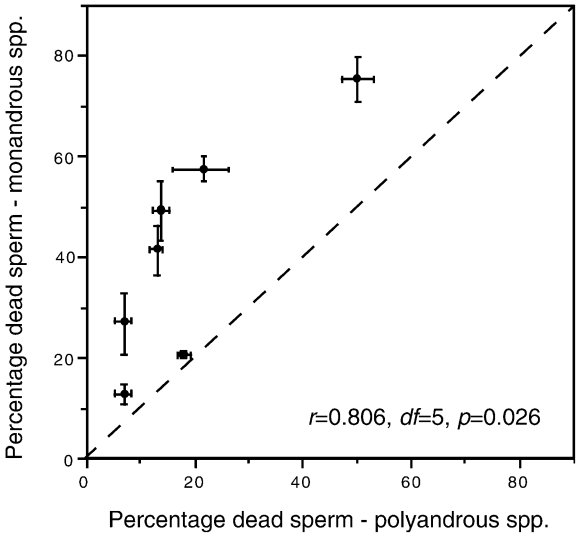

Figure 2. Relationship between the Percentage of Dead Sperm in Polyandrous and Monandrous Species

The symbols represent the arcsine-squareroot transformed means ± SE for each species pair. The dashed line represents the line of equality.

tially available for a number of vertebrates where it is possible to obtain replicate semen samples from males, e.g., [15–17], and it is already well established that consistent differences in fertilizing capacity exist among males, e.g., [1, 2, 5, 11]. The consistency of sperm viability in individual male insects remains to be tested.

Many studies of sperm competition in insects comprise two males sequentially inseminating the same female. In most species, the greatest proportion of eggs is fertilized by the second male and is referred to as P2. However, most studies show considerable variation in P2 (for a review, see [4]). Several factors, including the interval between inseminations, can account for some of this variation, but the variation in ejaculate quality that our study demonstrates may also help to explain some of this variation, see also [4].

In conclusion, we used a simple assay for recording the proportion of live sperm in a male's seminal vesicles or testes to show that male insects with relatively more intense sperm competition produce higher proportions of viable sperm. This suggests that sperm competition has shaped the quality of sperm in insects.

Experimental Procedures

Materials

We used a pairwise comparative approach [18] and compared insect taxa whose females are classed as polyandrous or monandrous, that is with and without sperm competition, respectively (using information in [19]). The seven species pairs (from four orders) comprised a mixture of wild and laboratory-bred animals with no consistent bias with respect to whether females were monandrous or polyandrous (see legend to Figure 1 for details). We recognize that the use of laboratory-bred animals might not be ideal, since extreme inbreeding might adversely affect sperm viability—although there is no evidence for this in insects. Our sample size is limited because few monandrous species exist [19], and it is difficult to obtain live specimens of these.

Sperm Assay

Males were anesthetized with CO_2 and dissected immediately. We removed the seminal vesicle (the male's sperm store) or the testes from sexually mature males and placed them in phosphate-buffered saline (PBS) at 5°C. We took sperm from the seminal vesicle, because this is the primary sperm storage site, but in the diptera this structure was not evident, and in these three species pairs we took sperm from the testes. Sperm were released from the tissue and diluted with PBS. Equal volumes of sperm and live/dead stain (LIVE/DEAD viability/cytotoxicity assay, Molecular Probes) were mixed in an eppendorf vial and incubated in the dark at room temperature for 45 min. We previously established optimal amounts of the live/dead stain reagents to be 0.25 μl calcein AM, 1 μl ethidium homodimer (EthD-1), and 0.5 ml PBS. The assay works by differential staining of live and dead cells. Live cells are characterized by intercellular esterase activity which causes the cell permeant calcein to produce a bright green fluorescence. EthD-1 enters dead cells through damaged membranes, binds to nucleic acids, and produces an intense red flourescence. Stained sperm were viewed with a standard flourescein excitation optical filter at 20× or 40×, depending on the size of sperm. We examined 500 sperm from each individual of most

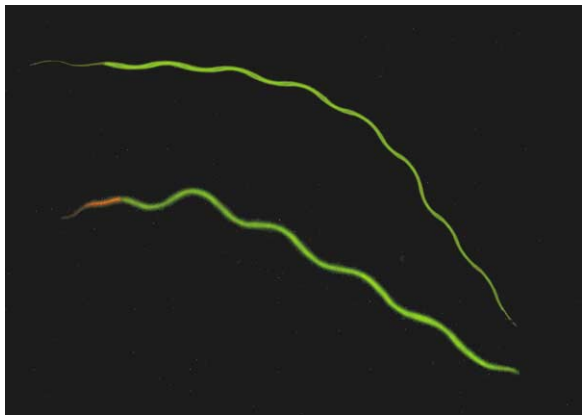

Figure 3. Photomicrograph of Two Stained Cockroach (*Nauphoeta cinerea*) Sperm with Their Heads Facing Left, Showing a Dead Sperm (Red Head) and above It a Live Sperm

species (fewer if numbers were limited) and recorded whether each sperm had a red head (dead) or was entirely green (live; Figure 3).

Statistical Analysis

Our analysis is based on data from seven pairs of species, each comprising 9 to 30 individuals: a total of 246 males and over 40,000 sperm. The data were analyzed using a logistic regression (generalized linear model with binomial errors and logit link) in which the effect of breeding system (monandrous or polyandrous) was determined only after accounting for differences between species pairs. Due to severe overdispersion, an empirical scale parameter was used, and F values were calculated rather than χ^2 values [20]

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