

The influence of male ejaculate quantity on female fitness: a meta-analysis

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ABSTRACT

Although the primary function of mating is gamete transfer, male ejaculates contain numerous other substances that are produced by accessory glands and transferred to females during mating. Studies with several model organisms have shown that these substances can exert diverse behavioural and physiological effects on females, including altered longevity and reproductive output, yet a comprehensive synthesis across taxa is lacking. Here we use a meta-analytic approach to synthesize quantitatively extensive experimental work examining how male ejaculate quantity affects different components of female fitness. We summarize effect sizes for female fecundity (partial and lifetime) and longevity from 84 studies conducted on 70 arthropod species that yielded a total of 130 comparisons of female fecundity and 61 comparisons of female longevity. In response to greater amounts of ejaculate, arthropod females demonstrate enhanced fecundity (both partial and lifetime) but reduced longevity, particularly for Diptera and Lepidoptera. Across taxa, multiply mated females show particularly large fecundity increases compared to singly mated females, indicating that single matings do not maximize female fitness. This fecundity increase is balanced by a slight negative effect on lifespan, with females that received more ejaculate through polyandrous matings showing greater reductions in lifespan compared with females that have mated repeatedly with the same male. We found no significant effect size differences for either female fecundity or longevity between taxa that transfer sperm packaged into spermatophores compared to taxa that transfer ejaculates containing free sperm. Furthermore, females that received relatively larger or more spermatophores demonstrated greater lifetime fecundity, indicating that these seminal nuptial gifts provide females with a net fitness benefit. These results contribute to our understanding of the evolutionary origin and maintenance of non-sperm ejaculate components, and provide insight into female mate choice and optimal mating patterns.

Key words: seminal fluid proteins, nuptial gifts, spermatophores, female fecundity, polyandry.

CONTENTS

I. Introduction	300
II. Meta-analysis methodology	301
(1) Selection criteria	301
(2) Methods	301
III. Results	302
(1) Overall effects on longevity and fecundity	302
(2) Differences between spermatophores and free ejaculates	303
(3) Differences due to experimental design	303
(4) Multiple matings: polyandrous <i>versus</i> repeated matings	303
IV. Discussion	304
(1) Overall effects on longevity and fecundity	304
(2) Differences between spermatophores and free ejaculates	304
(3) Differences due to experimental design	305
(4) Multiple matings: polyandrous <i>versus</i> repeated matings	305
(5) The impact of seminal nuptial gifts on female fitness	305

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V. Conclusions	305
VI. Acknowledgements	306
VII. References	306
VIII. Supporting Information	308

I. INTRODUCTION

Although the primary function of mating is gamete transfer, male ejaculates also contain numerous other substances that are produced by reproductive accessory glands (Leopold, 1976; Chen, 1984; Gillott, 1996, 2003; Simmons, 2001; Poiani, 2006). Recent work has identified many of the specific non-sperm components of ejaculates, which include numerous seminal fluid proteins, compounds with immunostimulant and antibiotic properties, as well as antipredator chemical defences (see Gillott, 2003; Poiani, 2006; Wolfner, 2007, for reviews). When transferred to the female, this complex cocktail of non-sperm materials is likely to have profound fitness implications for both sexes.

In arthropods, these substances not only assist in delivering and provisioning sperm, but also have diverse physiological and behavioural effects on females: reducing the likelihood of re-mating, stimulating egg production and oviposition, initiating sperm storage and/or release, and shortening lifespan (Chapman, 2001; Chapman & Davies, 2004; Wolfner, 2007; Chapman, 2008). Such effects have been most extensively studied in *Drosophila melanogaster* (reviewed in Wolfner, 2002, 2007, 2009) because of the powerful genetic and molecular techniques available for this model organism. Documented effects of *Drosophila* seminal fluid proteins (SFPs) on females include increased egg production (Soller, Bownes & Kubli, 1999), stimulation of ovulation and oviposition (Heifetz *et al.*, 2000), decreased receptivity to re-mating (Manning, 1962; Chen *et al.*, 1988; Chapman *et al.*, 2003), elevated sperm storage (Tram & Wolfner, 1999) and sperm utilization (Tram & Wolfner, 1999), altered feeding behavior (Carvalho *et al.*, 2006), and decreased lifespan (Fowler & Partridge, 1989; Chapman *et al.*, 1995). SFPs mediate a characteristic short-term response to mating shown by *D. melanogaster* females (Kalb, DiBenedetto & Wolfner, 1993), and some are also essential for a more persistent long-term response (Liu & Kubli, 2003; Peng *et al.*, 2005; Ravi Ram & Wolfner, 2007). SFPs have also been implicated as being crucial mediators of *Drosophila melanogaster* sperm competition (Harshman & Prout, 1994; Prout & Clark, 2000; Wigby *et al.*, 2009).

Although numerous studies have been conducted in arthropods to elucidate fitness costs and benefits, considerable debate persists about how male ejaculates affect female fitness. Some authors have argued that male ejaculates enhance female fitness by providing nutrients to females or offspring (Boggs, 1990; Vahed, 1998; Gwynne, 2008), while others have proposed that male ejaculates produce a net decrease in female fitness by manipulating female reproduction (Arnqvist & Nilsson, 2000; Gillott, 2003; Vahed, 2007; Wolfner, 2007). Previous attempts to synthesize extensive empirical results include narrative reviews and

meta-analyses that have focused primarily on the fitness effects of polyandry (Ridley, 1988; Simmons, 2005; Arnqvist & Nilsson, 2000). However, these syntheses have largely ignored other factors that also affect ejaculate quantity; such factors include male mating status (virgin or previously mated) and repeated matings by a single male. Other reviews have focused solely on the fitness effects of male nuptial gifts (Boggs, 1990, 1995; Vahed, 1998, 2007; Gwynne, 2008). The term “nuptial gift” encompasses a wide range of structures that are transferred to females during mating, and which may have either positive or detrimental effects on female fitness. Nuptial gifts can be divided into oral gifts, which are absorbed through the digestive tract (e.g. captured prey, secretions of male salivary and other glands), and seminal gifts (i.e. spermatophores) that are absorbed through the reproductive tract. As pointed out by Gwynne (2008), these two categories of nuptial gift may differ in how they affect female fitness. Because seminal gifts provide males with direct access to the female reproductive tract (Sakaluk, Avery & Weddle, 2006), they may be more likely to include manipulative substances that provide a benefit to males at a cost to female fitness (called “Medea gifts” by Arnqvist & Nilsson, 2000). A meta-analysis by Arnqvist & Nilsson (2000) included a comparison of how polyandry affects female fitness between species with and without nuptial “feeding”. Importantly, this study pooled species with oral gifts and seminal gifts together and thus could not test directly the Medea hypothesis prediction that male seminal gifts should reduce female fitness. In addition, because this previous meta-analysis was focused on polyandry several other important factors were not assessed, including distinguishing between polyandrous matings and repeated matings with the same male.

In the present study we conducted a comprehensive meta-analysis to quantify how male ejaculate quantity affects female fitness, which has not previously been done. We focused specifically on male ejaculated substances, defined here as any male-derived material transferred through genital contact to the female (oral gifts were excluded). Male ejaculates include both free ejaculates and spermatophores, which consist of sperm packaged within a structure manufactured by male reproductive glands (Mann 1984). We restricted this meta-analysis to arthropods based on the extensive empirical literature from this group. Meta-analysis provides a powerful tool that can objectively synthesize previous research results, allowing treatment effects to be quantified and effect sizes compared across multiple studies (Hedges & Olkin, 1985; Gurevitch & Hedges, 1993; Nakagawa & Cuthill, 2007). Previous meta-analyses have provided insight into several topics in behavioural ecology and evolution (Arnqvist *et al.*, 1996; Gontard-Danek & Moller, 1999; Vollestad, Hindar & Moller, 1999; Arnqvist & Nilsson, 2000; Torres-Vila &

Jennions, 2005). Using this meta-analytic approach, our goal was increased understanding of selective forces, such as sexual conflict, driving the evolution of reproductive traits and behaviors, including male spermatophores, female polyandry, and mate choice.

In this meta-analysis we evaluated several factors that could potentially influence the magnitude or direction of male ejaculate effects on female fitness. These factors include: (1) whether males transfer spermatophores or free ejaculates - if spermatophores represent delivery vehicles for manipulative compounds, we predict that receiving more ejaculate should reduce female fitness to a greater extent in spermatophore-producing species compared to those with free ejaculates. (2) Different experimental designs commonly used to evaluate effects, to help identify particular design artifacts and weaknesses. (3) Taxonomic affiliation (insect orders), which allowed us to examine whether different taxa show similar patterns in how females respond to ejaculate quantity. (4) Effects of polyandry *versus* repeated matings with the same male - this comparison yielded insight into the importance of direct *versus* indirect benefits, as only the former would provide indirect fitness benefits to females.

II. META-ANALYSIS METHODOLOGY

(1) Selection criteria

We conducted a literature search for relevant studies by querying *Scopus*, *ISI Web of Science*, and *Google Scholar* using keyword combinations that included polyandry, mating rate, spermatophore, nuptial gift and ejaculate size paired with female fitness or fecundity. We also included studies that were referenced in previous narrative reviews (Boggs, 1995; Ridley, 1988; Vahed, 1998) and meta-analyses on related topics (Arnqvist & Nilsson, 2000).

To be included, studies needed to meet several additional criteria: (1) The study included ≥ 2 treatments comparing females that received larger *versus* smaller quantities of ejaculated substances based on differences in known or inferred number of matings, or based on matings conducted with males that differed in their mating history. (2) The study reported at least one measure of female fitness based on fecundity (egg or offspring production); longevity was sometimes also reported. (3) The study reported means, some measure of variability and sample sizes for each group somewhere in the text, tables, or figures. We found 84 studies published from 1962 to 2008 that met all of these criteria; 80 of these studies were conducted on insects, while four were conducted on other arthropods (details of these studies are included in an online Appendix S1, see Section VIII).

(2) Methods

We classified each study according to several factors that could potentially influence how male ejaculate size affects female fitness, including whether males transfer sperm in

spermatophore packages or in free ejaculates, and taxonomic affiliation (Order). In addition, we also categorized studies based on five experimental designs commonly used to investigate effects of mating on female fitness. We classified these designs into comparisons of either: (1) Nonvirgin males *versus* virgin males: females received single ejaculates from either previously-mated or virgin males. (2) Small *versus* large spermatophore: females received single spermatophores from either virgin males or previously-mated males. (3) Single mating *versus* multiple matings: females received ejaculate from either a single mating or from multiple (≥ 3) matings. (4) Single mating *versus* double matings: females received male ejaculate from either a single mating or from two matings. (5) Less *versus* more ejaculate: treatments differed in how much exposure females had to males, but the mating rate and number of matings was unknown (e.g. females that were exposed to a single male for 24 h likely achieved fewer matings compared with females exposed to a single male for 48 h. For treatments in which females mated more than once, we further distinguished between studies in which females were assigned polyandrous matings (≥ 2 matings with different males) *versus* repeated matings (≥ 2 matings with the same male). Finally, we also distinguished between studies that measured fecundity measured over a female's entire lifetime or only part thereof. Whenever it was reported we used female lifetime fecundity, clearly the most comprehensive fitness measure, but many studies reported only partial fecundity. Although reproductive timing may also constitute an important aspect of female fitness (Brommer, Merila & Kokko, 2002; Reinhardt, Naylor & Siva-Jothy, 2009), most published studies did not include such data.

Based on the included studies, we obtained data for 130 comparisons of female fecundity and 61 comparisons of female longevity (some studies yielded more than one comparison). For each comparison we calculated Hedges' *d*, an unbiased weighted estimate of effect size that is typically used with continuous response variables and categorical predictors (Nakagawa & Cuthill, 2007). Hedges' *d* is calculated as the difference between a control and experimental group measured in standard deviation units (Gurevitch *et al.*, 1992). In this meta-analysis, the control was always defined as the group in which females received less male ejaculate (either through fewer matings or receiving a smaller spermatophore or ejaculate), while the experimental group was defined as the group in which females received more male ejaculate. Hedges' *d* was separately calculated for female fecundity (based on either egg or offspring production) and female longevity.

We used MetaWin 2.1 (Rosenberg, Adams & Gurevitch 2000) to calculate mean effect sizes weighted by sample size, and ran an initial analysis combining all studies to determine whether the overall effect sizes for female fecundity and longevity differed significantly from zero. We then examined differences in mean effect size for each factor described above using categorical random effects models, which incorporate both sampling error and a random component as contributors to effect size variation (Hedges & Olkin, 1985; Rosenberg *et al.*, 2000). In addition to mean effect

sizes, we also report 95% confidence intervals calculated using a bias-corrected bootstrapping approach (with 1,000 replicates used for re-sampling), along with the appropriate test statistics Q_t (total heterogeneity) and Q_b (between-group heterogeneity). If a significant overall difference was detected between groups, then we examined differences in mean effect sizes between specific groups using randomization tests based on 1,000 replicates. We consider Hedges' $d \sim 0.2$ to represent a small treatment effect, $d \sim 0.5$ to be a moderate effect, and $d \sim 0.8$ to be a large effect (Cohen, 1988; Moller & Jennions, 2002; Hagen, Connelly & Schroeder, 2007; Morris *et al.*, 2007; Nakagawa & Cuthill, 2007; Sara 2007).

We used several methods to check for publication bias, which arises when published studies are biased towards reporting significant differences. For each analysis, we calculated Rosenthal's fail-safe number and generated funnel plots using MetaWin (Rosenberg *et al.* 2000). Rosenthal's fail-safe number represents how many unpublished studies with effect sizes equal to zero would be required to negate an effect size significantly different from zero at the $\alpha = 0.05$ level of significance. Funnel plots provide a graphical check for publication bias (Wang & Bushman, 1998; Gurevitch & Hedges, 1999), and have been used widely as a method for ascertaining bias in meta-analyses (Gurevitch *et al.*, 1992; Arnqvist *et al.*, 1996; Gontard-Danek & Moller, 1999; Vollestad *et al.*, 1999). Both approaches indicated that no systematic sampling bias was evident for data on female fecundity. However, for female longevity these two methods for detecting bias differed: Rosenthal's fail-safe number was not robust, although the funnel plot indicated a lack of bias for these data.

III. RESULTS

(1) Overall effects on longevity and fecundity

When data were combined across all arthropod studies, treatments in which females received relatively more male ejaculate showed effect sizes that were significantly different from zero (*i.e.* the 95% confidence intervals exclude 0) for both female longevity and fecundity (Fig. 1). Across all studies, fecundity showed a significant increase when

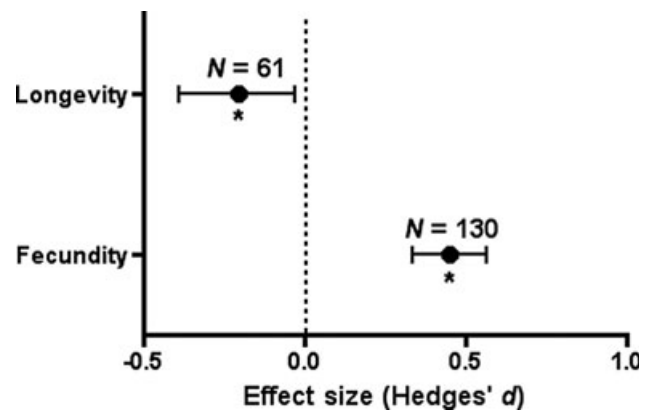


Fig. 1. Mean effect size (Hedges' d with 95% CI) for arthropod studies that examined the effects of receiving relatively more male ejaculate on female fecundity and longevity. Asterisks indicate effect sizes significantly different ($P < 0.05$) from zero (dotted line).

females received more male ejaculate (Fig. 1; Hedges' $d = 0.4542$, 95% CI: 0.3373 to 0.5658, d.f. = 129, $Q_t = 181.3$, $P = 0.002$). This positive effect of male ejaculate quantity on female fecundity was remarkably consistent across insect orders; all five orders showed mean effect sizes for fecundity that were significantly greater than zero (Table 1). Also, when we compared studies in which female fecundity was measured over only part of each female's adult lifetime with measurements over her entire lifetime, significantly positive effect sizes were seen for both fecundity measures (Fig. 2), with no significant difference between them ($Q_b = 0.904$, d.f. = 1, $P = 0.341$).

However, male ejaculate showed opposite effects on female longevity; when data were combined across all studies, longevity decreased significantly when females received more male ejaculate (Fig. 1; Hedges' $d = -0.2034$, 95% CI: -0.3770 to -0.0200 , d.f. = 60, $Q_t = 82.5$, $P = 0.029$). This decrease in female lifespan was most pronounced in Diptera and Lepidoptera, as mean effect sizes for longevity were significantly less than zero in these two insect orders (Table 1).

Table 1. Mean effect sizes (Hedges' d with 95% CI) and number of comparisons (N) classified by insect order that have examined the effects of receiving relatively more male ejaculate on female longevity and fecundity (effect sizes only reported for groups with ≥ 5 comparisons).

Order	Fecundity Effect size (95% CI)	Longevity Effect size (95% CI)
Orthoptera	0.5816 (0.2899 to 0.8960) $N = 12$	0.3612 (-0.1364 to 0.7542) $N = 7$
Lepidoptera	0.2646 (0.1073 to 0.4752) $N = 38$	-0.2560 (-0.5812 to -0.0088) $N = 22$
Heteroptera	0.3603 (0.0556 to 0.8131) $N = 8$	Insufficient data
Diptera	0.6605 (0.3702 to 1.0158) $N = 23$	-0.4501 (-0.6910 to -0.2330) $N = 6$
Coleoptera	0.4847 (0.2273 to 0.7168) $N = 31$	-0.3914 (-0.8241 to 0.0416) $N = 16$

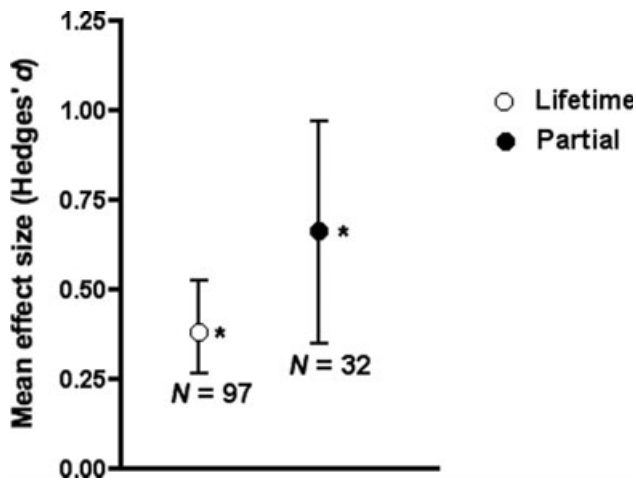


Fig. 2. Mean effect sizes (Hedges' d with 95% CI) of arthropod studies that examined the effects of receiving relatively more ejaculate on female fecundity categorized according to whether female fecundity was measured over female's entire lifespan or only a portion of it. Asterisks indicate effect sizes significantly different ($p < 0.05$) from zero.

(2) Differences between spermatophores and free ejaculates

When we compared taxa in which male ejaculate is packaged into spermatophores *versus* taxa where males transfer ejaculates containing free sperm, we found no significant difference in average effect size between these groups when females received more ejaculate (Fig. 3); this was true for both female longevity ($Q_b = 1.3$, d.f. = 1, $P = 0.262$) and fecundity ($Q_b = 0.4$, d.f. = 1, $P = 0.533$). When we compared lifetime fecundity for females that received relatively larger or more spermatophores across 30 species (Fig. 3), we found an overall positive effect size (Hedges' $d = 0.3320$, 95% CI: 0.1987 – 0.4807, $N = 55$ comparisons). This positive effect of larger/more spermatophores on lifetime fecundity was found for Coleoptera, Lepidoptera and Orthoptera (remaining orders had <5 comparisons).

(3) Differences due to experimental design

Many different experimental designs have been used to assess how relatively more male ejaculate influences female fitness, so we compared average effect sizes among these common study designs (Fig. 4). For female longevity, effect sizes did not differ significantly among study designs ($Q_b = 3.4$, d.f. = 4, $P = 0.488$). However, significantly negative effect sizes were seen in only two study designs (Fig. 4A): (1) virgin males *versus* non-virgin males, and (2) a single mating *versus* multiple (≥ 3) matings. For female fecundity, three study designs showed significantly positive effect sizes (Fig. 4B): (1) virgin males *versus* non-virgin males, (2) single *versus* multiple matings, and (3) single *versus* double matings. In addition, studies comparing single *versus* multiple matings had fecundity effect sizes that differed significantly from studies in which females were singly-mated to males

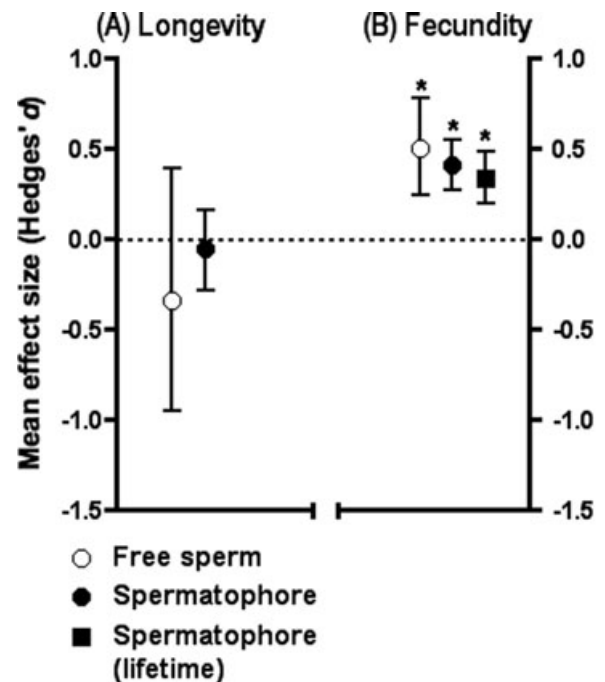


Fig. 3. Mean effect sizes (Hedges' d with 95% CI) for arthropod studies that examined the effects of receiving relatively more male ejaculate on: (A) female longevity (species in which the ejaculate is packaged into a spermatophore $N = 39$, species with free sperm $N = 10$), (B) female fecundity (species in which the ejaculate is packaged into a spermatophore $N = 67$, species with free sperm $N = 36$). Also shown is the effect size for lifetime fecundity for species transferring spermatophores ($N = 55$). Asterisks indicate effect sizes significantly different ($P < 0.05$) from zero (dotted line).

producing small *versus* large spermatophores ($Q_b = 7.4$, d.f. = 1, $P = 0.006$). When studies compared experimental treatments that differed in how much exposure females had to males but failed to control mating rates (less *versus* more in Fig. 4B), measured effect sizes for female fecundity varied widely.

(4) Multiple matings: polyandrous *versus* repeated matings

We further examined two variations on study designs in which singly-mated females were compared to multiply-mated females. When we compared studies in which females mated multiple times to different males (polyandrous matings) *versus* multiple times to the same male (repeated matings), we found significantly positive effect sizes for female fecundity using both designs (Fig. 5B), with no significant difference between them ($Q_b = 0.9$, d.f. = 1, $P = 0.34$). For longevity (Fig. 5A), however, these two multiple mating designs yielded significantly different effect sizes ($Q_b = 6.5$, d.f. = 1, $P = 0.011$), and only polyandrous matings showed a mean effect size that was significantly less than zero.

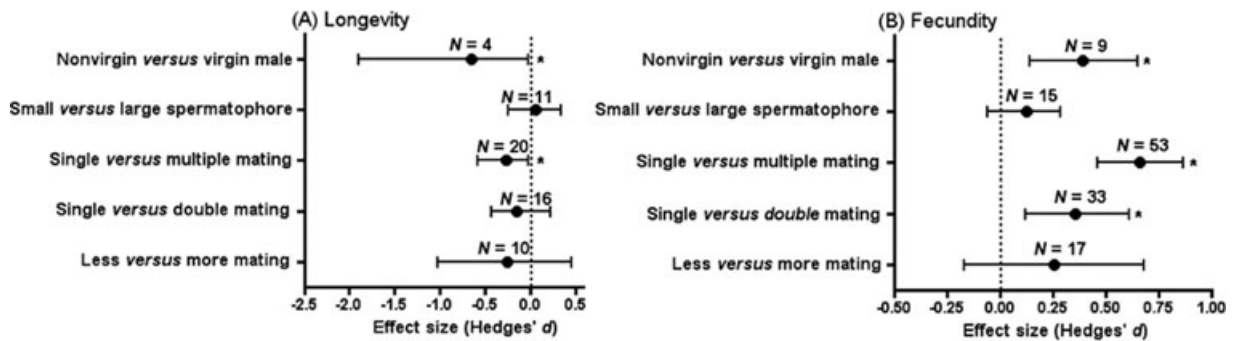


Fig. 4. Mean effect sizes (Hedges' d with 95% CI) classified by experimental design for arthropod studies that have examined the effects of receiving relatively more male ejaculate on: (A) female longevity, (B) female fecundity. Asterisks indicate effect sizes significantly different ($p < 0.05$) from zero (dotted line). For details of experimental categories see Methods (Section II.2).

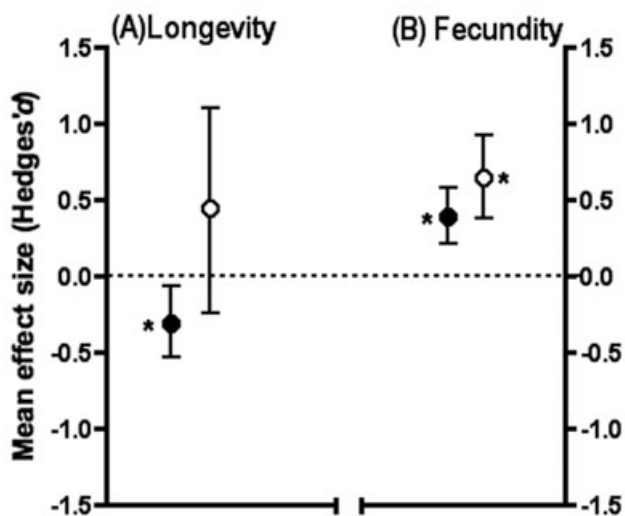


Fig. 5. Mean effect sizes (Hedges' d with 95% CI) for arthropod studies that have examined the effects of receiving relatively more ejaculate on: (A) female longevity (polyandrous matings $N = 34$, repeated matings with the same male $N = 7$), (B) female fecundity (polyandrous matings $N = 66$, repeated matings with the same male $N = 7$). Asterisks indicate effect sizes significantly different ($P < 0.05$) from zero (dotted line). Open circles in the figure represent repeated matings, closed circles in the figure represent polyandrous matings.

IV. DISCUSSION

(1) Overall effects on longevity and fecundity

These results reveal a general pattern that male ejaculates produce opposite effects on different female fitness components. Within several insect orders, positive effect sizes indicate that fecundity of arthropod females is significantly increased when they receive relatively more ejaculate. However, receiving more ejaculated substances often decreased female lifespan, as shown by significantly negative effect sizes. For the subset of studies that measured lifetime fecundity (59 species), significantly positive effect sizes demonstrate that females gain a net fitness benefit from receiving more

ejaculate. It would be worthwhile for future studies to focus on measuring lifetime fecundity, and also to report data concerning the timing of reproductive events (Brommer *et al.*, 2002; Reinhardt *et al.*, 2009).

These general results are consistent with work in *D. melanogaster* that has detailed how specific male seminal fluid proteins affect female behaviour and reproduction. Females that receive male ejaculate lacking Acp70A (a 36-amino-acid peptide also known as sex peptide) have greater longevity (Wigby & Chapman, 2005), are more willing to remate, and produce fewer eggs (Chen *et al.*, 1988 Soller *et al.*, 1999; Chapman *et al.*, 2003; Liu & Kubli, 2003). Another SFP, ovulin, induces ovulation in the first 24 h following copulation (Herndon & Wolfner, 1995; Heifetz *et al.*, 2000), while Acp62F has been linked to shorter female life span (Lung *et al.*, 2002; Mueller, Page & Wolfner, 2007). We still have much to learn about the functional role played by specific components within male ejaculates in most species, although recent work has begun to characterize seminal fluid proteins in other taxa (yellow fever mosquito, *Aedes aegypti*, Sirot *et al.*, 2008; *D. mojavensis*, Kelleher *et al.*, 2009).

(2) Differences between spermatophores and free ejaculates

We distinguished between species in which males transfer free ejaculates *versus* spermatophores, which are categorized as seminal nuptial gifts (Gwynne, 2008). For both categories, our meta-analysis revealed that more male ejaculate significantly increased female fecundity, and that effect sizes did not differ between these groups. This result differs from a previous meta-analysis that focused on polyandry: Arnqvist & Nilsson (2000) found that insects with nuptial feeding (this included both oral and ejaculated nuptial gifts) showed more pronounced positive effects of polyandry on both egg production and longevity compared to groups lacking nuptial feeding. One explanation for these differing results may be that orally ingested nuptial gifts are more likely to be nutritive in function, whereas seminal nuptial gifts might be more likely to contain specialized compounds targeted to receptors within the female reproductive tract (Poiani, 2006; Gwynne

2008). However, our meta-analysis results demonstrate that rather than reducing female fitness, seminal nuptial gifts instead have a significantly positive effect on female lifetime fecundity. This result thus argues against spermatophores arising through selection for more elaborate male ejaculates that enhance the delivery of manipulative compounds to females.

(3) Differences due to experimental design

Our results also show that different study designs can dramatically alter measured effect sizes. Only two study designs showed both significant positive effects on female fecundity coupled with significant negative effects on female longevity: virgin males *versus* non-virgin males, and a single mating *versus* multiple matings. Also, the wide discrepancy in effect sizes from studies in which the mating rate was not controlled (Fig. 4B) suggests that this is not an effective design for measuring how male ejaculates affect female fitness.

(4) Multiple matings: polyandrous *versus* repeated matings

Our results indicate that polyandrous females across many arthropod taxa show increased fecundity as compared to females with only a single mating. This overall fitness benefit of polyandry is consistent with Arnqvist & Nilsson's (2000) result showing higher offspring production for multiply mating females. Possible advantages of polyandry for females have been discussed extensively (e.g. Thornhill & Alcock, 1983; Choe & Crespi, 1997; Yasui, 1998; Hasson & Stone, 2009), and include both direct benefits (replenished sperm supply, ejaculate nutrients, enhanced paternal care, *etc.*) and indirect benefits (increased offspring fitness, avoidance of genetic incompatibility, increased genetic diversity) to females. While indirect benefits have attracted much attention (Yasui, 1998), our results support Arnqvist & Nilsson's (2000) suggestion that the near-ubiquity of female polyandry might be explained solely on the basis of direct benefits.

When we considered polyandrous and repeated matings separately, we found that both categories of multiple mating resulted in increased female fecundity, but only polyandrous matings significantly decreased female longevity. This suggests that interactions among ejaculates from multiple males may have costs for females. It is also possible that repeated matings by a single male might deplete any longevity-reducing components of male ejaculates, such that females with polyandrous matings receive a higher dose of these ejaculate components.

(5) The impact of seminal nuptial gifts on female fitness

Spermatophores are seminal nuptial gifts produced by male accessory glands in many invertebrate taxa (Mann, 1984). Some debate has arisen over how these seminal products affect female fitness (Vahed, 2007; Gwynne, 2008). Several

reviews have highlighted the negative impact on females of nuptial gifts, because they can reduce female mating rates below optimum levels (Arnqvist & Nilsson, 2000; Arnqvist & Rowe, 2005; Vahed, 2007). However, other reviews have suggested that nuptial gifts generally increase female fitness by providing direct benefits (Gwynne, 2008).

Our meta-analysis results demonstrate a net fitness benefit to females from seminal nuptial gifts, as across 30 species, females receiving larger/more seminal nuptial gifts showed positive effect sizes for lifetime fecundity. These results are consistent with several other lines of evidence suggesting that seminal nuptial gifts provide females with a net fitness benefit. In many species, seminal nuptial gift components are incorporated into female oocytes or utilized for defensive purposes, resulting in enhanced fecundity and increased lifespan (e.g. Boggs 1990, 1995; Brown, 1997; Vahed, 1998; Rooney & Lewis, 1999, 2002). Therefore, our meta-analysis results indicate that seminal nuptial gifts provide females in many taxa with a lifetime fecundity benefit in spite of associated longevity costs.

Although seminal nuptial gifts currently appear to provide a net fitness benefit to females in many arthropod species, they may have originated *via* sexual conflict to benefit males at the expense of female fitness (see Arnqvist & Rowe, 2005; Gwynne 2008 for reviews). Male accessory glands may have originated to produce compounds that exploit female sensory pathways and limit female re-mating rates (Arnqvist & Nilsson, 2000; Sakaluk, 2000; Arnqvist & Rowe, 2005; Fedorka & Mousseau, 2002, Enqvist 2007). Females may have then responded by evolving ways to cope with these compounds and derive a direct benefit by using them for somatic maintenance or reproduction (Arnqvist & Nilsson, 2000; Fedorka & Mousseau, 2002; Arnqvist & Rowe, 2005). Thus, the evolutionary origins of seminal gifts remain unclear, although in general they currently function to increase female fitness.

V. CONCLUSIONS

- (1) A comprehensive meta-analysis was conducted for studies on arthropods that have examined how male ejaculate quantity affects female fitness; 84 studies of 70 species yielded a total of 130 comparisons of female fecundity and 61 comparisons of female longevity. Across most arthropod taxa, females show significantly higher fecundity (both partial and lifetime) after receiving more male ejaculate. However, greater ejaculate quantity had the opposite effect on female lifespan, particularly for Diptera and Lepidoptera.
- (2) The overall effect size for the subset of 59 species that measured lifetime fecundity indicated that females gained a net fitness benefit from receiving more ejaculate, as their lifetime fecundity increased. Studies comparing multiply-mated to singly-mated females showed particularly large fecundity increases, indicating that single matings do not maximize female fitness.

Therefore, these results support the hypothesis that non-sperm components of male ejaculates provide a direct benefit to females.

- (3) The positive effect of multiple matings was seen whether females mated polyandrously (with different males) or repeatedly (with the same male). However, polyandrous females showed reduced longevity compared to females that had repeated matings, suggesting that interactions among multiple males ejaculates might have costs for females.
- (4) Our results also have implications for the evolution of seminal nuptial gifts. Our meta-analysis results showed greater lifetime fecundity for females that received relatively larger or more spermatophores, demonstrating that seminal nuptial gifts provide a net fitness benefit that is likely to offset any potential longevity costs. Additionally, we believe that seminal and oral nuptial gifts should be considered separately when evaluating their effects on female fitness.
- (5) We suggest that future empirical studies on this topic should control for past mating history of both sexes, record the exact number of copulations, and monitor lifetime egg and offspring production as well as female longevity. We also advocate more focused efforts to identify and characterize the functional role of particular non-sperm components within male ejaculates in species beyond *Drosophila*, as this will provide valuable insights into the evolutionary dynamics of sexual selection.

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VII. REFERENCES

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VIII. SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article.

Appendix S1. Table containing data used in calculation of effect sizes. For mating treatment: 1 = virgin *versus* non-virgin male, 2 = small spermatophore *versus* large spermatophore, 3 = single *versus* multiple mating, 4 = single *versus* double mating, 5 = less *versus* more mating. For ejaculate: 1 = free sperm, 2 = spermatophore, 3 = unknown. For duration of

experiment: 1 = lifetime, 2 = partial, 3 = not clear. For repeated *versus* polyandrous: 0 = not clear or not applicable, 1 = polyandrous, 2 = repeated. The control group is defined as the group in which females received less male ejaculate; the experimental group is the group receiving more ejaculate.

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