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Polyandry and Female Control: The Red Flour Beetle *Tribolium castaneum* as a Case Study

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ABSTRACT Females of many animal species are polyandrous, and there is evidence that they can control pre- and post-mating events. There has been a growing interest in consequences of polyandry for male and female reproductive success and offspring fitness, and its evolutionary significance. In several taxa, females exhibit mate choice both before and after mating and can influence the paternity of their offspring, enhancing offspring number and quality, but potentially countering male interests. Studying female mating biology and in particular post-copulatory female control mechanisms thus promises to yield insights into sexual selection and the potential of male–female coevolution. Here, we highlight the red flour beetle *Tribolium castaneum* (Herbst), a storage pest, as a model system to study polyandry, and review studies addressing the effects of polyandry on male sperm competitive ability and female control of post-mating events. These studies show that the outcome of sperm competition in the red flour beetle is influenced by both male and female traits. Furthermore, recent advances suggest that sexual conflict may have shaped reproductive traits in this species.

Sexual selection arises from interactions among conspecifics over reproduction: on one hand, intrasexual competition (usually among males for access to females or, after mating, their ova) and on the other hand, intersexual choice (usually with females being the choosy sex, Darwin, 1871). Sexual selection thus favors male traits that increase reproductive success in the context of male–male competition, and female traits that increase the ability to control mating and post-mating processes influencing the outcome of male–male competition and fertilization.

If females of a species mate with multiple males during one reproductive period, the fitness of males and females may be optimized by different values of traits expressed in this context, including reproductive physiology and behavior (Bateman, '48; Trivers, '72; Parker, '79; Rice and Holland, '97; Arnqvist and Rowe, 2005). Whereas males are selected to maximize their paternity (e.g., by preventing females from remating, Chapman et al., 2003), females may be selected to choose the best possible sire for their offspring (e.g., by remating with another male, Birkhead, 2000). Non-overlap of evolutionary optima for such traits (in the above example, for female remating) can thus result in sexual conflict. Such a conflict of interests between the sexes may translate into sexually antagonistic coevolution wherein individuals of each sex attempt to manipulate individuals of the other sex to maximize their own fitness interests, whereas simultaneously resisting manipulation from the opposite sex (Trivers, '72; Arnqvist and Rowe, 2005). Antagonistic co-evolution between the sexes is a significant force leading to divergence among populations and reproductive isolation (Rice, '96; Parker and Partridge, '98; Gavrilets et al., 2001; Hosken

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*Correspondence to: Aditi Pai, Department of Biology, 280 Science Building, Spelman College, 350 Spelman Lane, Atlanta, GA 30314. E-mail: apai@spelman.edu et al., 2001; Arnqvist and Rowe, 2002, 2005; Chapman et al., 2003).

Recently, the study of causes and consequences of female promiscuity (also called polyandry or multiple mating) has been gaining prominence in sexual selection research (Andersson, '94; Keller and Reeve, '95; Eberhard, '96; Jennions and Petrie, '97, 2000; Birkhead, 2000) as it promises to yield new insights into the evolution of reproductive traits (Eberhard, '96; Birkhead, 2000), male–female coevolution (Rice, '96; Holland and Rice, '99; Hosken et al., 2001), and speciation (Parker and Partridge, '98; Arnqvist et al., 2000; Gavrilets, 2000; Gavrilets and Waxman, 2002; Arnqvist and Rowe, 2005).

Here, we focus on post-mating processes, i.e. (i) competition among the sperm of different males for fertilization of a female's limited set of ova and (ii) cryptic female choice, i.e. the influence of female reproductive behavior, morphology or physiology on the outcome of sperm competition. Cryptic female choice may result in biased paternity in favor of males with certain traits (Eberhard, '96). Although sperm competition has been widely studied since first being formalized as a field of research in the 1970s (Parker, '70, '82; Blum and Blum, '79; Smith, '84; Birkhead and Møller, '98; Simmons, 2001), there has been only since the past decade an increasing interest in female control of post-copulatory processes (Knowlton and Greenwell, '84; Eberhard, '96), and in the interaction of male and female mechanisms (Wilson et al., '97; Clark and Begun, '98; Clark et al., '99). Post-copulatory mechanisms of female control have now been documented in a variety of taxa, ranging from vertebrates (Reyer et al., '99; Pizzari and Birkhead, 2000) to invertebrates (Eberhard, '97; Hellriegel and Bernasconi, '99). They include sperm extrusion or digestion (Eberhard, '85; Birkhead et al., '93; Haase and Baur, '95), and selective sperm storage (Siva-Jothy and Hooper, '95; Eberhard, '96; Hellriegel and Bernasconi, '99).

Insects in particular have been very useful as model systems for studying polyandry (Arnqvist and Nilsson, 2000), male determinants of sperm competition success (Birkhead and Møller, '98), female control (Eberhard, '94, '96, '97) and male–female interactions (Clark et al., '99). Here, we review studies with the red flour beetle *Tribolium castaneum* (Herbst) as model system, focus on the evidence for male–female interactions on post-mating events, address whether current findings support sexual antagonism in this system, and outline future directions.

MODEL SYSTEM

Tribolium castaneum (Coleoptera: Tenebrionidae) is a widely distributed storage pest in flour-mills, pantries, grain silos, etc. (Sokoloff, '74). Because of its short generation time and ease of culturing in the laboratory (Sokoloff, '74), it is a well-studied organism in developmental genetics (e.g., Brown et al., 2002) and in the context of pest control (e.g., Handler and Beeman, 2003).

Males as well as females mate with multiple partners (Sokoloff, '74; Lewis and Iannini, '95; Pai and Yan, 2003; Lewis, 2004) making it a good model system to study the evolution and consequences of multiple mating, sexual conflict over mating rates, and male and female traits influencing paternity under conditions of sperm competition. Males favor virgin and large females (Lewis and Iannini, '95; Arnaud and Haubruge, '99). Females too exhibit pre-copulatory mate choice for males based on a pheromone cue (Boake, '85; Lewis and Austad, '90), probably stemming from male-specific setiferous femoral glands (Bloch Qazi et al., '98b).

Sperm precedence is found to vary based on a wide range of factors both under male control (e.g., male copulatory courtship behavior: Edvardsson and Arnqvist, 2000), female control (e.g., female response to male pheromonal cues: Lewis and Austad, '94), as well as being affected by the interaction between male- and female-controlled processes (e.g., mating order: Wool and Bergerson, '79; Lewis and Austad, '90; Lewis and Jutkiewicz, '98; Bernasconi and Keller, 2001; Pai and Yan, 2002a; Nilsson et al., 2003; Lewis et al., 2005). This large variation in male siring success at sperm competition (sperm precedence: 0–100%) signals the importance of post-copulatory processes and female influence in this species. Indeed, probably the first study exploring variance components of sperm precedence, which could be attributed to differences among males and females, was conducted with *T. castaneum*, and likely played a key role for this field of research (Lewis and Austad, '90). Thus, overall this is a particularly useful species for studying questions relating to female control and promises to yield further insights into female influence as an evolutionary force.

PROPENSITY TO POLYANDRY AND INTENSITY OF SPERM COMPETITION

Female flour beetles may live on average up to 6 months (Sokoloff, '74) and their fertile period can exceed 5 months (Sokoloff, '74), during which females lay up to >300 eggs (Mean 128, SE 9.36) and readily remate (A. Pai, unpublished data). However, the propensity to remating and polyandry varies both within (Pai and Yan, 2003) as well as among populations of flour beetles (Nilsson et al., 2002, 2003; Attia and Tregenza, 2004; Pai et al., in press). For example in the c*SM* strain, females exhibit rapid remating and can copulate with up to 10 males within 1 hr (Pai and Yan, 2003), but in the *TIW*1 strain females are highly resistant to remating and do not remate in the 1 hr following initial copulation (Pai et al., in press; Table 1). Although polyandrous behavior creates a situation where sperm from different males co-occur in the female tract and compete for female ova (Parker, '84), variation in the degree of female multiple mating is likely to strongly influence the intensity of this sperm competition (Parker, '84; Simmons and Siva-Jothy, '98). In addition to the degree of polyandry itself (number of mating partners), the rate of remating which, influences inter-mating intervals, determines whether sperm from different males co-occur in the female tract, affects the age of the competing sperm, and thus impacts the outcome of sperm competition (Simmons and Siva-Jothy, '98). Among-strain variation in the propensity to polyandry and in average inter-mating intervals suggests that selection intensity for male and female adaptations for sperm competition and cryptic female choice are also likely to vary among beetle populations. Moreover, female flour beetles possess sperm storage organs (Bloch Qazi et al., '96; Lewis and Jutkiewicz, '98; Fedina and Lewis, 2004; Bernasconi et al., 2006), where the sperm of different males can be stored and used for fertilization for up to 3 months (Good, '33; Sinha, '53; Schlager, '60; Surtees, '61; Bloch Qazi et al., '96, '98a). Clearly, long-term sperm storage intensifies sperm competition.

Females apparently exert at least some control over mating rates, as is suggested by female ability to reject or accept a potential mate. This includes behaviors that suggest resistance to mating, such as rapidly moving away from a male that is attempting copulation, and backward movement to slip away from underneath a male attempting to mount (Pai and Yan, 2003). It is also likely that by moving rapidly and dislodging a mounted male (Bloch Qazi, 2003; Pai and Yan, 2003), female beetles are able to control length of the copula, which might be important in determining the outcome of sperm competition. Indeed, some studies found significant correlation between copula duration and paternity (e.g., Edvardsson and Arnqvist, 2000). On the other hand, females also appear to solicit matings by approaching males in other instances (Pai and Yan, 2003).

In addition, females prefer mating with, or display more biased paternity in favor of certain males based on male cues that females can perceive before or during copulation. One such cue for female pre-copulatory mate choice is the pheromone 2–4 dimethyl decanal (Boake, '85, '86; Obeng-Ofori and Coaker, '90; Lewis and Austad, '94). This compound is an aggregation pheromone but also serves as a sexual attractant (Obeng-Ofori and Coaker, '90). Although females prefer certain males based on this pheromonal cue, the fitness consequences of this choice for female and offspring fitness remain unknown (Boake, '85, '86). Another cue for female choice is male size, whereby larger males were significantly more likely to obtain fertilizations in one study (Lewis and Austad, '90), but not in two other studies

TABLE 1. Variation in female propensity to mating with multiple males (polyandry) in Tribolium castaneum *laboratory (cSM, TIW1, NDG11,PRUZ) and field (ADM, PIERCE) strains, assessed as the number of mating partners in 1 hr observation period*

Strain	Average number of mating partners	Range	Reference
c*SM* (day 1)	3.8	2–7	1
c*SM* (day 2)	6.0	2–10	1
*NDG*11	4.1	1–12	2
*TIW*1	1.0	1–1	2
PRUZ	3.5	1–8	2
ADM	2.0	1–4	3
PIERCE	2.5	1–5	3

Females were placed with a male at the start of the observation period in a mating arena. Once the pair had finished copulating, the first male was replaced with a new one. This process was repeated for the 1 hr period. Note that c*SM* females were non-virgin and observed on two different days. Females of the other strains were virgins at the start of the experimental period.
1 = Pai and Yan (2003); 2 = Pai et al. (in press); 3 = A. Pai, unpublished.

(Edvardsson and Arnqvist, 2000; Bernasconi and Keller, 2001). This suggests that male body size might be another cue for female mate choice, at least in some populations. Finally, females may show bias against infected males as revealed by lower sperm precedence of males infected with the rat tapeworm parasite (Yan and Stevens, '95).

FITNESS CONSEQUENCES OF POLYANDRY

Female mating with multiple males, mate choice and influence over paternity shares clearly result in a conflict of interests between the sexes over the outcome of sperm competition. Whereas male interests lie in maximizing the share of paternity, female interests lie in choosing the best possible sire for their offspring (Birkhead, 2000), or in remating to increase genetic diversity among their offspring (Bernasconi et al., 2003), or to avoid fertilizations by related males, incompatible males, or males carrying selfish genetic elements (Atlan et al., 2004), so as to increase offspring number and fitness (Bernasconi et al., 2004).

Polyandry may benefit females either directly, by providing additional sperm or nutrients in the ejaculate, and by eliciting higher offspring production (Arnqvist and Nilsson, 2000), or indirectly, by enhancing the fitness of their offspring via paternal genes in the form of better offspring survival, attractiveness, higher genetic diversity, etc. (Jennions and Petrie, 2000; Bernasconi and Keller, 2001). There is evidence that female flour beetles derive both direct and indirect benefits of polyandry (Lewis and Austad, '90; Nilsson et al., 2002; Pai and Yan, 2002b, 2003; Pai et al., 2005). Indirect, or genetic, benefits of female multiple mating include higher reproductive success of male offspring (Bernasconi and Keller, 2001; Pai and Yan, 2002b) and higher egg-to-adult survival of eggs produced by offspring from promiscuous mothers (Pai and Yan, 2002b). The mechanisms of this fitness increase are yet unclear. However, it is clear that different laboratory populations exhibit variability also in the costs and benefits associated with polyandry (Pai et al., in press). Thus, there is significant intraspecific variation in how female mating behavior affects offspring fitness.

Polyandry clearly impacts male fitness also. The reproductive success of an individual male, i.e. his share of paternity, decreases with increasing number of competing males. Because, due to last-male advantage, males obtain a higher share of paternity when mating with virgin females as opposed to already-mated females, they show preference for virgin females (Lewis and Iannini, '95). However when mated to polyandrous females, several factors including mating order (Wool and Bergerson, '79; Lewis and Austad, '90; Lewis and Jutkiewicz, '98; Bernasconi and Keller, 2001; Pai and Yan, 2002a; Nilsson et al., 2003; Lewis et al., 2005), intensity of male copulatory courtship (Edvardsson and Arnqvist, 2000), female response to male pheromonal cues (Lewis and Austad, '94), as well as male and female genotypes (Pai and Yan, 2002a; Nilsson et al., 2003) influence a male's success at siring offspring. As a result, male sperm competition success varies considerably in this species. For 11 pairs of rival males mated with replicate females the shares of paternity varied between 40 and 86% (Lewis and Austad, '90) and comparable variation is found also for per-copulation P_2 values (Lewis and Jutkiewicz, '98). The availability of newly developed molecular markers (Pai et al., 2003; Zhong et al., 2003, 2004, 2005; Demuth et al., in press), in addition to the heritable phenotypic markers (available through the Tribolium Stock Centre, US Grain Marketing Research Laboratory, Manhattan, KS, USA) used for inferring paternity in classical double mating experiments, should help to characterize the variability of paternity shares for a larger and more realistic range of polyandry (i.e. beyond two males) and in natural populations. Moreover, more studies using quantitative genetics approaches may help to further our understanding of genome × genome interactions in this system.

MALE AND FEMALE DETERMINANTS OF POST-COPULATORY SPERM COMPETITION SUCCESS

In addition to the above-mentioned copulatory courtship behavior (Edvardsson and Arnqvist, 2000) and olfactory attractiveness (Lewis and Austad, '94), males may have evolved additional traits that are potential adaptations to increase their post-copulatory reproductive success. For instance, the brush-like structure on the male aedeagus (intromittent organ), or sperm size and number variation, may play a role in post-copulatory processes (Arnaud et al., 2001b,c). During mating, the male deposits a spermatophore in the bursa copulatrix and sperm are translocated to the spermatheca. Males can transfer $>100{,}000$ sperm per copulation, but only about 4% reach the spermatheca, which can store roughly 7,500 sperm. Thus, the spermatheca is filled to capacity after two matings and when females remate, stored sperm are partly displaced (Lewis and Jutkiewicz, '98). The great discrepancy between the number of inseminated and stored sperm (which are likely those used for fertilization) suggests that investment in sperm expenditure may be a male strategy to increase the probability that his sperm will be retained in the ''fertilization pool''. Because red flour beetle populations are found to diverge for siring success (Nilsson et al., 2002, 2003; Attia and Tregenza, 2004; see below), it is possible that different male traits might affect male fertilization success in different populations.

Male adaptations to increase siring success are likely to interact with female perception. Males whose pheromone cue were more attractive to females in assays, subsequently obtained higher sperm precedence (P_2) than less attractive males (Lewis and Austad, '94), suggesting that these beetles have a post-copulatory bias mechanism in favor of sperm from the preferred males, and/or that male ''signals'' target female ''receptors'' associated with reproductive physiology. Another study demonstrated a correlation between the intensity of male copulatory courtship and male share of paternity when females were allowed to perceive this behavior (Edvardsson and Arnqvist, 2000). By contrast, no such correlation was apparent when females were experimentally prevented to perceive male copulatory courtship (by shortening the legs that males rub along the female body during copulatory courtship). A later study confirmed the importance of male copulatory courtship as determinant of male paternity success, yet in combination with female behavior observable during mating (Bloch Qazi, 2003). In particular, female quiescence behavior during copulation predicted the timing and numbers of sperm transferred, and explained greater variation in siring success (Bloch Qazi, 2003). Behavioral studies that involve interactions between individuals are complex, because it is difficult to disentangle male and female components. Indeed, males and females likely influence each other's behavior during mating, making it difficult to experimentally isolate effects.

Females have been observed to eject the sperm they receive by extruding the spermatophore (Lewis and Jutkiewicz, '98), and this is consistent with the fact that a proportion of observed copulations fail to result in offspring production (Bloch Qazi et al., '96; Bernasconi and Keller, 2001; G. Bernasconi, unpublished data). Possibly, spermatophore ejection may be a way for females to control sperm precedence, and it would be interesting to examine in greater detail this phenomenon in future studies. Another possible mechanism for female control over paternity after copulation is differential sperm movement and storage (Eberhard, '96). As in other insect species (Hellriegel and Bernasconi, '99), in *T. castaneum* also, several studies indicate that females have an active role in controlling sperm movement during or after copulation (Bloch Qazi et al., '98a; Bloch Qazi, 2003; Fedina and Lewis, 2004). In these studies, females were anesthetized to interfere with muscular control over the reproductive tract. This experimental approach demonstrated that interference with female muscular control significantly affected sperm numbers in different parts of the female reproductive tract, providing direct evidence for female control of sperm movement (Bloch Qazi et al., '96,'98a; Lewis and Jutkiewicz, '98).

Variation in spermathecal morphology is another possible source of female influence in determining paternity (Fedina and Lewis, 2004; Bernasconi et al., 2006). The spermatheca of *T. castaneum* is a complex organ. It consists of a muscular chamber with usually three (± 1) long, convoluted compartments (tubules, Fig. 1, see also Bernasconi et al., 2006) connected to the bursa copulatrix by a common duct (Sinha, '53; Surtees, '60, '61; Bloch Qazi et al., '98a; Fedina and Lewis, 2004). This morphology may allow selective storage of the sperm of different males in different tubules (Hellriegel and Ward, '98). Moreover, there is substantial variation among individuals in spermathecal shape as it results from coiling, length, and width of tubules (Bernasconi et al., 2006). However, it is unknown whether this variation correlates with intraspecific variation in the propensity to polyandry and sperm competition intensity. Interestingly, a recent study found that sperm precedence patterns differed between doubly mated females that were not able to control sperm movement and storage due to anesthetization and control females, confirming the role of females in biasing paternity of their offspring (Fedina and Lewis, 2004). Moreover, siring success of the second male to mate decreased with increasing spermathecal tubule volume (Fedina and Lewis, 2004). This suggests that spermathecal size and shape may possibly evolve to decrease last-male advantage, which may be beneficial to females for example if genetic

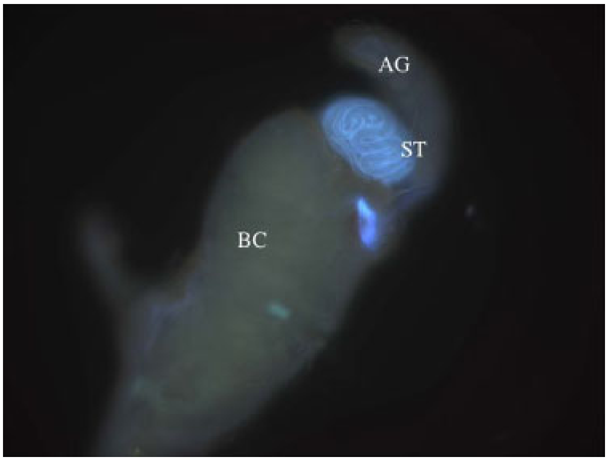

Fig. 1. The spermatheca (sperm storage organ) of *Tribolium castaneum* females (fluorescence microscopy, G. Bernasconi). ST = spermatheca, AG = accessory gland, BC = bursa copulatrix.

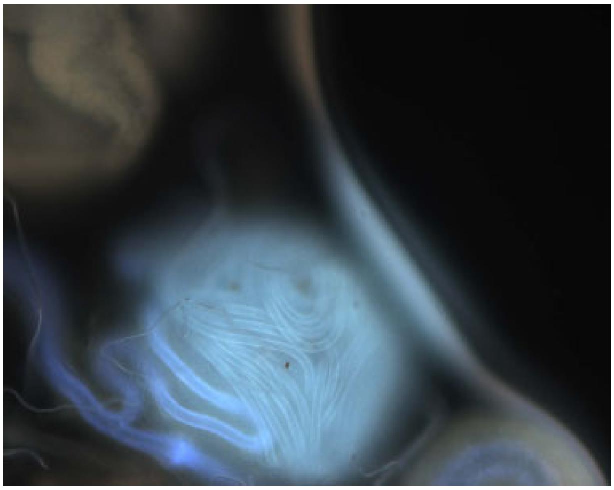

Fig. 2. The spermatheca (sperm storage organ) of *Tribolium brevicornis* females (confocal laser scanning microscopy, image dimensions: 214 μm × 214 μm; G. Bernasconi).

diversity of the offspring increases fitness (Bernasconi et al., 2003, 2004). Although an independent study failed to find evidence for a correlation between spermathecal size and shape with paternity (with only a trend for spermathecal volume, Bernasconi et al., 2006), this may be due to low statistical power, differences among strains, and/or differences in design. A promising novel direction would be to investigate evolution of size and shape of the sperm storage organs across species, and to correlate this to interspecific variation in polyandry, sperm competition intensity, and effects of multiple mating on female fitness. In the genus *Tribolium* alone, there is great variation in spermathecal morphology (G. Bernasconi and L. Arnaud, unpublished data). For instance in *T. brevicornis*, there are numerous, very thin tubules (Fig. 2), whereas other species in the genus have less complex spermathecae (G. Bernasconi and L. Arnaud, unpublished data).

EVIDENCE FOR SEXUALLY ANTAGONISTIC COEVOLUTION

A prediction from sexual conflict is local co-adaptation between the sexes (Arnqvist and Rowe, 2005; see also Rowe et al., 2003; Pizzari and Snook, 2003). A number of studies reveal that the genotype of males and females can affect important components of pre- and post-copulatory processes, including female willingness to mate, female odor preference, sperm precedence, egg-laying rates, lifetime egg production, and female lifespan (Table 2).

Female willingness to mate depended on the genetic background of the female, the male, and the interaction between them (Graur and Wool, ’82; Nilsson et al., 2002, 2003; Attia and Tregenza, 2004). The genetic background of males and females influenced female preference in a study examining female odor preference for males from same or different genetic backgrounds (Boake and Wade, ’84).

Male success at sperm competition also depends on whether they stem from the same, or a different strain as the female: several independent studies that have examined male sperm precedence (P_1 and/or P_2) also found this trait to be affected by male genotype, female genotype and/or the interaction between them (Lewis and Austad, ’90; Bernasconi and Keller, 2001; Pai and Yan, 2002a; Nilsson et al., 2003). Pai and Yan (2002a) found first male sperm precedence was highest with females of the same genetic background but found no clear pattern of male–female interaction with respect to second male sperm precedence, suggesting that offence and defence ability may rely upon different mechanisms (see also Bernasconi and Keller, 2001).

Moreover, the interaction between male and female genetic background significantly influenced components of female fitness such as oviposition rate immediately after a mating, lifetime offspring production, and female lifespan (Nilsson et al., 2002, 2003; Attia and Tregenza, 2004; Table 2). Similar results have been obtained for *Drosophila melanogaster* (Aguade et al., ’92; Clark et al., ’99; Fiumera et al., 2005). Altogether,

TABLE 2. Influence of male and female genetic background revealed by inter-population beetle crosses on various mating related traits in Tribolium castaneum

Traits examined	Male influence	Female influence	Male × female influence	Source
Pre-copulatory processes				
Female response to male pheromone cue		X		Boake and Wade ('84)
Mating probability	X	X	X	Nilsson et al. (2002)
Female reluctance to mate			X	Nilsson et al. (2003)
Copulatory processes				
Copulation duration	X	X	X	Nilsson et al. (2003)
Post-copulatory processes				
Success of copulation (based on whether or not females produced offspring)				Attia and Tregenza (2004)
P_1 (paternity share of first of two males to mate with a female)		X	X	Pai and Yan (2002a)
		X		Nilsson et al. (2003)
P_2 (paternity share of second of two males to mate with a female)	X		X	Lewis and Austad ('90)
	X			Pai and Yan (2002a)
	X	X	X	Nilsson et al. (2003)
Effect of mating on female offspring production in first week after copulation	X	X	X	Attia and Tregenza (2004)
Effect of mating on female lifetime offspring production	X	X	X (in combination with mating frequency)	Nilsson et al. (2002)
Life-history traits				
Effect of mating on female lifespan	X	X		Nilsson et al. (2002)

Pre-copulatory processes (female response to male pheromone, female probability of mating, female reluctance to mate), copulatory processes (duration of copulation), post-copulatory processes (success of copulation, male sperm precedence, female short-term and lifetime offspring production) and life history traits (effect of mating on female lifespan) are shown. "X" indicates significance at $P<0.05$. Note that a significant male × female interaction signals a strong likelihood of post-copulatory female controlled processes (Pitnick and Brown, 2000).

these studies reveal that male and female reproductive traits have diverged, thus indicating heritable variation in traits that determine mate choice, male sperm competition success, and the impact of polyandry on female fitness, and suggest that post-copulatory sexual selection drives reproductive divergence in *T. castaneum*. Importantly, interactions between male and female genotype clearly constitute strong evidence for female control of post-copulatory processes (Pitnick and Brown, 2000).

In congruence with the above-mentioned results of inter-population crosses suggestive of local co-adaptation between the sexes possibly resulting from sexual conflict (Pizzari and Snook, 2003; Rowe et al., 2003; Arnqvist and Rowe, 2005), other experimental evidence also supports sexually antagonistic co-evolution in this species. A study of the fitness consequences of polyandry revealed a negative correlation between fitness of F_1 males and females produced by polyandrous mothers (Pai and Yan, 2002b). Interestingly, whereas the F_1 males from polyandrous mothers showed higher fitness than those from monandrous mothers, the F_1 females from polyandrous mothers were less fit than those from monandrous mothers in a competitive environment (Pai and Yan, 2002b). The possibility that this opposite pattern of fitness of sons and daughters may be due to paternal genes is intriguing because it suggests that polyandry may have genetic costs (i.e., producing less fit daughters) to females, consistent with conflict of interests between males and females (Chippindale et al., 2001).

FUTURE DIRECTIONS

Several decades of research on the reproductive biology of *T. castaneum* have revealed that this is a highly promiscuous species, displaying a number of pre- and post-copulatory traits in both males and females that can influence the outcome of sperm competition. This species thus provides a case study demonstrating the importance not only of male adaptations to sperm competition, but also of cryptic female choice (Eberhard, '96), and contributes evidence for the idea that male and female reproductive success in polyandrous species likely depends on epistatic interactions between mating partners (Wolf et al., 2000). This fulfils the conditions for co-evolution between the sexes, and initial evidence supports the notion that many of the traits involved in post-copulatory processes may in fact be sexually antagonistic. A number of interesting questions however remain to be addressed.

Benefits to female of cryptic choice and remating

Although female control of sperm movement and storage has been demonstrated, the fitness benefits of this behavior (if any) have not been established. Edvardsson and Arnqvist (2005) examined whether females mated to males they perceived as attractive produced more sons than daughters, compared to females mated to males prevented from performing copulatory courtship, but did not find any significant support for sex-ratio allocation by females. Edvardsson and Arnqvist (2006) also examined whether females, by biasing paternity in favor of males with more vigorous copulatory courtship, produce offspring of higher viability (i.e. derive indirect benefits) and found no evidence for that hypothesis either. The relationship between female control and its fitness consequences, including on offspring vigor and viability, therefore remains to be determined. For example, it would be interesting to explore whether cryptic female choice avoids fertilizations by related males, and thus the costs of inbreeding. Similarly, it would be interesting to explore the fitness consequences of variation in female propensity to polyandry. Although fitness benefits on sons' sperm competitiveness have been found (Bernasconi and Keller, 2001), other aspects need to be explored. For instance, polyandry presumably results in increased genetic variation within cohorts of offspring and may thus also affect the prevalence of sib cannibalism, an important factor for population dynamics in this species.

Clearly, the fitness consequences of cryptic female choice are crucial to elucidate which selection forces shape male–female coevolution in flour beetle populations. If cryptic female choice is beneficial to females, male–female coevolution is likely to be cooperative, wherein the reproductive interests of the sexes coincide. Alternatively if it is not beneficial, perhaps the male signals that lead to higher share of paternity through cryptic female choice might represent a manipulation of the female into mating at the cost of her own reproductive interest (sexual conflict).

Prevalence of polyandry and extent of multiple paternity

To date no published study has measured the prevalence and extent of multiple paternity within broods under natural conditions. We do not know whether all of a female's mates have a share in the paternity of her offspring, and there are no estimates of the variance in male reproductive success under realistic ranges of mating partners. *Tribolium castaneum* females mate with more than two males in most populations that have been examined thus far (Nilsson et al., 2002; Pai and Yan, 2002b, 2003; Pai et al., 2005, in press) yet, most published studies of sperm precedence have only examined the outcome of sperm competition between two males (for example Lewis and Austad, '90, '94; Yan and Stevens, '95; Haubruge et al., '99; Bernasconi and Keller, 2001; Pai and Yan, 2002a; Nilsson et al., 2003; Fedina and Lewis, 2004, but see Wool and Bergerson, '79; Lewis and Jutkiewicz, '98; Arnaud et al., 2001a). The extent of female influence when sperm competition is among three or more males needs to be determined especially because this is likely to be a more accurate reflection of conditions in natural populations. Paternity analysis using molecular markers such as microsatellites (Pai et al., 2003; Demuth et al., in press) thus promise to reveal significant clues about determinants of paternity shares and the intensity of post-copulatory sexual selection. Moreover, the availability of a variety of molecular markers (Beeman and Brown, '99; Pai et al., 2003; Zhong et al., 2003, 2004, 2005; Lorenzen et al., 2005; Demuth et al., in press) makes it now possible to investigate polyandry and paternity shares without confounding effect from the genetic background of the phenotypic markers used thus far to score paternity (Wool and

Bergerson, ’79; Lewis and Austad, ’90, ’94; Bernasconi and Keller, 2001; Pai and Yan, 2002a; Nilsson et al., 2003). Clearly, the limitation of using mutant strains for such a purpose is that male × male and female × male genotype (including, possibly, loci linked with the phenotypic markers) can influence sperm precedence (Lewis and Austad, ’90; Nilsson et al., 2002, 2003; Pai and Yan, 2002a; Attia and Tregenza, 2004).

Variation in male signals and mechanisms for cryptic female choice

Although a variety of male signals (pheromones, copulatory courtship) and female mechanisms (control of sperm storage, variation in spermathecal morphology, copulatory behavior) have been identified, additional traits may play a role. Given the differences among strains and populations (Nilsson et al., 2002, 2003; Attia and Tregenza, 2004) it is likely that different traits may be important in different populations. Moreover, although within populations antagonistic effects may balance, some traits involved in sexual conflict may become apparent in between-population crosses. Inter-population crosses clearly reveal divergence in male and female traits affecting siring success. However, the mechanistic basis for the differential ability of females from different populations to manipulate post-copulatory processes remains to be elucidated. Thus an important future direction is to identify novel traits and explore in greater detail and in multiple populations all known traits that create male × female genotype interaction. In addition, interspecific comparisons of traits and relevant population parameters (e.g., prevalence of polyandry) within the genus *Tribolium* (see Figs. 1 and 2), or within the Tenebrionidae, may reveal evolutionary trends.

It is possible that male ‘‘signals’’ and female ‘‘receptors’’ are chemical in nature, as in the fruitfly *Drosophila melanogaster* (Chapman et al., 2003). Exploring the chemical nature of the spermatophore in *T. castaneum* will possibly unveil mechanisms that affect post-copulatory reproductive success. Molecular approaches in identifying male and female reproductive genes have been very successful in other species (Panhuis and Swanson, 2006; Panhuis et al., 2006). For example, new studies in *Drosophila* have identified candidate genes for female reproductive proteins in addition to the previously documented genes for male reproductive proteins (Panhuis and Swanson, 2006). Using the candidate gene approach may thus be an important avenue of future research in other model systems, including the red flour beetle. Genomic tools such as analysis of male or female reproductive secretions, expressed sequence tag (EST) sequencing, and microarray analysis are likely to be fruitful in identifying new factors involved in male and female interactions (Clark et al., 2006), and parallel approaches in different taxa are important to address the generality of the consequences of polyandry. *Tribolium castaneum* is highly suitable as a novel model organism especially in light of the new genomic tools available in this organism (Brown et al., 2003) and the existing knowledge of the reproductive biology of various Tenebrionids.

In conclusion, there is a large body of evidence for cryptic female choice and male adaptations to sperm competition in the highly polyandrous red flour beetle, with evidence in support of sexual conflict. This background of knowledge provides ideal conditions to make this species a novel test case for the generality and importance of sexual antagonism in shaping reproductive traits. Substantial advances can be expected from applying molecular tools to explore the extent of multiple and effective paternity within natural broods, and genomics to characterize mechanisms, including chemical signals in male spermatophores as well as female receptors in the reproductive tract.

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