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Chemical communication during mating of the harpacticoid *Tigriopus japonicus*

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The importance of contact and diffusible pheromones in the reproductive biology of the harpacticoid *Tigriopus japonicus* was studied. When given a choice, males preferred developmentally advanced conspecific female partners over less mature or congeneric females. Males judged female attractiveness on a relative scale, based on the locally available females. The attractiveness of a female copepodid was reduced with non-fatal proteolytic treatment, but only if normal females were also present. To sample the available females, males repeatedly grabbed the caudal rami and terminal urosome segment of potential partners before committing themselves to guarding one female. Males occasionally dropped their copepodid partners. Releases increased in frequency in water conditioned from virgin adult females and adult males, decreased in mated-female conditioned water and were unaffected by copepodids or their treated water. The waning attractiveness of a recently mated female was tracked over 16 hours. Relationships between *Tigriopus japonicus* adults appeared to involve both contact and diffusible pheromones. No evidence of a diffusible copepodid pheromone was uncovered.

Keywords: copepod; mate guarding; contact chemoreception; mate recognition; surface glycoproteins

1. INTRODUCTION

Chemical communication may influence many facets of a zooplankter's existence, including swarming, diel vertical migration, changes in life history traits, morphology, and avoidance or pursuit of predators, prey, and mates (Larsson & Dodson 1993). Members of the harpacticoid copepod genus *Tigriopus* use chemical signals in intergenerational and intersexual interactions. Ovigerous *T. japonicus* females at high densities of 40 animals ml^{-1} delay the hatching of their attached eggs (Kahan *et al.* 1988). Eggs removed from the female commence hatching immediately, even if the unharmed mother is still present. These data suggest that an inhibitory substance is passed from the female genitalia to the egg sac. Once hatched, first stage *Tigriopus nauplii* are susceptible to cannibalism by ovigerous females (Lazzaretto *et al.* 1990), although not by their own mothers (Lazzaretto & Salvato 1992). Kin recognition is probably mediated by a chemical compound present in the developing embryos. After maturation, virgin adult females release a diffusible mate-location pheromone (Lazzaretto *et al.* 1990) that attracts males and is species-specific, varying among different populations (Lazzaretto *et al.* 1994).

Evidence for diffusible mate-location pheromones is common across the Copepoda (Lonsdale *et al.* 1998). Soluble compounds are thought responsible for mate-seeking behaviour in some calanoids (see, for example, Katona 1973; Griffiths & Frost 1976; Jacoby & Youngbluth 1983; Chow-Fraser & Maly 1988), cyclopoids (Uchima & Murano 1988), harpacticoids (Lazzaretto *et al.* 1990, 1994) and a siphonostomatoid (Ritchie *et al.* 1996). Although attraction occurs from a distance, recognition of the developmental or reproductive state of the female often occurs upon contact. Watras (1983) found that *Diaptomus leptopus* males pursued all conspecifics equally, but would select gravid females and reject non-gravid females after handling. Antennular inspection of a female's caudal rami and urosome precedes spermatophore transfer by male *Labidocera aestiva* (Blades & Youngbluth 1980). The parasite *Lepeophtheirus salmonis* manipulates copepodids before establishing precopula with those closer to maturation (Ritchie *et al.* 1996). Surface chemicals probably comprise at least one element of these recognition systems.

Although a contact sex pheromone has yet to be purified from a crustacean, recognition molecules from other marine species have been characterized. Surface glycoproteins emerge as a common recognition mechanism in such diverse fertilization systems as sea urchins, bivalve gametes (Keller & Vacquier 1994; Focarelli & Rosati 1995), and a rotifer (Snell *et al.* 1995). Surface glycoproteins have been found on representatives of the three free-living orders of copepods (Snell & Carmona 1994), including *Tigriopus japonicus* (Kelly & Snell 1998). Glycoprotein distribution was correlated with structures important to that sex or developmental stage. Adult females from all species tested showed the densest glycoprotein concentrations at the urosome, where sexual attention from males is usually focused. Adult male *T. japonicus* displayed high glycoprotein density and diversity on their antennules, which are used in manipulating females for copulation or mate guarding. Juvenile female *T. japonicus* exhibited glycoproteins at the prosome and the urosome, both of which are contacted by males initiating precopula (Kelly & Snell 1998).

Table 1. *Abbreviations for experimental animals or conditions*

(All animals are *Tigriopus japonicus* unless otherwise noted.)

abbreviation	definition
M	adult male removed from precopula with a third stage copepodid
CII, CIII, CV	female second, third, or fifth stage copepodid removed from precopula
vCVI	virgin adult female; fifth stage copepodids were removed from precopula and allowed 3 d to mature
nvCVI	non-virgin adult female; females carrying eggs about to hatch (red egg sacs) were isolated overnight until the eggs hatched
M-CII	intact pair consisting of a male guarding a second stage female copepodid
CIIIC	female third stage *Tigriopus californicus* copepodid removed from precopula with conspecific male
$M{\cdot}H_2O$, $CII{\cdot}H_2O$, $CV{\cdot}H_2O$, $vCVI{\cdot}H_2O$, $nvCVI{\cdot}H_2O$	conditioned water; animals of the specified class placed at a density of 20 animals ml^{-1} for at least 4 h

Evidence of the functional significance of these molecules comes from behavioural assays in which surface glycoproteins are masked by incubation with complementary carbohydrate-binding proteins (lectins). Female *Coullana canadensis* (Harpacticoida) treated with lectins were less likely to be found in precopula (Lonsdale *et al.* 1996). Male *T. japonicus* mate-guarding behaviour was inhibited after treatment with the lectin from *Triticum vulgaris*, which suggests female copepodids carry a glycoprotein, containing glucosamine or N-acetyl glucosamine residues, which promotes mate guarding and may announce her developmental stage (Kelly & Snell 1998).

Male discrimination of the female developmental stage is necessary if males are to maximize their reproductive fitness. Time spent in precopula can vary from 1 to 15 days depending on the developmental state of the female (Lazzaretto *et al.* 1994). Mate guarding precludes copulation, but free-swimming males can fertilize multiple females over several days (Burton 1985). The opportunity costs of missed matings would be reduced if females could be chosen that were closer to their terminal moult. *Tigriopus californicus* males are found most often clasped to fifth-stage copepodids, and will abandon younger copepodids for more mature ones (Burton 1985).

The aim of the present study was to test the ability of male *Tigriopus japonicus* to maximize their reproductive fitness by selecting conspecific partners close to maturation, and investigate the cues used in choosing or changing partners, especially the importance of contact and diffusible pheromones in copepodids, virgin adult females and mated females.

2. MATERIALS AND METHODS

(a) *Experimental conditions and subjects*

All experiments were done at 25 °C in 15 p.p.t. artificial sea-water (Instant Ocean salts) unless otherwise noted. *Tigriopus japonicus* was cultured under these conditions. *Tigriopus californicus* was cultured in 35 p.p.t. artificial seawater at 20 °C; animals were removed from culture and allowed to acclimatize to experimental conditions overnight (12–16 h) before use. Frequency of precopula among *T. californicus* is unaltered by the overnight acclimatization. The percentage of *T. japonicus* males making a choice to guard or mate a normal female is relatively uniform, with 84±9% (mean±s.d.) of males from all experiments selecting a partner. Both species were fed a mixed algal diet of *Tetraselmis suecica*, *Cryptomonas* sp., and *Isochrysis galbana* twice a week. Abbreviations and descriptions of experimental animals and conditions are given in table 1.

(b) *Male preference experiments*

To ascertain if male *T. japonicus* preferentially choose conspecific or more developmentally advanced females, we conducted male preference experiments in which 20 or 40 males were individually placed with two females in 1 ml of seawater in a 48-well tissue culture plate. The females were either two CIII, two CIIIC, CIII+CIIIC, CIII+CVI, or CIII+vCVI (see table 1). Observations on frequency of precopula (male grasping female prosome with both antennules) or copulation (ventral–ventral abdominal contact with male urosomal vibration) were made after 2 h for the species choice test, or every 5 min for 1 h for developmental stage tests. vCVIs were observed continuously and scored as mated for the time-period in which copulation was initiated.

We examined more closely the means of male mate-choice by observing 30 males, each with two females (CIII+CIII, CIII+CV, or CIII+vCVI) under a dissecting microscope (20×). The number of times each female was grabbed (male antennular contact with the urosome, often with a visible pause), and the latency to guarding or copulating were quantified. Only those males making a choice within 10 min were used in the analysis. In the small volumes dictated by microscope observation, if a behaviour is not seen in 10 min, it is unlikely to be observed under longer conditions.

(c) *Chemical disruption of precopulatory signals*

To elucidate the chemical nature of the precopulatory signals, male *T. japonicus* were presented with a choice between two females (two untreated control females, two chemically treated females, or one treated and one control

Table 2. *Enzyme and detergent treatments on CIII females*
(U are units of enzyme activity defined by the manufacturer.)

treatment	activity	control	experimental conditions
10 U Pronase E	general protease	seawater	30 °C, 1 h
40 U Chymotrypsin	endoprotease at aromatic residues	seawater	30 °C, 1 h
2 U PNGase F	removes N-linked oligosaccharides from glycoproteins	2.5% enzyme buffer in seawater (buffer = 100 mM phosphate, 5 mM NaN_3, 25 mM EDTA, 50% glycerol)	30 °C, 16 h
2% n-octyl β-glucopyranoside	non-denaturing detergent	seawater	25 °C, 90 s

female) in a male preference experiment described above. All chemicals were purchased from Sigma Chemical Company, and treatments are described in table 2. The guarding decisions of 80 males were quantified after 2 h for each treatment.

(d) ***Male–male bioassay***

The effect of female-conditioned water from CV, vCVI, and nvCVI females on the grabbing tendency of males was investigated by observing two males in 250 µl of the treated water at 20 × magnification. The first male to grab the urosome of the other was watched for 10 min, and the number of subsequent grabs was counted. A total of ten males were observed for each treatment.

(e) ***Stability of M-CII pairs***

To determine the propensity of males to drop a current partner in the presence of other animals or their conditioned water, five M-CII pairs were placed in 250 µl of seawater with five animals of various sex–age classes, or their conditioned water. After 2 h, the number of intact pairs, new pairs or mated females was counted, and females were examined under 100 × magnification for spermatophore transfer. The experiment was repeated 7–10 times for each treatment, and the results for each treatment were pooled and compared with a seawater control with a *G*-test contingency analysis. One replicate from each treatment was video-taped, to gain an insight into events which might precipitate the release of a CII.

(f) ***Attractiveness of nvCVI***

The attractiveness of nvCVI females for further copulation attempts was explored in this experiment. One male and one vCVI female were observed in 250 µl of seawater under 20 × magnification. To ensure the female was initially attractive, the pair was discarded if copulation was not achieved in 10 min. Spermatophore transfer was verified by observation under 100 × magnification. Because copulation was inhibited under bright light, light was filtered to remove wavelengths below 600 nm. The females were placed individually in 1 ml of seawater until remated with a fresh male in 15 min, or 2, 4, 8, or 16 h. Before remating, the condition of the previously transferred spermatophore was reassessed. If the second male did not attempt to copulate within 10 min, he was removed and another male was introduced for 10 min. At the end of the experiment, the female was again checked for transferred spermatophores. In addition to copulation attempts and spermatophore transfers, we recorded the number of male grabs, the latency to a copulation attempt, and the time spent in copulation from the first ventral–ventral contact to release.

(g) ***Neutral red dye***

For experiments requiring the discrimination of morphologically identical animals, half of the animals of each treatment were dyed in 0.01 mg ml^{-1} of Neutral Red dye for 1 h (Anstensrud 1989). Because males showed a statistical bias ($p < 0.05$ for a *G*-test contingency analysis) towards either dyed or undyed animals (one instance each), two replicates of the PNGase disruption experiment were discarded.

(h) ***Statistics***

The *G*-test contingency analysis was used to analyse the distribution of males performing a behaviour with different-type females, whereas a one-way ANOVA was used to detect the effect of treatments on a continuous variable, such as the number of male grabs, or latency to copulation (Sokal & Rohlf 1995).

3. RESULTS

When presented with either *T. japonicus* or *T. californicus* copepodids, male *T. japonicus* found them to be equally attractive ($G = 0.630$, $p = 0.4272$) (see figure 1). However, males will preferentially choose conspecific females above heterospecifics when given a choice ($G = 5.277$, $p = 0.0216$). Males were also found to distinguish among conspecific females, preferring more developmentally advanced partners (figure 2). At the end of 1 h with a CIII and a CV copepodid, 9 out of 40 males were guarding CIII females compared with 24 out of 40 males guarding CV females ($G = 11.947$, $p = 0.0005$). Most of the pairs formed were stable to the end of the observation period. Only six males dropped their partners, with three dropping a CIII and three dropping a CV. Males also choose vCVI over CIII copepodids ($G = 8.906$, $p = 0.0028$) as their initial partner (figure 2*b*). About 39% of the recently mated males went on to guard the younger female, whereas around 22% of the mated males recopulated with the same female.

Males were observed individually to reveal more about the nature of their choice. When faced with two CIII

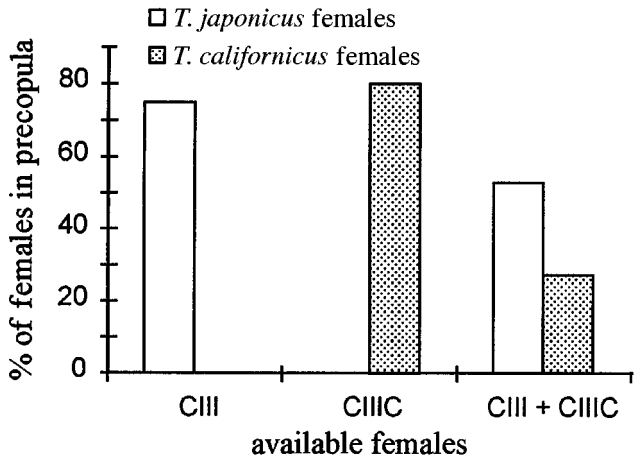

Figure 1. Ability of *T. japonicus* males to recognize conspecific females. The percentage of *T. japonicus* and *T. californicus* females taken into precopula is given for males presented with two females of each species separately, or together. Female abbreviations are given in table 1. Males preferred conspecifics when available ($G=5.277$, $p=0.0216$).

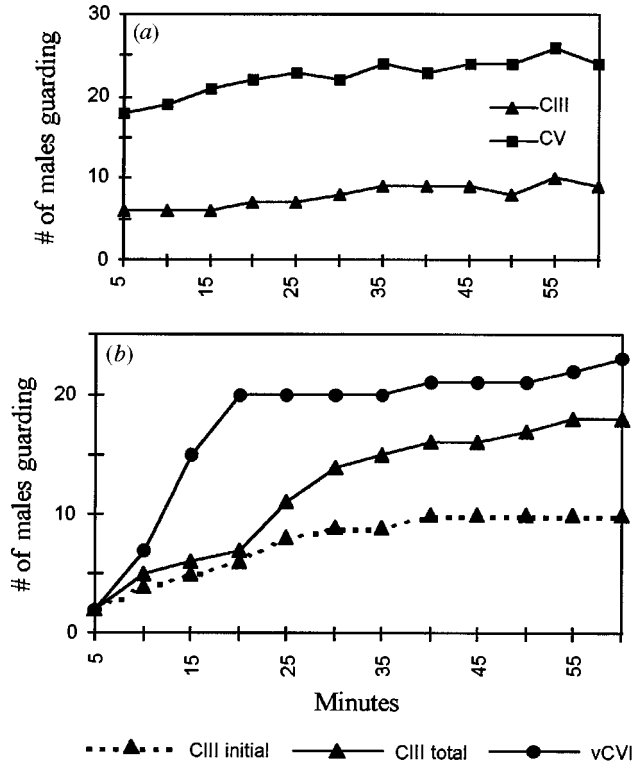

Figure 2. Ability of *T. japonicus* males to recognize more mature females. A total of 40 males were presented with two females of different developmental stages, and the number of males guarding each copepodid stages (or copulating with an adult) is given in 5 min increments for 1 h. (*a*) CIII and CV females. (*b*) CIII and vCVI females. Because males can mate with a vCVI and then guard a CIII, both the number of males choosing to guard CIIIs initially and the total number of males guarding CIIIs are given.

females, the male appeared to choose at random, usually after investigating each female by grabbing her on the caudal rami or urosome (figure 3*a*). Males continued to investigate CIII females in the presence of CV or vCVI females. Comparisons of the number of male grabs to the

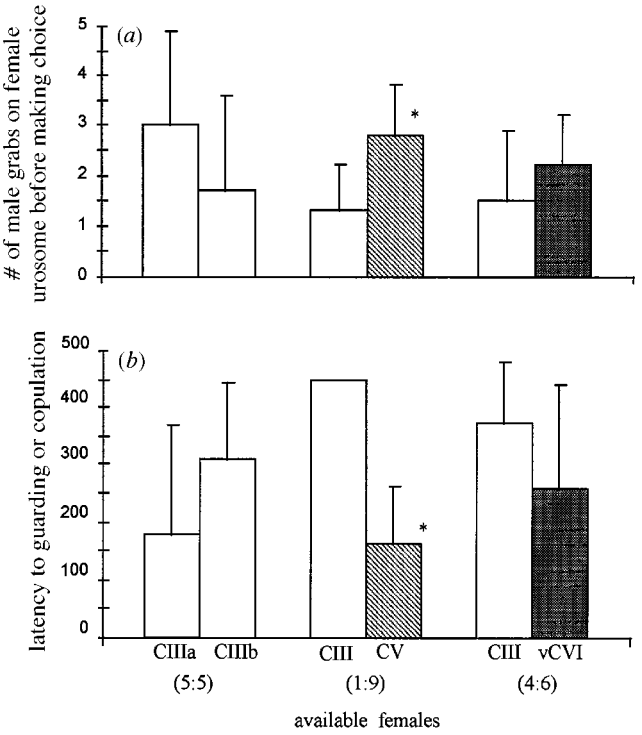

Figure 3. Effect of female developmental stage on mate-selection behaviour of males. The number of males choosing each stage is given as a ratio beneath the bar. An asterisk indicates a significant ($p<0.05$ with an ANOVA) discrepancy between the two stages. (*a*) Number of antennule grabs on each female urosome. (*b*) Latency to mate guarding (CIII, CV) or copulation (vCVI).

three stages of females revealed that CV females were explored more often than CIII females ($G=11.441$, $p=0.0053$), but vCVI females were not ($G=1.690$, $p=0.210$). Similarly, males were quicker to guard CV females than CIII females ($G=4.543$, $p=0.0445$), but displayed no increased interest in selecting vCVI females ($G=0.169$, $p=0.6859$). Overall, males appeared to grab the caudal rami or urosome of females with their antennules as a means of gauging female developmental stage, and tested more than one female before committing themselves to guarding or mating.

If chemical signals on the body surface of females are important in this discrimination, they could be disrupted by enzyme degradation. Although females subjected to the general protease Pronase E or the endoprotease Chymotrypsin are equally attractive when presented separately, they are discriminated against when males are given a choice between treated and untreated females (figure 4) ($G=19.547$, $p<0.0001$ for Pronase E; $G=6.166$, $p=0.0130$ for chymotrypsin). Males did not distinguish untreated females from those treated with PNGase F or n-octyl β-glucopyranoside.

In addition to investigating contact chemoreception, the existence of a diffusible sexual signal was also assayed. The potency of female-conditioned water in altering male behaviour was dependent upon the condition of the female (figure 5). vCVI·H_2O elicited increased grabbing behaviour over CV·H_2O, nvCVI·H_2O, and a seawater control. vCVI·H_2O was not sufficient to induce male–male guarding or copulation.

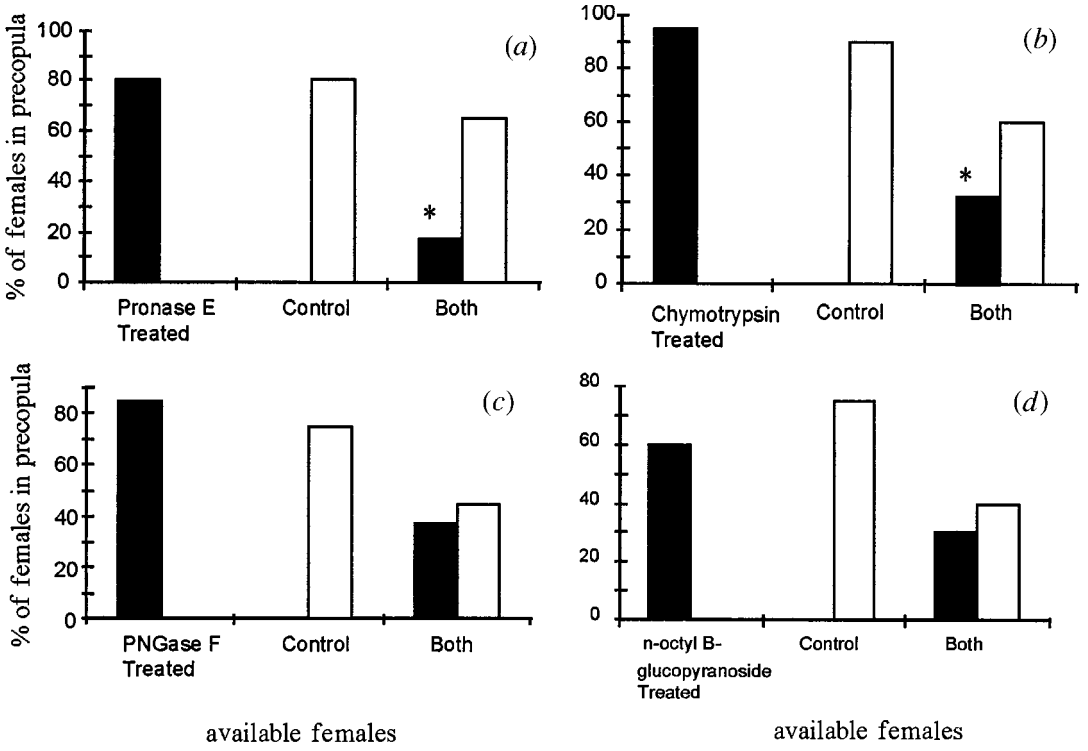

Figure 4. Effect of enzymes and a detergent on attractiveness of *T. japonicus* CIII females as precopula partners. The per cent of CIII females taken into precopula is given for males presented with two chemically treated females, two untreated females, or one of each type. An asterisk indicates the treated female is chosen significantly ($p < 0.05$ in a *G*-test contingency analysis) less often than the control. Females treated with (*a*) Pronase E; (*b*) Chymotrypsin; (*c*) PNGase F; (*d*) n-octyl β-glucopyranoside.

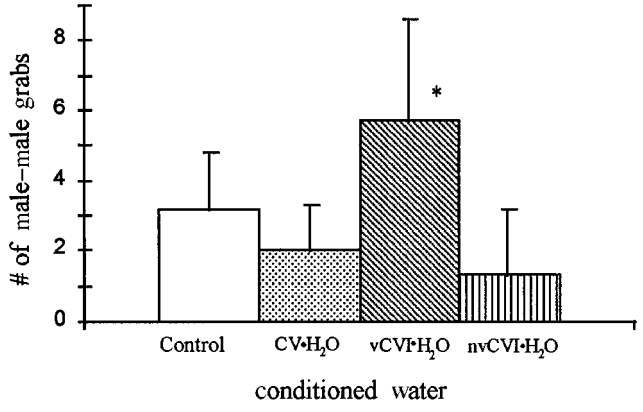

Figure 5. Effect of *T. japonicus* female conditioned water on male–male interactions. The number of antennular grabs by one male on another's urosome is given for males placed in pairs in water conditioned by females of three separate life stages. An asterisk indicates significantly ($p < 0.05$ with ANOVA) enhanced activity over the seawater control. Abbreviations are given in table 1.

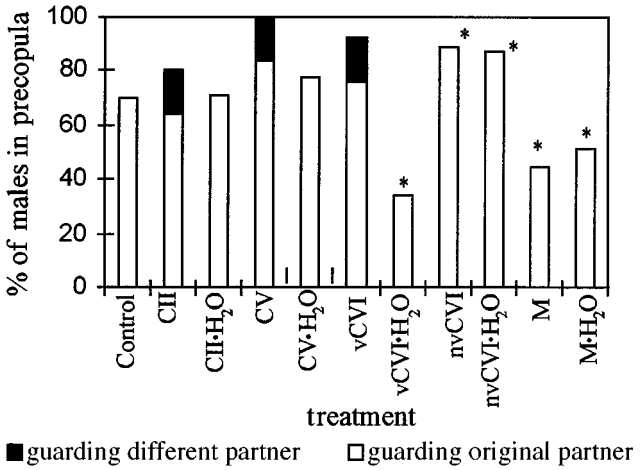

Figure 6. Influence of sex and developmental stage on disruption of M-CII pairs in precopula. The per cent of males maintaining or changing partnerships after incubation for 2 h with females of different developmental or reproductive states, adult males, or their treated water is given. An asterisk indicates significant ($p < 0.05$ in a *G*-test contingency analysis) effect from control. Abbreviations are given in table 1.

Mating decisions are not necessarily irrevocable. In plain seawater, around 30% of males dropped their CII partners within 2 h (figure 6). The presence of added CII females, CV females, or their conditioned water did not cause additional males to release their partners. Although the presence of vCVI females did not increase the likelihood of male release, almost 66% of males placed in vCVI·H_2O relinquished their grasp. The presence of males or male-conditioned water was just as effective in precipitating releases as vCVI·H_2O. Conversely, the presence of nvCVI females or their conditioned water significantly increased the retention of CII partners.

The transformation of adult females from attractive to undesirable after mating was followed over 16 h (table 3). At each original mating two spermatophores were transferred, and both were discharged but present prior to any subsequent mating attempts. The spermatophore must therefore discharge within 15 min. With females at 4 or

Table 3. *Effect of time since original female copulation on second copulation attempts by new males*
(Latency to copulation and time in copula are given in seconds.)

time since original copulation	number of antennular grabs	% attempted copulation	latency to copulation attempt	time in copula	% spermatophore transfer
original mating	1.9±0.9	100% (20/20)	105.7±64.3	132.9±77.0	100
15 min	2.1±0.8	87.5% (6/8)	266.4±211.9	142.9±118.0	75
2 h	2.0±0.8	85.7% (6/7)	316.8±161.4	283.0±232.1	57
4 h	5.4±6.2	40.0% (2/5)	343.8±362.7	194.0±161.2	0
8 h	3.7±3.2	20.0% (1/5)	7	187	0
16 h	5.2±2.6	40.0% (2/5)	648.0±82.0	116.5±126.6	0

more hours postcopulation, males began to increase the number of times they grabbed females, while concurrently decreasing the number of mating attempts. The latency to copulation or attempts at copulation were longer for all previously mated females. Males attempted to copulate with females at 15 min and 2 h postcopulation as often as virgin females, but successful spermatophore transfer declined for those females mated 2 h previously. The time in copula was increased only for those females at 2 h post-copulation. The original spermatophores were no longer present on almost 77% (10 out of 13) of all remated females. It is not clear if they were intentionally or coincidentally removed by the second male in his mating endeavours. All secondary spermatophores were placed correctly at the genital pore and observed to discharge. No male was successful in transferring a spermatophore to a female at four or more hours postcopulation.

4. DISCUSSION

Tigriopus japonicus males are similar to those of other copepod species which select conspecific (Lonsdale *et al*. 1988; Maier 1995; Frey 1996) and more developmentally advanced or receptive females (Watras 1983; Burton 1985; Anstensrud 1992; Frey 1996; Ritchie *et al*. 1996) as partners. Despite relatively strong biases, many of their preferences are only discernible when a choice is presented between two disparate females. Similarly, *Coullana canadensis* (Harpacticoida) males will guard CII and CV females at the same frequency if presented separately, but completely reject CII females if CV females are available (Frey 1996). These results imply that males gather information about several potential partners before investing in a female, and that a particular type of female does not carry an absolute level of attractiveness, as is sometimes assumed (Grafen & Ridley 1983). Male *Corophium volutator* amphipods handle a mean of about five females before mate guarding (Forbes *et al*. 1996). Ritchie *et al*. (1996) described mate-testing behaviour for the parasitic copepod *Lepeophtheirus salmonis* in which males initiate and then quickly terminate precopula with a female before establishing more permanent guarding. This behaviour occurred more often with younger females, and it was suggested that contact chemoreception aided the males in distinguishing female developmental stage.

Mate discrimination in *T. japonicus* also appears to rely on contact chemoreception. When choosing partners, males investigated (grabbed) their caudal rami and terminal urosome with their antennules. These structures correspond to sex–age-specific lectin-binding sites on *T. japonicus* (Kelly & Snell 1998), supporting the hypothesis of a surface glycoprotein acting as a recognition signal. In many copepod species, only caudal approaches by the male are successful (Blades & Youngbluth 1980; Watras 1983; Durbaum 1995), and lectin-binding studies consistently revealed the caudal ramus as a potential site for glycoprotein signal concentration (Snell & Carmona 1994). The discrimination signals on *T. japonicus* CIII copepodids were disrupted with proteolytic enzymes, but treated females were still desirable to males given no other choice. Female *Tigriopus* may exhibit a basal level of attractiveness conveyed by morphology or swimming wake (Strickler & Bal 1973; Yen & Strickler 1996), supplemented by species- and stage-specific contact chemical signals. The enzyme PNGase F had no effect on female attractiveness, but this enzyme is not always efficient on non-denatured proteins known to contain N-linked oligosaccharides (Ohlendieck *et al*. 1993).

Once a commitment to guarding is made, the pair is relatively stable. However, even in untreated seawater, males release CII females at a moderate rate. This rate is not influenced by the presence of additional CII or older copepodids, or their conditioned water. Thus, copepodids probably do not produce a diffusible pheromone. It seems that males guarding young juveniles will occasionally drop them and then retest the available females. Non-tenacious males were seen to re-grasp females of the same stage as those just released. Thus the males were not in a physiological state where females of a certain stage were unattractive. Possibly, in addition to producing a pair-initiation pheromone, females exude a pair-continuation pheromone that increases in strength as the female matures. One could predict that male-CII pairs would have a relatively higher, and male-CV pairs a relatively lower, incidence of spontaneous separation even in untreated seawater. Anstensrud (1992) found that the number of male *Lernaeocera branchialis* (Pennellidae) with stable pairing increased if the partner was closer to her terminal moult.

Just as the putative pair-initiation signal grants a relative rating of attractiveness, the strength of the putative pair-continuation signal depends on the local environment. Conditioned water from virgin adult females, mated adult females and adult males each altered the

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degree of copepodid retention. The presence of mated females or their conditioned water increased male tenacity, whereas the addition of males or their treated water precipitated the drop of many copepodids. This enhanced release rate suggests the influence of male–male competition in mating decisions. That male-conditioned water was as potent as male presence means direct male–male conflict is unnecessary for competitive interaction. Boxshall (1990) states that mate guarding would be a selective advantage when receptive females are rare, such as with male-biased sex ratios. For *T. japonicus* males, the costs of guarding a very young juvenile in the face of intense male competition probably outweigh the benefits (see Wen (1993) for a discussion in amphipods). The additional costs could be increased risk of injury or displacement. It is predicted that older copepodids, with their lower associated mate-guarding cost (i.e. time remaining in precopula), would be held more tenaciously in the presence of additional males.

Virgin adult *Tigriopus* females release a species-specific diffusible pheromone (Lazzaretto *et al*. 1990, 1994). Virgin female-conditioned water increased the testing activity of males, but did not induce them to attempt to copulate with each other. Thus, the soluble compound is probably a localization pheromone, with final recognition of mates occurring on contact. Virgin female-conditioned water also caused males to drop young copepodids, presumably to find the older female, but the females themselves were ineffectual. The reason for this is unclear, but the gradual increase of the released chemical must not provide the same sensory impact as that experienced by males transferred to conditioned water. The receptor(s) for these diffusible pheromones must be accessible even while the male is mate guarding, and may be located on structures outside of the antennules (Griffiths & Frost 1976).

Production of the virgin female diffusible pheromone may cease immediately upon her mating (Lonsdale *et al*. 1998). Delays in male copulation attempts are observed within 15 min postcopulation, although the frequency of copulation attempts and successful transfers remain the same. Attempts remain high for at least 2 h after mating, but the success of sperm transfer declines. By 4 h postcopulation, the few attempted copulations are all unproductive. These results suggest a physical block is forming by 2 h, with associated chemical changes by 4 h. Because there is no additional moult, the mechanism of pheromone alteration is not obvious. Mated females may secrete pheromones *de novo*, or produce a degradative enzyme. The effect of female resistance to a second mating attempt was not quantified.

All secondary spermatophores transferred were observed to discharge their contents, but their contribution to future offspring is unknown. Previous reports stating that *Tigriopus* females only mate once (Burton 1985; Lazzaretto *et al*. 1994) were based on females beyond the brief window for second spermatophore transfer. Males copulating twice with the same female were observed in the mate preference assay, but the natural prevalence of this behaviour is yet to be determined. A male could secure his paternity by postcopulatory mate guarding (Durbaum 1995), but this behaviour was not seen in our assays with one male and one female. Because males can immediately guard copepodids after mating, and can perceive the presence of other males, males may increase their reproductive fitness by limiting postcopulatory mate guarding to conditions of male–male competition.

In summary, male *Tigriopus japonicus* employs a complicated, plastic system to maximize reproductive potential. Females are deemed attractive or unattractive relative to other available females, probably through male sensing of chemicals on the body surface of females. These chemicals are species- and sex-specific, acting as contact pheromones. They are probably glycoproteins located on the caudal rami or terminal urosome of all animals whose conformation may change during the life cycle. Copepodids do not produce a soluble pheromone, but adults release a diffusible chemical indicative of their sex and mating status. This difference between copepodids and adults may reflect different selective pressures at various life stages (Havenhard 1995; Kelly & Snell 1998). Recently mated females quickly lose their attractiveness, although there is a brief period when they can be remated.

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