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Sperm dynamics and cryptic male choice in tephritid flies

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Variance in female quality can result in males discriminating females either through precopulatory or via postcopulatory choice. Male cryptic choice can be exhibited through copulation duration and strategic ejaculation. Female quality can also affect sperm storage distribution. Here, we studied sperm allocation in three tephritid flies with contrasting life histories and multiple sperm storage organs, *Anastrepha ludens* (MX flies), *Anastrepha obliqua* (WI flies) and *Anastrepha spatulata* (AS flies). In addition, for MX flies, we assessed the effect of female potential fecundity, size and diet on sperm allocation and the effect of female age on sperm storage distribution. Sperm distribution during copulation differed between species, which may be explained by their oviposition strategies. MX males mating with more fecund and younger females had shorter copulation durations. Female quality also influenced sperm storage patterns, as well-fed females stored more sperm in long-term sperm storage organs such as the ventral receptacle compared to malnourished females. Detailed studies on how female quality affects sperm transfer and sperm storage asymmetry will further our understanding on cryptic male choice.

It is now widely acknowledged that females are not the only sex that is choosy. Male choice has now been demonstrated in a variety of animal species (Droney & Thaker, 2006; Engqvist & Suaer, 2001). Males can choose females during both pre- and postcopulatory processes (Edward & Chapman, 2011). In species where there is no paternal care, ejaculate costs in spermatogenesis (Dewsbury, 1982; Wedell, Gage, & Parker, 2002) and seminal fluid production (Hayward & Gillooly, 2011) are thought to favour 'cryptic male choice'. Thus, males are expected not only to discriminate among females as mates, but also to modulate investments in copula duration and ejaculates, depending on female quality (Bonduriansky, 2001; Engqvist & Sauer, 2003; Galvani & Johnstone, 1998; Janicke, Kesselring, & Schärer, 2012; Kelly & Jennions, 2011; Simmons, 2001).

Copula duration and sperm transfer can vary with male quality parameters such as size, diet, previous mating experience and age (e.g. Taylor & Yuval 1999; Teng & Zhang, 2009). Female traits such as nutrition, age, size, mating status and fecundity can also modulate copula duration and sperm transfer (Kelly & Jennions, 2011). Indeed, recent studies have highlighted the importance of female effects in understanding sperm storage patterns (Lüpold et al., 2013).

Resources allocated during copulation, such as sperm, are expected to vary if males can gauge female quality (Janicke et al., 2012; Reinhold, Kurtz, & Engqvist, 2002). For example, *Drosophila melanogaster* males deliver significantly more sperm to mated, large or young females than they do to virgins, small or old females (Lüpold, Manier, Ala-Honkola, Belote, & Pitnick, 2011). Other qualities that males could be assessing are female fecundity (e.g. Teng & Zhang, 2009). Male evaluation of these parameters could continue during copulation, influencing the duration of the copula and sperm allocation patterns.

Furthermore, for species with multiple and different types of sperm storage organs, there can also be plasticity in how sperm are distributed and stored within the female (Curril & LaMunyon, 2006; Nakahara & Tsubaki, 2007; Pitnick, Markow, & Spicer, 1999). Sperm allocation patterns could help females bias paternity and utilize sperm efficiently (Fedina & Lewis, 2004; Hellriegel & Bernasconi, 2000; Otronen, Reguera, & Ward, 1997), while in multiply mated females, sperm precedence may depend on the interaction between male and female genotype (reviewed in Pai & Bernasconi, 2008). However, an understanding of how female quality influences plasticity in copulation duration and sperm storage is still incipient and remains poorly understood.

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Here, we sought to elucidate the dynamics of sperm storage in tephritid flies by asking the following three questions. (1) What is the relationship between copulation duration and sperm storage in species with short or relatively long copula durations and contrasting life histories? (2) Can males adjust the amount of sperm transferred according to female potential fecundity? (3) How do female age, size and diet affect copulation duration and sperm storage? For the first question, we took advantage of a truly unique opportunity to compare sperm dynamics in three species with contrasting life histories and copulation durations, the Mexican fruit fly, *Anastrepha ludens* (hereafter MX flies), the West Indies fruit fly, *Anastrepha obliqua* (hereafter WI flies) and *Anastrepha spatulata* (hereafter AS flies) (Table 1). WI flies are egg limited, meaning females cannot produce enough eggs to exploit all available hosts (Díaz-Fleischer & Aluja, 2003). In addition, they can be characterized as having a short life span, a very concentrated reproductive window, high daily egg production (Liedo, Carey, Celedonio, & Guillen, 1992), host-dependent oocyte maturation (Aluja, Díaz-Fleischer, Papaj, Lagunes, & Sivinski, 2001), a numerous but short-lived native ancestral host and a relatively long female sexual refractory period (Aluja, Rull, Sivinski, Trujillo, & Pérez-Staples, 2009). Thus, as this species needs to oviposit quickly, we predicted that proportionally more sperm would be stored in short-term storage organs such as the ventral receptacle. MX flies in contrast, have a longer life span, a longer reproductive window and lower daily egg production (Liedo et al., 1992), and oocyte maturation does not depend on host presence (Aluja et al., 2001). Their native ancestral hosts are available for longer periods and the female sexual refractory period is shorter (Aluja, Rull, Sivinski, et al., 2009; Díaz-Fleischer & Aluja, 2003). Thus, we expected proportionally more sperm stored in long-term sperm storage organs such as the spermathecae. For AS flies, we expected similar patterns to WI flies, as their native hosts are only present for short periods. To address male adjustment in the ejaculate and copulation duration according to female fecundity, we contrasted a polyphagous species (MX flies) with a monophagous species (AS flies). We predicted that in polyphagous species with many hosts available, males would ejaculate more sperm compared to the monophagous species. In addition, in MX flies, we evaluated the effect of female quality parameters, such as age, size and diet on copula duration and sperm storage. We predicted that more sperm would be transferred to high-quality females. We expected malnourished females to have fewer sperm than well-fed females immediately after copulation as a result of cryptic male choice discriminating against malnourished females. Twelve hours after copulation ended, we expected differential sperm storage patterns between females to be more the result of female influences than of male influences. We also predicted well-fed females to store proportionally more sperm in the ventral receptacle (VR), where it would be readily available for oviposition, compared to malnourished females, which may not have energy reserves available to oviposit.

METHODS

Study Species

Copula duration varies widely among species of tephritid fruit flies (Aluja, Piñero, Jácome, Díaz-Fleischer, & Sivinski, 2000). We chose the WI, MX and AS fruit flies with a range of average copulation durations from 47 to 320 min and varying life history characteristics (Table 1). WI and AS flies are similar in their oviposition strategies compared to MX flies. Even though WI is currently a polyphagous species, its native and ancestral hosts are fruits of *Spondias* (Anacardiaceae trees) that mature quickly and synchronously (Díaz-Fleischer & Aluja, 2003). AS flies oviposit in 'guayabillo' *Schoepfia schreberi* that are available only for approximately 1 month during the year (López-Ortega et al., 2013). Both WI and AS flies need to oviposit quickly into their native host plants, because they are available only for a brief time (Aluja, Herrera, Lopez, & Sivinski, 2000; Díaz-Fleischer & Aluja, 2003; López-Ortega et al., 2013). In comparison, MX flies have more time to oviposit into their native hosts, yellow chapote, *Casimiroa greggii*, as the fruiting period is more prolonged (Thomas, 2012). MX females produce eggs continuously, dumping eggs if no hosts are available (Aluja, Birke, Guillén, Díaz-Fleischer, & Nestel, 2011).

Table 1
Life history characteristics of three tephritid flies*

Natural history	*Anastrepha ludens* (MX fly)	*Anastrepha obliqua* (WI fly)	*Anastrepha spatulata* (AS fly)
Female sexual maturation	Does not depend on host presence	Depends on host presence	?
Sexual maturation period (days)	10–15	7–13	±70
Mean (±SE) copulation duration	73.4±6.6	47.01±0.9	205±53†
Daily male mating frequency	1	3	1
% Females remating	20	20	? (probably monandrous)
Average sexual refractory period (days)	12	17	?
Clutch size (eggs)	1–40	1	1
Reproductive period	Long	Short	Short‡
Native host availability	Stable (2–3 months)	Highly ephemeral (2–3 weeks)	Ephemeral (1 month)‡
Host breadth	Polyphagous	Polyphagous	Monophagous
Life expectancy	51.7±2.2♀, 71.9±6.6♂	39.9±22.4♀, 38.5±21.7♂	?
Predicted sperm storage patterns	More in SP	More in VR	More in VR

?: data not available. SP: spermathecae; VR: ventral receptacle.
* After Aluja, Herrera, et al. (2000); Aluja, Piñero, et al. (2000); Aluja, Ordano, et al. (2009); Aluja, Rull, Sivinski, et al. (2009) and Liedo et al. (1992) unless otherwise noted.
† Present study.
‡ López-Ortega et al. (2013).

Sperm competition is limited in all three species. Both MX and WI females have long sexual refractory periods after mating during which females oviposit (Aluja, Ordano, et al., 2009; Table 1). For AS flies, remating is unknown. All three species are synspermatogenic (Boivin, Jacob, & Damiens, 2005), as males produce sperm throughout their lives. Females have three spermathecae (one doublet and a singlet) and a ventral receptacle (VR), which is the site for short-term sperm storage and is also the fertilization chamber (Fritz, 2004; Twig & Yuval, 2005). For MX flies, previous studies have shown that females prefer to mate with older, sexually experienced males (Pérez-Staples, Martínez-Hernández, & Aluja, 2010), while copulation duration varies with the degree of genetic relatedness (Aluja, Rull, Pérez-Staples, Díaz-Fleischer, & Sivinski, 2009). In a related tephritid, females have considerable control over copulation termination (Pérez-Staples, Weldon, Radhakrishnan, Prenter, & Taylor, 2010). Thus, we predicted that parameters of female quality could further influence copulation duration and sperm storage.

Experimental Procedures

Wild MX flies were collected from infested *Citrus auriantum* (cultivar Cucha) from Martinez de la Torre and Misantla in the State of Veracruz, Mexico. Wild WI flies were collected from *Spondias*