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Female Reproductive Cycle and Sexual Conflict over Precopulatory Mate-guarding in *Thermosphaeroma* (Crustacea, Isopoda)

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Abstract

In species with time-limited opportunities for insemination, precopulatory mate-guarding is expected to coevolve with the duration of female reproductive cycles. Despite this adaptation to female characteristics, it may also be advantageous for males to adjust the duration of guarding with respect to sex ratio because the benefits of guarding are dependent on the availability of females. If female fitness is reduced because of guarding, male guarding behavior leads to intersexual conflict. We studied these aspects of male mate-guarding behavior in two closely related, thermal-spring isopods (*Thermosphaeroma*). First, guarding duration showed species specificity which was related to the duration of reproductive cycle: cycle length for females and duration of guarding by males in *T. milleri* were twice as long as in *T. thermophilum*. Second, males in both species adjusted their guarding duration with sex ratio, guarding longer when a competing male was present. Third, in *T. thermophilum*, ovarian development began immediately after the birth of the previous brood and continued through guarding, sexual molt and post-molt periods until oviposition, whereas in *T. milleri*, ovarian development was largely postponed until the post-molt period. Because guarding during ovary provisioning periods may be costly for females, we tested the existence of intersexual conflict over guarding duration in *T. thermophilum*. We compared the duration of guarding of control pairs with those of pairs in which either male guarding ability or female ability to resist guarding was reduced experimentally. Guarding durations for manipulated and control males were equal, but manipulated females were guarded longer, suggesting that conflict exists and that females can effectively shorten guarding duration by their behavior. Moreover, we suggest that selection in the context of intersexual conflict may play an important role in the evolution of delayed oviposition and sperm-storage organs in mate-guarding crustaceans.

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Introduction

In most sexually reproducing species, females limit the reproductive rates of populations because of their greater parental investment in reproduction compared with males (Trivers 1972: Clutton-Brock & Vincent 1991). Female availability thus limits male fitness, and the spatio-temporal distribution of receptive females determines the success of behavioral strategies males use to obtain mates (Emlen & Oring 1977). Male mating strategies may therefore be viewed as adaptations to female reproductive physiology and behavior, with which they must coevolve (Rice 1996: Chapman & Partridge 1996). The sexual difference in reproductive rates typically produces competition among males, thus, male mating strategies are also influenced by the competitive environment.

Owing to sex differences in reproductive rates, mating strategies maximizing male and female fitness usually differ. Thus, when a mating strategy employed by one sex imposes fitness costs on members of the other, intersexual conflict arises. A typical conflict situation occurs when males attempt to mate and females resist these attempts (Parker 1979). Such conflicts of interest may be more the rule than the exception since polygyny, a common male strategy, often imposes costs for females (e.g. Parker & Thompson 1980: Lifjeld & Slagsvold 1991: Rowe et al. 1994: Clutton-Brock & Parker 1995: Eens & Pinxten 1995: Eggert & Sakaluk 1995: Kaitala & Wiklund 1995: Warner et al. 1995: Westneat & Sargent 1996).

Mate-guarding by males can lead to intersexual conflict if guarding by males reduces female fitness (Parker 1974, 1979: Jormalainen & Merilaita 1993, 1995: Rowe et al. 1994: Yamamura & Jormalainen 1996), as may occur when guarding limits feeding opportunities or increases the risk of predation for females (Strong 1973; Gwynne 1989: Jormalainen et al. 1994). In aquatic crustaceans, the evolution of precopulatory mate-guarding has been attributed to the time-limited receptivity for copulation during the female molt: monopolizing a female before receptivity is a competitive strategy by which males can ensure their presence when insemination can occur (Parker 1974: Grafen & Ridley 1983). The models analyzing precopulatory mate-guarding from the male perspective (Grafen & Ridley 1983: Yamamura 1987) predict that males should invest more time in guarding when there is more competition for females.

In this paper we explore species specificity, plasticity and the potential for intersexual conflict over the timing and duration of precopulatory mate-guarding in two species of *Thermosphaeroma* isopods (Sphaeromatidae, Dynameninae). Molecular phylogeny indicates that *Thermosphaeroma* is a monophyletic clade with marine ancestry, and that the species used in this study, *T. milleri* and *T. thermophilum*, are the two most ancestral species (Davis 1996). All *Thermosphaeroma* species described (n = 8) are or were endemic to geographically isolated thermal springs which appear to have imposed unique sets of selective pressures on their inhabitants since the late Cretaceous Period (Bowman 1981: Shuster 1981a,b: Jormalainen & Shuster 1997: Davis 1996). *T. milleri* (Bowman) inhabits a series of springs in north central Chihuahua, Mexico; *T. thermophilum* (Cole & Bane) inhabits a single spring near Socorro, NM, USA, and is considered

as an endangered species (Federal Register 1977). Both species are iteroparous and reproduce year-round (Shuster 1981a; V. Jormalainen & S. M. Shuster, unpubl. data). Moreover, earlier observations of precopulatory guarding in *T. thermophilum* suggest that sexual conflict over this guarding may exist (Shuster 1981b).

We begin by describing female reproductive cycles for both species. We identify the relative potential for intersexual conflict in the female reproductive cycle by documenting the degree to which male guarding attempts overlap with the period of ovarian maturation. Next, we measure guarding duration, and demonstrate its species specificity, as well as phenotypic plasticity with respect to sex ratio. In *T. thermophilum*, we test for the existence of intersexual conflict by manipulating the abilities of males and females to influence mate-guarding duration, and show that conflict exists.

Methods

Laboratory Rearing and Determination of Female Reproductive Cycle

Isopods were collected from their native springs in Jun. and Dec. 1995, placed in insulated containers and transported to Northern Arizona University, Flagstaff, AZ, USA. Experiments were conducted in aquaria filled with water from each species' native spring and equipped with aeration, heating (26–30°C) and water circulation with filtration. Males were identified by their possession of penes (Bowman 1981, 1985); females were identified by their possession of: 1. developing ovaries (pregravid); 2. marsupia containing embryos (gravid); or 3. empty marsupia (post gravid). Gravid females and most males were individually maintained in 55 cm^3 cylindrical containers equipped with removable net walls at both ends. The remaining specimens were maintained in mixed-sex groups in $16.0 \times 13.5 \times 12.5$ cm fine mesh containers from which embryo-bearing females were collected and placed into individual containers. All animals were fed with flaked fish food (a mixture of Tetramin, shrimp flake, and vegetarian fish food).

Animals were examined at least once weekly and gravid females were examined daily or every other day after parturition began. The ovarian condition of post gravid females, viewed ventrally, was noted immediately after parturition and with each successive examination was scored as one of three classes: 1a. no ovary (no ovary visible); 1b. some ovary (visible as a yellow mass dorsal to the hepato-pancreas, but not extending posteriorly beyond the fifth peraeonal segment); 1c. large ovary (visible as a yellow mass filling the anterior part of the body cavity and extending well below the hepatopancreas; at this stage distinct ova could be seen). Preliminary dissections revealed that when females were allowed access to males, females acquired sperm masses in each oviduct, observable as a whitish mass visible through the ventral cuticle and beneath genital openings located near the base of each fifth walking leg.

After parturition, which took place over 2–4 d, post-gravid females were used in the experiments described below. In these experiments females molted and became inseminated, and shortly afterward were transferred back to individual

containers where their fate was followed through oviposition and parturition. or death. The timing and duration of pre- and post-molt periods and incubation as well as timing of ovarian development and sperm storage for females of both *T. thermophilum* and *T. milleri* were constructed from these observations.

Manipulation of Operational Sex-ratio

We examined the effect of operational sex ratio. i.e. the number of sexually receptive females to males. on male guarding duration in both isopod species using two treatments. In the first treatment. two randomly chosen adult males and one post-gravid female were placed in fine mesh containers (12 × 12 × 8 cm for *T. thermophilum*: 16 × 14 × 13 cm for *T. milleri*). and submerged in aquaria. In the second treatment. two post-gravid females and one adult male were placed in similar containers. Females used in these experiments had just (within one to two days) completed their parturition in isolation. but were otherwise chosen randomly: males had been isolated in individual containers for at least 1 wk. Males in each triplet were individually marked 1 wk before experiments were begun by clipping the propodus and dactyl from either the left or right fifth peraeopod. *T. thermophilum* females were marked by clipping the exopod tip of either the left or right uropod. All individuals were chilled on crushed ice for 1–2 min before clipping. *T. milleri* females were identified by unique variations in their dorsal pigmentation patterns. All animals were measured (to the nearest 0.125 mm) and weighed (fresh weight to the nearest 0.1 mg) when each triplet was established. Containers were provided with food twice weekly.

We examined containers for evidence of molts and/or guarding pairs twice daily between 6 and 12 h and 18 and 24 h. Pairs were often sequestered beneath the gravel substrate and were identified with as little disturbance as possible. Isopod molts usually occur in two phases: the posterior half of the cuticle. below the 4th pereiopod. is shed first and the anterior half of the cuticle is shed next. Copulation can take place after the posterior part of the cuticle is shed (Shuster 1981b: Wilson 1991). The duration of precopulatory guarding was defined as the time between the start of *continuous guarding* (i.e. guarding that was observed during at least two successive inspections). and the shedding of the posterior cuticle by females at the sexual molt. Thus defined. 'guarding duration' identifies the moment in the female molt cycle when female becomes acceptable to males as guarding partner, i.e. it reflects the 'guarding criterion' of the males (cf. Grafen & Ridley 1983). However. 'guarding duration' is not exactly the same as the 'guarding criterion' because this concept does not consider female behavior and female resistance may prevent males pairing with her although she would otherwise be acceptable from the male viewpoint. The actual guarding duration was slightly longer when guarding continued until the molt of anterior cuticle and shorter if paired individuals changed partners (after the start of guarding. males sometimes usurped females from one another).

Manipulation of Male and Female Behavior

To study the existence and resolution of sexual conflict over the initiation of mate guarding in *T. thermophilum*. we established three groups of male–female

pairs: 1. a control group comprised of unmanipulated males and females: 2. a male manipulation group in which male guarding abilities were decreased by clipping the dactyls from the peraeopods 2–5 (males use all of their peraeopods, especially the distal dactyls, to hold females during mate-guarding and removal of these structures was assumed to be an effective way to decrease male guarding performance if the female resists guarding attempts): 3. a female manipulation group in which the female's ability to resist guarding attempts were decreased by anesthesia, i.e. by immersion in a 12% alcohol and spring water solution for 1 h daily until precopulatory guarding began. Anesthetization of females in this way was sufficient to immobilize females for a few hours with no negative effects on their survival: females appeared to fully recover before treatment the next day. Although females gradually recovered from anesthesia over a 24-h period and thus were not completely immobilized between treatments, the ability of anesthetized females to resist guarding appeared to be markedly reduced for hours. Osmotic stress and a muscular blocking agent, have previously been shown to decrease female's ability to resist in an isopod (Jormalainen & Merilaita 1995). While anesthetizing females in group 3, the females from the other two groups were separated from their partners for the same period in order to control for the possible effect of separating pairs.

As in previous experiments, all trials were conducted in $12 \times 12 \times 8$ cm mesh containers, all females used in these trials had been isolated in individual containers for the duration of their gravidity, and had completed parturition within 24 h before the start of each trial.

Results

Female Reproductive Cycles

Following parturition, females in both species underwent a premolt period which averaged 6.5 d (SD = 1.1, n = 66) in *T. thermophilum* and 15 d (SD = 3.4, n = 54) in *T. milleri* (Fig. 1: difference between species: ANOVA $F_{1,11} = 379$, p < 0.0001). After this interval, females of both species underwent the sexual molt (Fig. 1). Duration of the molt, i.e. the time during which the shedding of both the posterior and anterior parts of the cuticula were completed, was ≈ 12 h for *T. thermophilum* and 24 h for *T. milleri*. After the completion of the sexual molt, a post-molt period of about 1.5 wks (*T. thermophilum*) or 4 wks (*T. milleri*) followed (Fig. 1: ANOVA $F_{1,48} = 150$, P < 0.0001). During this period ovarian development was completed and sperm from copulation were stored within females (Fig. 1). Following ovarian maturation eggs were deposited into ventral brood pouches, where embryos developed, and from which juveniles (mancas) were released before a new molt cycle began (Fig. 1). As with all the other periods, the duration of incubation was longer in *T. milleri* (Fig. 1, ANOVA $F_{1,46} = 77.7$, P < 0.0001).

The female reproductive cycle differed between species in four ways. 1. Absolute cycle duration for *T. thermophilum* females (Fig. 1: mean 45–56 d, depending on the number of molts before oviposition) was about half the duration for *T.*
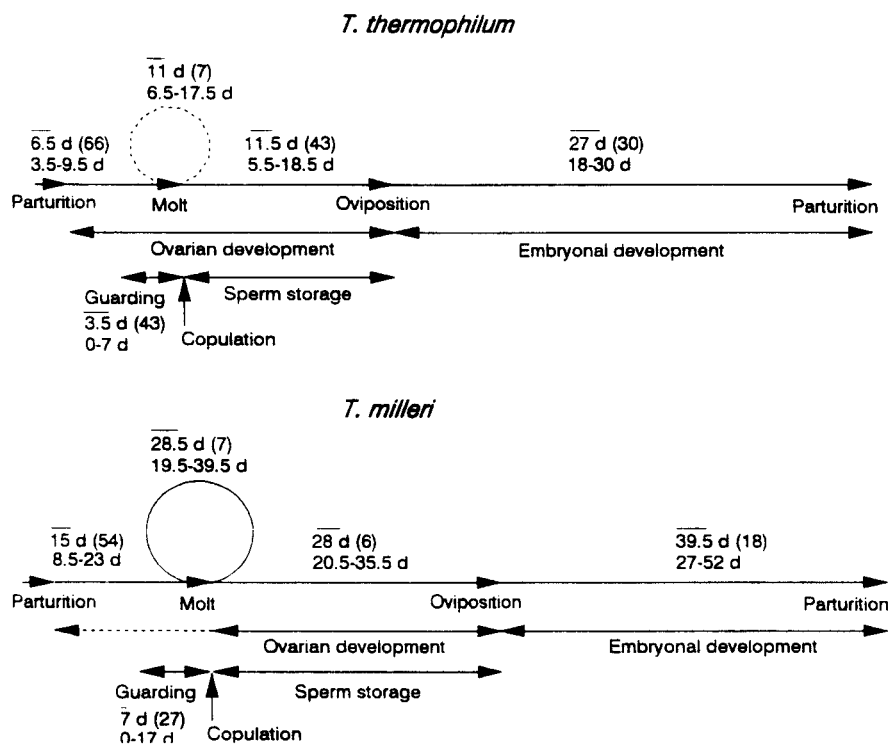
T. thermophilum

11 d (7)
6.5-17.5 d

6.5 d (66)   11.5 d (43)   27 d (30)
3.5-9.5 d   5.5-18.5 d   18-30 d

Parturition   Molt   Oviposition   Parturition

Ovarian development   Embryonal development

Guarding   Sperm storage

3.5 d (43)
0-7 d   Copulation

T. milleri

28.5 d (7)
19.5-39.5 d

15 d (54)   28 d (6)   39.5 d (18)
8.5-23 d   20.5-35.5 d   27-52 d

Parturition   Molt   Oviposition   Parturition

Ovarian development   Embryonal development

Guarding   Sperm storage

7 d (27)
0-17 d   Copulation

Fig. 1: The female reproductive cycle for *Thermosphaeroma thermophilum* and *Thermosphaeroma milleri*. The mean duration (in days), number of replicates in parentheses, and maximum and minimum values for various periods are given. The molt itself takes about 12 h in *T. thermophilum* and 24 h in *T. milleri*. The guarding duration is the mean of male- and female-biased sex ratios from the experiment summarized in Fig. 2

milleri females (Fig. 1: mean 82.5–111 days). 2. The occurrence of two molts between parturition and oviposition was common in *T. milleri*, but rare in *T. thermophilum*. Few females in *T. thermophilum* (seven out of 50) and about half the females in *T. milleri* underwent a second molt after the first post-molt period instead of oviposition (seven out of 13; difference between the species in the frequency of secondary molts: $G = 8.30$, $df = 1$, $p < 0.01$). In *T. milleri*, females that underwent a secondary molt instead of ovipositing were smaller than those who proceeded directly to oviposition (fresh weight (mg), mean $\pm$ SD: 18.1 ± 4.7, $n = 7$ and 26.8 ± 7.5, $n = 5$, respectively; ANOVA $F_{1,10} = 6.17$; $p < 0.05$). We found no such size difference in *T. thermophilum* (mean $\pm$ SD: 5.31 ± 0.8, $n = 7$ and 6.01 ± 1.4, $n = 44$, respectively; ANOVA: $F_{1,49} = 1.81$, ns). 3. The timing of ovarian development differed between species: ovarian development began earlier in *T. thermophilum*, since ovaries in an early developmental stage (1b: see above) were visible immediately after parturition, whereas in *T. milleri*, ovaries were not visible

before the sexual molt (Fig. 1). Female *T. milleri* undergoing secondary molts had some ovary visible after the second molt. 4. The relative duration of the period between the sexual molt and oviposition of the whole cycle was longer in *T. milleri*, thus providing more time for ovarian maturation after the sexual molt compared with *T. thermophilum* (Fig. 1: mean $\pm$ SD: 34.2 $\pm$ 6.98%, n = 6, and 25.9 $\pm$ 5.84%, n = 44, respectively; ANOVA $F_{1,48}$ = 10.2, p < 0.01).

Effect of Operational Sex-ratio on Guarding Duration

Within species, there was no significant effect of sex ratio on the latency to begin guarding (i.e. the number of days between the start of the experiment and the appearance of guarding pairs. ($\bar{x}$ $\pm$ SD: *T. thermophilum* 2.79 $\pm$ 1.97 vs. 2.77 $\pm$ 1.63, $F_{1,46}$ = 0.00, ns; *T. milleri* 7.46 $\pm$ 4.45 vs. 7.03 $\pm$ 2.12, two-way ANOVA: $F_{1,28}$ = 0.12, ns). Before continuous guarding males in both species often attempted to guard females. Once continuous guarding began, the duration of precopulatory guarding was nearly twice as long in *T. milleri* as in *T. thermophilum* (Fig. 2: two-way ANOVA $F_{1,62}$ = 30.3, p < 0.001) and in both the species, guarding duration was influenced by sex ratio. Males in both species guarded females longer in the presence of another male than they did when two females and no other males were present (Fig. 2: two-way ANOVA $F_{1,62}$ = 12.2, p < 0.001; species × sex–ratio interaction: $F_{1,62}$ = 2.97, ns). Furthermore, comparison of guarding duration for the 1:2 and 2:1 female to male groups (Fig. 2) with guarding duration for a 1:1 female to male control group of the sexual conflict experiment (see below; Fig. 3) in *T. thermophilum* showed that males guarded females longer under male-biased sex ratios than under even or female-biased sex ratios (contrasts after significant one-way ANOVA 1:2 vs. 1:1 $F_{1,52}$ = 13.53, p < 0.001; 2:1 vs. 1:1 $F_{1,52}$ = 2.44, ns). Thus, increased guarding duration was a specific response to the number of males present.
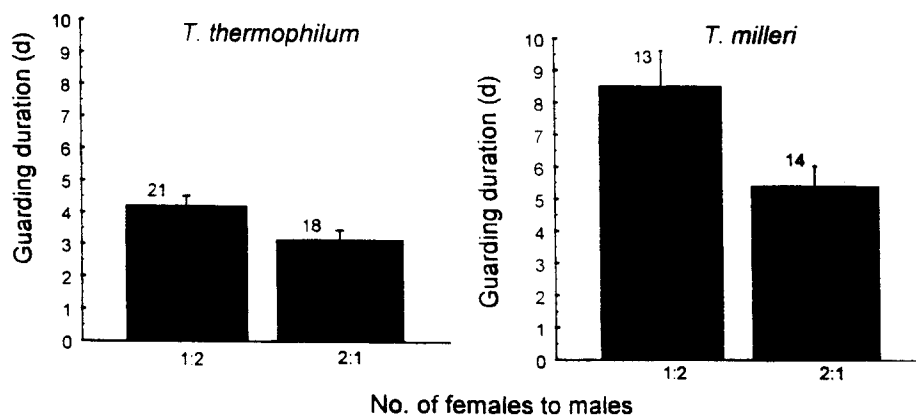

Fig. 2: Guarding duration (mean and SE, in days) for *Thermosphaeroma thermophilum* and *Thermosphaeroma milleri* in 1:2 and 2:1 female to male sex ratios. See text for the definition for guarding duration. The number of replicates is given on top of each bar
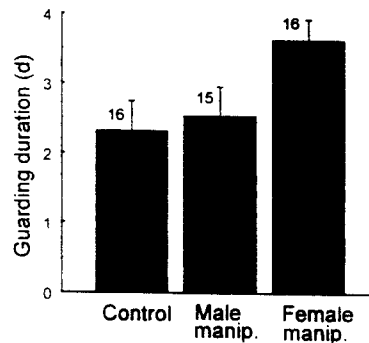
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Fig. 3: Guarding duration (mean and SE. in days) of male–female pairs in the control, male manipulation and female manipulation groups of the sexual conflict experiment in *Thermosphaeroma thermophilum*

Under female-biased sex ratios. males in both species tended to initiate pre-copula first with the more mature female. i.e. the individual in each pair of females with less time remaining before her sexual molt (*T. milleri* 10 out of 13. df = 1, G = 3.98. p < 0.05; *T. thermophilum* nine out of 14. df = 1. G = 1.16. ns). No preference for female size was found (*T. thermophilum*. larger female selected in nine out of 19 cases. df = 1. G = 0.053. ns; *T. milleri*. larger female selected in four out of 14 cases. df = 1. G = 2.66. ns).

In male-biased triplets. the male that initiated guarding first was the larger of the two males in *T. thermophilum* (16 out of 22. df = 1. G = 4.72, p < 0.05) but not in *T. milleri* (eight out of 13. df = 1. G = 0.70. ns). However. females were guarded by both males in eight out of 22 of trials in *T. thermophilum* and in six of 13 trials in *T. milleri*. suggesting that usurpation of guarded females by other males may be common. Such takeovers have been observed previously in the field (Shuster 1981b). as well as in laboratory stock containers. Exchanges of male partners occurred in both directions with respect to male body size in both species. However, the male partner present at the female's sexual molt was more often the larger male (*T. thermophilum*. 14 out of 22. df = 1. G = 1.66. ns; *T. milleri*. 10 out of 13. df = 1, G = 3.98. P < 0.05). Larger males started guarding females earlier than smaller males in *T. milleri* (male weight–guarding duration correlation: r = 0.46. p < 0.05, n = 27). but no such relationship was found in *T. thermophilum* (r = 0.10. ns, n = 38).

In *T. thermophilum*, guarding most often continued until females had shed both the posterior and anterior cuticle (30 cases of 34). In *T. milleri*. males ceased guarding with equal frequency after the posterior (seven cases of 18) and anterior (11 cases of 18) cuticles were shed. This difference between the species in timing the end of guarding either before or after anterior molt was statistically significant (G = 4.97. df = 1. p < 0.05).

Sexual Conflict

Statistically significant differences in guarding duration between the three treatments were found in *T. thermophilum* in the sexual conflict experiment (ANOVA $F_{2,44} = 3.25$, p < 0.05): Experimentally manipulated (clipped) males did not differ from the control (Fig. 3; ANOVA, male-manipulation vs. control contrast $F_{1,44} = 0.16$, ns). However, in the group in which the females' ability to resist guarding attempts was experimentally reduced, guarding was, on average, 57% longer than in the control group (Fig. 3; ANOVA, female-manipulation vs. control contrast $F_{1,44} = 5.70$, p < 0.05). There were no differences between the groups in the total duration of the experiment (time between the start of the experiment and posterior molt of the female mean ± SD: control 5.09 ± 1.64, n = 16; male manipulation 4.93 ± 1.58, n = 15; female manipulation: 4.59 ± 1.38, n = 16); thus, female manipulation had no effect on the molting rate of females. No correlations of male or female size on guarding duration were found in any of the treatment groups. These results show: 1. that males aim for a longer guarding duration than they normally obtain; and 2. that females, by their behavior, can effectively shorten the guarding duration.

Discussion

Female Reproductive Cycles and Timing of Key Events

Molt cycle characteristics of *T. milleri* and *T. thermophilum* differed in several respects, despite their close evolutionary relationship (Davis 1996). The total cycle length in *T. milleri* females was twice that of *T. thermophilum*, perhaps owing to the larger average size of *T. milleri* individuals (S. Shuster & G. Davis, unpubl. data). The timing of reproductive allocation also differed between the species: ovarian development in *T. milleri* occurred mainly after the sexual molt, whereas ovarian development in *T. thermophilum* preceded and overlapped with this event. Half of all *T. milleri* females, particularly small individuals, failed to oviposit after insemination and instead underwent a secondary molt, while in *T. thermophilum*, secondary molts were uncommon and unrelated to female size. This difference implies a differential allocation of energy to reproduction and growth between these species and *T. thermophilum*, in particular, appears to have accelerated its reproductive cycle relative to *T. milleri*.

Opportunities for fertilization are discrete in time in both species and guarding precedes copulation. In most isopods, copulation takes place at the molt (Manning 1975; Shuster 1981b; Ridley 1983; Wilson 1991) and fertilization, together with oviposition, follows immediately (e.g. in *Asellus aquaticus*, Asellota, Manning 1975, 1980; *Idotea baltica*, Valvifera, Borowsky 1987; *Paracerceis sculpta*, Shuster 1989). However, *Thermosphaeroma* females retain sperm masses in their reproductive tract between copulation and oviposition (Jormalainen et al. 1998), events that are separated by several days to several weeks in these species.

Clearly, sperm storage allows females to experience molting and oviposition, as well as copulation and fertilization, as separate events (Birkhead & Møller 1993;

Alexander et al. 1997). Separating molting from oviposition may be beneficial if the costs of molting process interfere with ovary provisioning. However, females who prolong the interval between sexual receptivity for copulation and oviposition, and delay ovarian development until after mating is complete. may also avoid costs of mate guarding during ovary provisioning. Guarding is likely to be costly in *Thermosphaeroma* since guarding males sequester themselves. while holding their potential mates. beneath the habitat substrate for several days before mating occurs, thereby preventing females from free foraging (Shuster 1981a).

Duration of Precopulatory Guarding

The ancestral phylogenetic position of *T. milleri* relative to *T. thermophilum*, together with the species-specific length of female reproductive cycles, provide a test of a general assumption of theoretical models of mate guarding (Grafen & Ridley 1983; Yamamura 1987; Yamamura & Jormalainen 1996): Evolutionarily stable guarding criteria (sensu Grafen & Ridley 1983) are expected to be proportional to the length of female reproductive cycles. an expectation that is met in these *Thermosphaeroma* species. Although the duration of mate-guarding for *T. milleri* was about twice as long as that observed for *T. thermophilum*, in both species males guarded females for about 50% of the premolt period and for less than 10% of the entire cycle. This suggests that male mate-guarding criteria in *Thermosphaeroma* have evolved primarily in response to patterns of female molt cycles.

Certain details of mate guarding in these species were also consistent with the predictions from the theoretical models concerning the effects of male–male competition (Parker 1970; Grafen & Ridley 1983; Yamamura 1987). First, half of all *T. milleri* males terminated their bouts of guarding before females had shed the anterior half of their cuticle, i.e. before the sexual molt was complete, whereas *T. thermophilum* males routinely guarded their mates until females had completed their sexual molt. Since copulation is possible as soon as the posterior cuticle is shed and until after the anterior cuticle is shed (Wilson 1991), *T. thermophilum* males appear to engage in precopulatory as well as short postcopulatory guarding. This is an expected response to high male densities and the threat of sperm competition. In *T. thermophilum* sex ratios (males:females) vary between 1.5 and 5.5 while the densities may reach 210 individuals/100 cm² (Shuster 1981a), which are much higher than observed for *T. milleri* (V. Jormalainen & S. Shuster. pers. obs.).

Second, males in both species adjusted their guarding criterion (i.e. they started guarding at different points of the female premolt cycle) depending on local sex ratios. This interpretation follows from the combined results: 1. that guarding duration was longer under male-biased than under female-biased sex ratios; and 2. that latency to begin mate-guarding did not differ between the sex ratio treatments. Under female-biased sex ratios. males could decide which one of the two available females to guard as well as when to attempt to start guarding. Thus, shorter guarding durations under female-biased sex ratios follow from male decisions to

guard the one of the two females that was closer to her sexual molt. and to start guarding her later in her reproductive cycle than in the male-biased sex ratio. If males only had chosen the more mature female. without adjustment in guarding criterion, we would not expect differences in guarding duration between sex ratios. but instead, shorter latency in female-biased sex ratio. Conversely, if longer guarding durations resulted from female responses to a higher level of harassment under male-biased sex ratios. we would expect shorter latency to guarding in this treatment. Furthermore. we showed in *T. thermophilum* that the increase in guarding duration was a specific response to the number of males. while the number of females, as such. had no effect on guarding duration.

Models of precopulatory mate-guarding predict increasing guarding duration with increasing male bias in sex ratio (Grafen & Ridley 1983; Yamamura 1987). However, these evolutionary models do not explicitly analyze behavioral plasticity. Our experiment showed that males are evidently able to evaluate both the female reproductive state and the competitive environment and. to some degree. adjust their guarding criterion in response to that information.

Sexual Conflict over the Timing and Duration of Guarding

In *Thermosphaeroma*. especially in *T. thermophilum*. the overlap of guarding periods with ovarian development generates the potential for intersexual conflict over guarding duration. because guarding appears to interfere with female feeding and provisioning of developing ovaries. Increased guarding duration among pairs with anesthetized females in *T. thermophilum* demonstrates that intersexual conflict exists: males aim for longer guarding periods than they can achieve with unanesthetized females. *Thermosphaeroma* females. like other sphaeromatids. can conglobate (i.e. assume a spheroid shape), thereby making themselves difficult to hold. as well as impossible to copulate with, and were often seen escaping from males.

Manipulation of males' ability to hold females had no effect on guarding duration. Thus. the duration of guarding observed in the male manipulation and the control groups appears to represent the guarding duration that females allow. i.e. the resolution of sexual conflict in favor of female interests.

This interpretation may seem contrary to our observation that males adjusted guarding duration according to the competitive environment. However. behavioral conflict and its resolution can be viewed as dynamic processes in which both sexes evaluate potential conflict costs. as well as the benefits of winning the contest. and adjust the levels of persistence and resistance accordingly (Yamamura & Jormalainen 1996). The longer guarding in male-biased sex ratios would then indicate that males put more effort in monopolizing a female when competitors are present. Since the natural sex-ratio of *T. thermophilum* remains highly male-biased throughout the year (Shuster 1981a). we suspect that conflict may be even more pronounced in nature.

In conclusion. the duration of precopulatory guarding in *Thermosphaeroma* isopods is affected by several factors. First. guarding duration appears to be primarily adapted to the length of female reproductive cycle. Second. guarding

duration shows behavioral plasticity: males evidently can evaluate the relative
intensity of male–male competition and adjust their guarding criterion accordingly.
Third. male and female interest on guarding duration differ as males aim for longer
guarding durations than females allow. Such sexual conflicts may arise if mate-
guarding overlaps with the period of provisioning developing ovaries. as occurs in
T. thermophilum. Mate guarding costs to females could favor delayed oviposition
so that ovarian development can take place after males have completed their
guarding and mating attempts. as occurs in *T. milleri*. To accomplish this. females
must temporally separate insemination and oviposition. which may favor the
females' ability to store sperm. as occurs in all known *Thermosphaeroma* species
(S. M. Shuster. pers. obs.). Sexual conflicts have been suggested to fuel coevol-
utionary arms races between the sexes. and therefore to accelerate evolution of
traits related to reproduction (Chapman & Partridge 1996: Rice & Holland 1997;
Alexander et al. 1997). Thus. we hypothesize that sexual conflict and selection
favoring females who can avoid it may play an important role in the evolution of
delayed oviposition and sperm-storage organs.

Acknowledgements

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