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REPRODUCTIVE ANATOMY, PRECOPULATORY MATE GUARDING, AND PATERNITY IN THE SOCORRO ISOPOD, *THERMOSPHAEROMA THERMOPHILUM*

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We examine the reproductive anatomy of both sexes of the isopod *Thermosphaeroma thermophilum* using SEM and light microscopy, and study the occurrence of multiple paternity using allozyme electrophoresis. Female reproductive openings are found ventrally, under the cuticular plate covering the body between the fifth and seventh pereonites. Receptivity for copulation is short during the sexual moult. Sperm from copulation are stored within the oviducts, close to the ovary, for up to two weeks before fertilisation and oviposition into ventral brood pouches. Sperm are not stored between broods. Male genitalia consist of penes with erectile extensions, and curved, channelled *appendices masculinae*. We suggest that *appendices masculinae* either channel sperm from the penes to oviduct openings located beneath the cuticular plate, or are used to push aside the cuticular plate for penile intromission. Paternity analyses suggest that multiple paternity is uncommon and that precopulatory guarding is an effective mate monopolisation strategy for males. We discuss the evolution of reproductive anatomy in the context of intersexual conflict, and suggest that conflict resolution may play a prominent role in the evolution of temporally restricted receptivity, male mate guarding, sperm storage, and delayed oviposition.

Keywords: Reproductive anatomy; mate guarding; sperm storage; sperm competition; sexual conflict

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INTRODUCTION

Male strategies for mate acquisition are constrained by the reproductive anatomy and physiology of females. Precopulatory mate guarding, common in aquatic crustaceans, is one such male strategy (Parker, 1974), whose evolution has been attributed to two distinct female characteristics. First, time-limited opportunities for copulation appear to favour male mate guarding before female receptivity begins. Such physical monopolisation of females ensures a male's presence during the brief period in which a guarded female is actually receptive (Parker, 1974; Ridley, 1983; Grafen and Ridley, 1983). Second, the structure of the female reproductive tract and its effects on the fate of successive ejaculates may favour mate guarding. When several males copulate with a female, i.e. sperm competition occurs, yet the first male to mate sires most or all of the progeny, males are expected to guard females before the receptive period begins to ensure their position as the first mate (Parker, 1970; Brown *et al.*, 1997).

The evolution of male strategies and structures which improve mating success, may in turn select for counter adaptations in females, especially if male strategies impose costs on female fitness (Parker, 1979; Rice and Holland, 1997). Possible examples of such female adaptations may include resistance against mating attempts (Strong, 1973; Rowe *et al.*, 1994; Jormalainen and Merilaita, 1995), hiding from males (Clutton-Brock and Parker, 1995), pheromonal signalling of receptivity (Dunham, 1978), and sperm storage, i.e. temporal separation of copulation and fertilisation (Birkhead and Møller, 1992: Alexander *et al.*, 1997; Jormalainen and Shuster, 1998). Evolution of reproductive characteristics, especially increasing genitalic complexity, is thought to be driven by mutual counter adaptations by both sexes to each others traits (Rice and Holland, 1997; Alexander *et al.*, 1997). The rate of evolution of such traits has been suggested to be reinforced by intersexual conflicts, arising from dissimilar fitness maximisation strategies between the sexes (Parker, 1979; Rice and Holland, 1997).

Recent results suggest that mate guarding in aquatic crustaceans is characterised by sexual conflict (reviewed in Jormalainen, 1998). In both *Idotea baltica* (Jormalainen and Merilaita, 1995) and *Thermosphaeroma thermophilum* (Jormalainen and Shuster, 1998) male and female interests over guarding duration are in opposition as males aim to initiate guarding earlier than females allow. By resisting, females effectively shorten guarding duration. Thus, sexual conflict in these species has the potential to favour more effective guarding tactics by males as well as more effective escape tactics by females, depending on the relative costs and benefits of such struggles to each sex.

In isopods, in which precopulatory guarding and multiple insemination are known to occur (Manning, 1975; Sassaman, 1978; Shuster, 1981b; 1989; Jormalainen, 1998), moulting is biphasic and female receptivity is usually restricted to the sexual moult. During this moult, sexual receptivity begins when mature females shed the posterior half of their cuticle and ends when females shed the anterior cuticle. Copulation takes place at the moult, and fertilisation and oviposition into ventral brood-pouch follows (e.g. *Asellus aquaticus*, Manning, 1975; *Idotea baltica*, Borowsky, 1987; *Saduria entomon*, Leonardsson, 1986; *Paracerceis sculpta*, Shuster, 1991). The manner of sperm transfer and storage is poorly known for most taxa (Wilson, 1991). Sperm storage occurs in some Oniscoidea and Asellota, and possibly in some Sphaeromatidae (Veuille, 1978; Wilson, 1986; 1991). Data on sperm competition patterns in isopods is scarce (but see Sassaman, 1978; Shuster, 1989).

In this study we describe the anatomy of male and female genitalia and the occurrence of sperm storage, and suggest a mechanism for sperm transfer in a freshwater sphaeromatid isopod *Thermosphaeroma thermophilum* (Cole and Bane). Using variable allozyme loci we examine the occurrence of multiple mating under conditions approximating those found in nature. Lastly, we discuss the evolution of temporally limited receptivity, sperm storage and delayed oviposition in the context of intersexual conflict and in light of our results for this species.

MATERIAL AND METHODS

Study Species

T. thermophilum, an endangered crustacean species, is endemic to a single thermal spring in Socorro, New Mexico, USA (Federal Register, 1977). In this stable, thermal environment, reproduction occurs year-round (Shuster, 1981a). Local population density varies within the spring, but may reach 210 individuals per 100 cm^2 when individuals emerge from sediments to feed at dawn or dusk. Samples collected at this time show that population sex ratio is consistently male biased, varying between 1.5 and 5.5 (Shuster, 1981a). Females are iteroparous. The duration of the female moult cycle is about 45 days. Copulation takes place during the sexual moult. Oviposition occurs within 18 days after the sexual moult, and incubation is complete within one month. After parturition a pre-moult period of less than 10 days follows. During this period males guard females (Jormalainen and Shuster, 1998). Guarding ends after copulation and completion of the moult, i.e. shedding of the anterior half of the exoskeleton (Shuster, 1981b; Jormalainen and Shuster, 1998).

Sampling and Laboratory Rearing

Isopods were collected from their native spring in Socorro, NM, on 17th September 1995, 18th March and 6th May 1996. Animals were maintained at Northern Arizona, Flagstaff, AZ within 55 cm^3 cylindrical plastic containers equipped with removable net walls at both ends to allow water circulation. Isopods were fed with both vegetarian fish food and brine shrimp flakes. Cylinders were submerged in 40 l aquaria maintained at 30°C with aeration and undergravel filtration. Gravid females were maintained individually until parturition, after which they were used in the mate guarding/ sperm competition experiment (see below), or paired with a male until the sexual moult and then prepared for microscopy. Other details of the female moult cycle, obtained from this same rearing, are described elsewhere (Jormalainen and Shuster, 1998).

Microscopy

Individuals used for scanning electron microscopy (SEM) and light microscopy were preserved into 2% glutaraldehyde fixative in 0.1 M sodium cacodylate buffer (pH 7.2). The time from their sexual moult and reproductive stage (non-gravid – egg bearing), as well as the existence of paired whitish masses seen on the ventral side between the fifth pair of pereiopods were recorded for females. Since these masses appeared after the sexual moult in females maintained with a male, we suspected the masses to be sperm. Surface structures were examined from the SEM photographs. Samples were prepared for SEM by dehydrating whole isopods in a graded series of ethanol (20, 50, 95, 100, 100, and 100%), critical point drying, and gold coating. Internal anatomy was studied from 1 μm cross-sections, after post-fixation with osmiumtetraoxide–kaliumferrocyanide solution, moulding into plastic, and staining with toluideblue.

Electrophoresis

As part of other experiments (Shuster and Jormalainen, unpublished) we identified the genotypes of all laboratory-maintained isopods at 5 enzyme loci from tissue contained in a single walking leg. Amputation of the right or left 7th pereiopod was varied among males to enhance our ability to distinguish individuals in the mate guarding experiment. We homogenised individual pereiopods in 0.05 M Tris-HCl pH 7.5 buffer and visualised allozyme variation using starch gel electrophoresis (PGM, PGI, MDH1, ME, 6PGDH; for gel buffer systems and staining procedures, see Sassaman, 1989).

Mate Guarding and Paternity

We arranged triplets of two males and one female in mesh containers (12 × 12 × 8 cm) submerged in larger aquaria as described in Jormalainen and Shuster (1998). Females used in this experiment had given birth to their previous brood in individual containers before the start of the experiment as described above, and were expected to undergo a sexual moult within two weeks. Males were maintained in individual containers and identified by enzyme genotype at least one week before the start of the experiment. We chose the two males for nine of the triplets so that genotypes differed at one or more loci. Pairs of males in each triplet were identified by the leg removed for allozyme analysis (left or right).

The triplets were examined twice per day to record precopulatory guarding and the female moult. Males were removed after the female had completed her moult. The female was moved into a smaller mesh container and maintained until the birth of juveniles. When the juveniles were released, they as well as the 5th pereiopod of the female were placed into individual wells of an ELISA plate with 25 μl Tris-HCl 7.5 buffer and frozen at −80°C until samples were screened for the same loci as the males. Because of mortality during the relatively long incubation period, only four broods were analysed.

The probability of paternity for males in each triplet was determined by comparing the observed allele frequencies with those expected if each locus exhibited Mendelian inheritance and if each male had sired either all or half of the brood. Although the low frequency of allelic variation in *T. thermophilum* prevented us from performing crosses to confirm Mendelian segregation at PGI, MDH1, and 6PGDH, variation at these loci among field collected animals conforms to Hardy–Weinberg expectations (Shuster and Jormalainen, unpublished) and thus suggests Mendelian inheritance at these loci.

RESULTS

Genital Anatomy

The male external genitalia consist of paired *penile papillae* (penes; Fig. 1) and *appendices masculinae* (Fig. 1(a)). The *penile papillae*, bearing the

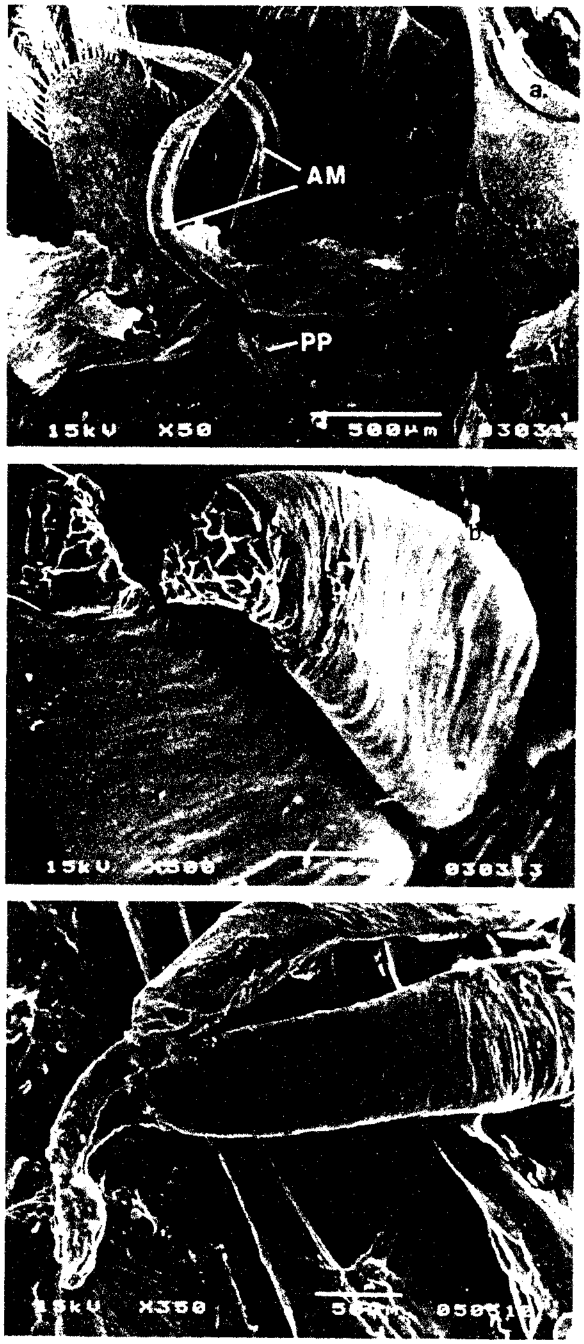

FIGURE 1 External male genitals of *T. thermophilum*. Figure (a) shows the *penile papillae* (PP), located ventrally on the seventh pereonite, and *appendices masculinae* (AM), the endopodal extensions of the second pair of pleopods. Pereiopods, the first pair of pleopods and the exopodal pleopods are removed. The *penile papillae* have a soft tip (b), through which a funnel-like extension erects (c).

openings of the sperm duct (*vas deferens*), are located ventrally, in the midline of the posterior end of the 7th segment (pereonite). *Appendices masculinae* are modified endopods of the second pair of pleopods. Each appendix forms a curved, channelled rod with a narrowing sharp tip (Fig. 1(a)). The *penile papillae* have a soft pinnacle, through which the *papillae* can erect a funnel-like extension, as revealed by SEM of one male whose extensions were fixed in a protruded position (Fig. 1(c)).

In females, no openings of reproductive tract are visible either on the ventral or dorsal surface of the animal. Immediately following, as well as preceding, the sexual moult, a cuticular plate covers completely the ventral side of the body between 5th and 7th pereonites (Fig 2(a)). The curved, anterior edge of this plate extends to the 4th pereonite. However, this cuticular plate appears soft, and its edge is not tightly attached to the anterior part of the cuticle, thus, revealing a possible route into female genitalia (Fig 2(b)). Furthermore, the plate has two grooves which appear thinner and softer than the surrounding cuticle, each starting close to the anterior plate margin and curving posterio-laterally (Fig. 2(a) and (b)). These grooves may accommodate male *appendices masculinae* during copulation. A few days after the sexual moult, and after oviposition, the cuticular plate appears harder, and these grooves are no longer visible (Fig. 2(c)).

Sperm Storage

Sperm masses were found within the female reproductive tract during the period between the sexual moult and oviposition (Figs. 3 and 5). After oviposition, no sperm masses were found (Fig. 6) implying that sperm is stored only for the forthcoming brood and a new copulation is needed for each successive brood. Laboratory maintenance of females in individual containers revealed the same result: none of eight females maintained alone through the duration of the sexual moult became gravid. Instead, ovaries of isolated females turned into soft masses 10–20 days after the sexual moult. Sperm were located within the female reproductive tract within the fifth pereonite (Fig 3(a)). The openings to the female reproductive tract appeared to be located beneath the cuticular plate in fifth pereonite (Fig. 4).

Sperm masses found in cross-sectioned females confirmed our supposition that whitish masses we saw by a light microscope through the cuticle of live individuals were sperm. Of 24 non-gravid females collected from the field and thereafter reared individually in aquaria, all oviposited within 5 to 15 days. 19 were scored by light microscopy as carrying sperm masses. None

FIGURE 2 Ventral side of a female *I. thermophilum* a few hours after the completion of the sexual moult (a), and a close up of the edge of the cuticular plate covering the posterior side of the body (b). The plate appears hard and thick after oviposition (c). Pereiopods are removed in order to reveal the ventral surface

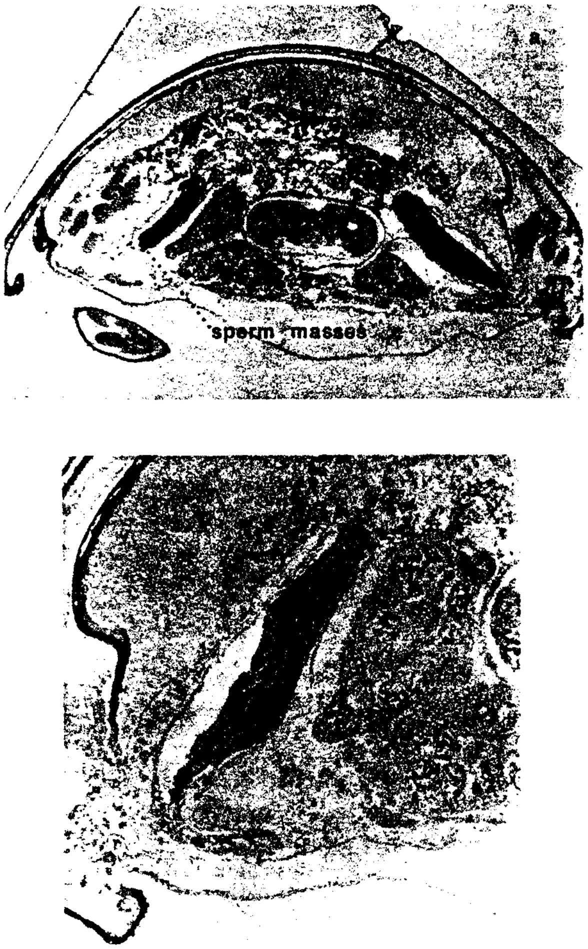

FIGURE 3 Cross-sections of a female two days after copulation and completion of the sexual moult, but before oviposition. Paired sperm-masses were found between 5th and 6th pereiopod, 40 × (a). A close-up of the sperm mass on the left, cross cut by the 5th pereiopod, 100 × (b).

of these 24 females underwent a moult in the laboratory, which suggests that they had moulted and copulated in the field before sampling. Although most females examined carried sperm in each oviduct, sometimes only single sperm mass was seen. Sperm masses were plate-like, appearing narrow when viewed along the longitudinal axis (Fig. 3) and rounded from the side (Fig. 5). Sperm within masses appeared to be grouped in dozens of elongated clusters, or spermatophores, as is generally found in isopods (e.g. Fig. 5(b)).

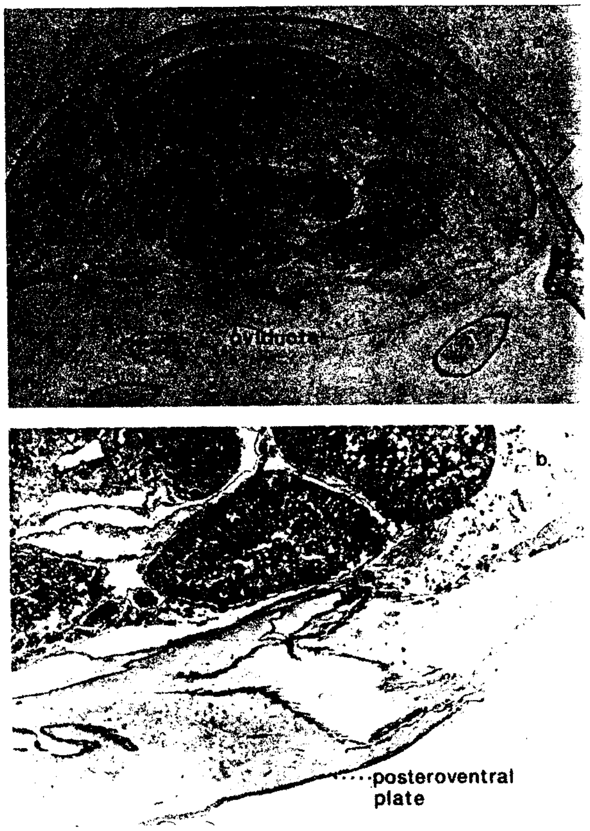

FIGURE 4 Cross-sections of the same female as in Fig. 3, cut between 4th and 5th pereiopod showing oviducts. 40 × (a), and the oviduct opening under the posteroventral plate, close to the 4th pereiopod, 100 × (b).

After oviposition, developing embryos were found within brood-pouches (Fig. 6). These brood-pouches were clearly internal; there was a wall between the embryos and the outermost cuticula (Fig. 6).

Mate Guarding and Paternity

In three of the four cases in which two males were maintained with one receptive female, the paternity of all offspring could be assigned to one of the males (Table I). In all of these cases, the guarding male sired all progeny; the other male was never seen guarding the female.

In the last case, both males were observed guarding the female; male #1 was observed guarding first, and male #2 after him. Male #1 was seen

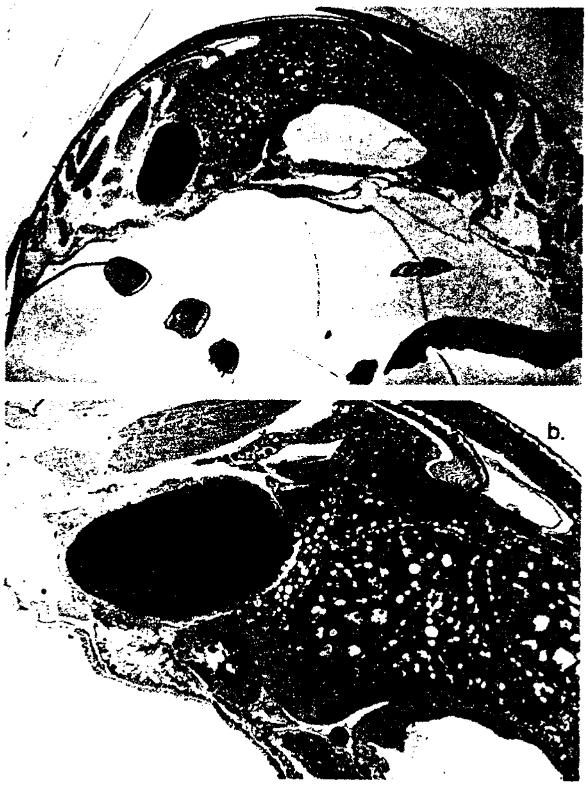

FIGURE 5 Cross-section of a female nine days after copulation and completion of the sexual moult, but before oviposition, taken at an angle so that the left end of the picture is from the 5th pereonite and the right one from the 1st pereonite, 40 × (a), and a close-up of the sperm mass, 100 × (b).

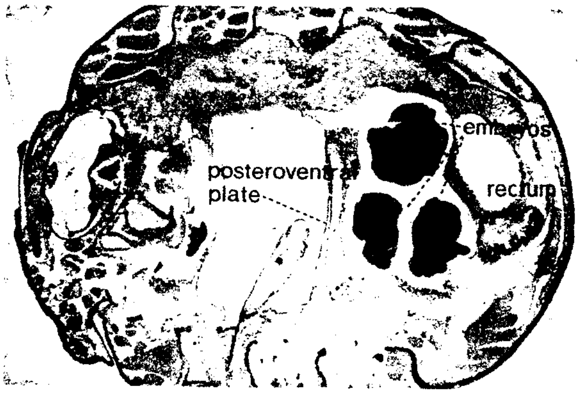

FIGURE 6 A horizontal cross-section of a female carrying embryos at early stages. At the anterior side of the animal, on the left, the dissect-plane cuts the head, and at the posterior side of the animal, on the right, the cross-section pierce through the 5th pereonite. The animal has assumed a slightly spheroid shape, and, thus, there appears a hole in the middle of the cross-section.

TABLE I Parent and offspring genotypes based on electrophoretic variation of various enzymes, and χ^2 statistics testing for hypotheses of all offspring coming from either one of the males

Enzyme	*Female*	*Male1*	*Male2*	*No. of offspring*		*Offspring from Male1*		*Offspring from Male2*	
						χ^2	P	χ^2	P
PGI	1/2	2/2*	1/2	1/1	—				
				1/2	21				
				2/2	13	1.88	0.17	11.8	<0.01
6PGDH	1/1	1/1*	1/2	1/1	24				
				1/2	—				
				2/2	—	perfect fit		24	<0.001
6PGDH	1/1	1/2*	1/1	1/1	6				
				1/2	10	1.00	0.32	no fit	
MDH1	1/2	1/2*	2/2*	1/1	—				
				1/2	15				
				2/2	8	7.70	<0.05	2.13	0.14

*Indicates that the male has been seen guarding the female when being close to her moult.

guarding the female on the last evening before her moult. However, male #1 was not siring the offspring, at least not alone (Table I). The probability of sole paternity by male #2 (Table I) was equal to the probability that each male sired half of this female's brood ($\chi^2 = 3.98$, df $= 2$, $P = 0.14$). Although male #2 may have sired all of this female's progeny, we cannot eliminate the possibility of mixed paternity in this case. Thus, mate guarding appears to be an effective male strategy for paternity assurance in this species. However, multiple insemination may occur if guarding is unsuccessful.

DISCUSSION

Male Anatomy

Microscopy of isopod seminal vesicles indicates that isopod sperm are immobile and organised into elongate bundles, i.e. spermatophores, where each contain several spermatozoa (Cotelli *et al.*, 1976). The mechanism of transfer of these non-motile spermatozoa from male to female genitalia is unknown. However, Wilson (1991) has suggested that the *appendices masculinae* may serve as a channel for transferring sperm from the tips of the penes to the female genital pores. In *Asellus* and *Jaera*, highly modified *appendices masculinae* do penetrate the female during copulation (Veuille, 1978; Wilson, 1991).

In *T. thermophilum* males, each *appendix masculina* has a spiny outer surface and contains a channel-like depression on its medial surface. In many male sphaeromatids, as well as in other *Thermosphaeroma* species, penes are separate, slender, and each taper to a non-erectile apex. (Rioja, 1950; Cole and Bane, 1978; Bowman, 1981; 1985; Shuster, 1987; Kensley and Schotte, 1989). The penes of *T. thermophilum* males appear erectile into a tube-like extension which may facilitate transfer of sperm from the penes into the *appendices masculinae*; these latter structures may be held together and funnel sperm into the female genital pores as suggested by Wilson (1991). Alternatively, the *appendices masculinae* may be used to push aside the cuticular plate covering the reproductive openings of females to permit sperm transfer by the penes directly. We know of no other descriptions of erectile penes in the Isopoda, but in *Porcellio scaber* there exist two sphincters at the terminal end of *ductus deferens*, which has been suggested to inject the bulk of sperm into female genitalia (ref. in Cotelli *et al.*, 1976). It may well be that the penes extensions we found and the sphincters of *Porcellio*, in fact, are homologous organs.

Female Anatomy

The genital openings in most isopod females are found ventrally on the fifth pereonite, and are occasionally fused with the base of pereiopods (Wilson, 1991). In *T. thermophilum* the openings of oviduct were not fused with coxae, but instead open beneath the posterior cuticular plate. Harrison (1984) called this plate the posterior pocket, and suggested that embryos are incubated directly beneath the plate. However, our microscopy revealed that embryos do not lie directly under the plate, but are instead enclosed within invaginated brood-pouches as occurs in other sphaeromatids (see Iverson, 1982; Harrison, 1984). These brood-pouches are also visible by light microscopy on live individuals for some time after parturition (V. Jormalainen, personal observation).

Sperm masses consisting of several spermatophores were found within the female reproductive tract after the sexual moult. Sperm masses appear to have been injected into oviduct as far as possible. We observed no specific cuticular structure, or spermatheca, as found in Asellota (Wilson, 1986; Brusca and Wilson, 1991), although the oviduct was expanded into a disk-shaped configuration to accommodate the sperm mass. All sperm were used for the current brood, as none were found within gravid females, and females that were isolated from males during their sexual moult did not produce eggs, but instead absorbed their ovaries.

Female Receptivity

Receptivity for copulation in *T. thermophilum* females is most likely restricted to the sexual moult. In several isopods, insemination is restricted to the period during the moult when the female exoskeleton is soft (Ridley, 1983; Wilson, 1991). Furthermore, in many species, the oostegial plates that form the marsupium cover the female genital pores at the moult (e.g. Ref. in Thompson and Manning, 1981; Borowsky, 1987). *T. thermophilum* females lack oostegites, but the hardening of the ventral posterior cuticular plate may prohibit intromission soon after the moult is completed. This is further supported by behavioural observations on males, who guard females eagerly before the moult, but soon lose their interest in post-moult females (Jormalainen and Shuster, 1998).

Sperm Storage

Retention of sperm within female genitalia for over two weeks (Jormalainen and Shuster, 1998) and the expansion of the oviduct to accommodate spermatophores shows that sperm storage exists in *T. thermophilum*. However, this process remains distinct from the extended storage of sperm over successive broods (refs in Cotelli *et al.*, 1976), as well as the existence of a specific structure, or spermatheca, for sperm storage (Veuille, 1978; Wilson, 1986).

Sperm storage in *T. thermophilum* suggests that ovaries are not yet mature for fertilisation at the time of the moult and copulation. Jormalainen and Shuster (1998) called this *delayed oviposition*, because in many isopods moulting, copulation and oviposition occur within hours of each other (e.g. Haahtela, 1978; Borowsky, 1987; Wilson, 1991). We suggest that delayed oviposition may evolve in response to male mating strategy in this species. Mate guarding lasts up to seven days in the laboratory, and may last even longer in the field (Jormalainen and Shuster, 1998; V. Jormalainen and S. Shuster, personal observation). Because guarding occurs simultaneously with ovarian maturation (Jormalainen and Shuster, 1998), guarding may interfere with the females' ability to provision energy and nutrients for their developing ova, thereby imposing a fecundity cost. Because the increase in ovarian size is most pronounced during the last few days preceding ovulation, delaying oviposition may be an efficient way to temporally separate guarding and ovary provisioning periods, and eliminate most of the guarding cost. Sperm storage ability is a necessary precondition for separating copulation from the fertilisation.

Precopulatory Mate Guarding and Paternity

Two hypotheses have been proposed to explain the evolution of precopulatory mate-guarding. The first hypothesis is based on the fate of sperm in the female reproductive tract. Depending on whether the female reproductive tract favours the sperm of the first or the last male to mate, pre- or postcopulatory mate guarding, respectively, is expected to evolve. For example, guarding patterns in insects, which usually do not show firmly limited receptive periods, have been attributed to variable sperm precedence patterns (Ridley, 1990; Choe and Crespi, 1997). This model assumes that multiple mating is common (Parker, 1970; Birkhead and Parker, 1997). Birkhead and Pringle (1986) studied sperm mixing patterns in *Gammarus pulex*, an amphipod species with precopulatory guarding and highly limited receptivity for insemination. In the laboratory they encouraged females to copulate with two successive males, and found that the first male fertilised about 90% of the eggs. They suggested that sperm precedence pattern explains the evolution of precopulatory guarding in this species (see also Birkhead and Parker, 1997).

The other proposed context for the evolution of precopulatory guarding is time-limited female receptivity (Parker, 1974; Wickler and Seibt, 1981; Ridley, 1983; Grafen and Ridley, 1983; Yamamura, 1987). According to this hypothesis, males monopolise females before the start of receptivity to ensure their presence when copulation is possible. Consistent with this hypothesis, in diverse animal taxa, Ridley (1983) found a strong association between the existence of short receptivity and precopulatory guarding. Multiple mating is not assumed to occur in this hypothesis because momentary receptivity itself makes it unlikely.

Our results suggest that males which successfully guard females sire all of the guarded female's progeny. But, because of our low sample size and possible differences in the intensity of male harassment in the field compared to our laboratory conditions, we can not make firm conclusions on the occurrence of multiple paternity in the field. However, in this species, the brevity of female receptivity reduces the likelihood that males other than the guarding male will successfully copulate. Guarding males may also avoid other males by burrowing under the substrate with females (Shuster, 1981b; Jormalainen and Shuster, 1998). If males lose control of their mate before female receptivity ends, multiple paternity may occur. Multiple insemination and sperm mixing are known in *Porcellio scaber* (Sassaman, 1978) and in *Paracerceis sculpta* (Shuster, 1989). In all of these crustaceans, including *T. thermophilum*, no evidence of sperm precedence of the first male to copulate exists. Both the duration of receptivity and the structure of female reproductive tract with respect to fate of successive ejaculates may thus play a role in the evolution of mate-guarding. Which one is more important depends largely on how commonly multiple mating occurs in the field.

To conclude, we propose that the evolution of male and female reproductive traits in *T. thermophilum* is shaped by sexual conflict arising from the differing fitness maximising strategies between the sexes (see Parker, 1979; Alexander *et al.*, 1997; Jormalainen, 1998). Male strategies of achieving matings must evolve as a response to female characteristics. Female reproductive traits themselves, like the characteristics of receptivity and the reproductive tract will, in turn, evolve in response to changes in male mating strategies. We suggest the following evolutionary scenario for the traits we described: Temporally limited receptivity for copulation may be ancestral in isopods, but could have evolved as a way to diminish mating costs due to continuous harassment by males. Limitation of receptivity is achieved by matching it temporally with the moult and by hiding reproductive openings under the hardening cuticular plate. This, in turn, is likely to select for enhancements in male genital structures, like erectile penises and *appendices masculinae*. Short receptivity should further reinforce selection for precopulatory guarding in males. Extended precopulatory guarding, in turn, may again have imposed fecundity costs for females, as suggested by female resistance for guarding attempts (Jormalainen and Shuster, 1998), and generated selection to avoid such cost. We suggest that the ability to store sperm and delayed oviposition, which together allow temporal separation of guarding and ovary provisioning periods, may represent such female counter strategies.

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