Copulation, mating system and sexual dimorphism in an Australian millipede, *Cladethosoma clarum*

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**Abstract.** Investigations of millipede mating dynamics have focussed primarily on Old World or North American species. In contrast, data for the diverse and abundant polydesmid millipedes of Australia are lacking. Here I examine copulation, behaviour and sexual dimorphism in an Australian millipede, *Cladethosoma clarum*. In this species, copula position is best described as 'in parallel'. Both females and males mated with two or more individuals of the opposite sex, and average copulation duration was 4.7 min. Behavioural observations revealed differences between the sexes in patterns of behaviour. Specifically, females devoted most of their time to feeding, whereas males were more mobile and were observed walking more often than females. The sexes also exhibited marked sexual dimorphism in that females exhibited greater body width and body mass compared with males, whereas males had longer legs relative to females. In millipedes, longer legs allow an individual to walk faster. Consequently, male *C. clarum* appear to be capable of relatively rapid locomotion. As Australian millipede densities are spatially unpredictable, the increased mobility of males likely reflects an active mate-location strategy. Furthermore, the occurrence of multiple mating by females suggests that male leg length may have evolved as an adaptation for rapid mate location under scramble competition polygyny.

**Introduction**

Millipedes are generalist decomposer organisms comprising a significant component of the litter and upper soil fauna in a wide range of environments. Through mechanical breakdown of decaying litter, millipedes facilitate microbial activity and enhance decomposition and nutrient turnover (Dangerfield and Telford 1989; Hopkin and Read 1992). To date, more than 12 000 species have been described within 16 distinct orders (Sierwald and Bond 2007). Recent estimates, however, suggest that a further 70 000 species may remain undiscovered, taking the total number of extant species to over 80 000 (Hoffman 1979; Blower 1985). Despite their ecological importance and the suggested speciose nature of the class, millipedes remain an understudied group. Furthermore, studies dealing with non-taxonomic aspects of millipedes are relatively uncommon, with behavioural studies being particularly scarce (Hopkin and Read 1992).

A handful of studies have examined the dynamics of mating in millipedes. These studies, however, have primarily focussed on a limited number of species within three orders: Spirostreptida, Spirobolida and Julida (e.g. Carey and Bull 1986; Mathews and Bultman 1993; Telford and Dangerfield 1993; Barnett 1997; Cooper and Telford 2000). In these species, copulation involves the transfer of sperm using secondary sexual structures – the gonopods – and copular position may be described as either 'coiled' or 'parallel' (Cooper and Telford 2000). Copulation duration is highly variable, both within and between species, lasting from several minutes to several hours (Cooper and Telford 2000). In the species for which most is known, *Alloporus uncinatus*, males prolong copulation duration in response to an increasingly male-biased operational sex ratio, a response that has been interpreted as a form of mate guarding (Telford and Dangerfield 1990). Finally, females appear to engage in multiple copulations with the same and/or different males in a few of these species (*Ommatoiulus moreletii*: Carey and Bull 1986; *Craspedosoma transsilvanicum*: Tadler 1993; *Alloporus uncinatus*: Telford and Dangerfield 1993).

Despite the fact that mating systems can be diverse (e.g. mammals: Clutton-Brock 1989; birds: Bennett and Owens 2002; insects and arachnids: Choe and Crespi 1997), even within narrow taxonomic groupings (e.g. dunnock, *Prunella modularis*: Davies and Lundberg 1984; Davies 1992; desert spider, *Agelenopsis aperta*: Singer and Riechert 1995), the mating dynamics of these few species have been used to support broad generalisations regarding the mating system of millipedes (e.g. millipedes are promiscuous). However, mating systems remain poorly described in many millipede groups, including species in the order Polydesmida (see Mukhopadhyaya and Saha 1981; Snider 1981; Bhakat et al. 1989; Tanabe and Sota 2008 for exceptions). In fact, mating behaviour has not been described for any Australian polydesmid species. Within the Polydesmida, female multiple mating has been documented in an Indian population of the species *Orthomorpha coarctata* (Mukhopadhyaya and Saha 1981). However, evidence that females receive enough sperm to fertilise all their eggs from a single mating (e.g. *Polydesmus inconstans*: Snider 1981; Hopkin and Read 1992) has led some...
to suggest that females within this order copulate only once (Ramel 2008).

Distributed worldwide, the Polydesmida is the largest order of millipedes (Hopkin and Read 1992) and polydesmids appear to comprise the greatest proportion of the known Australian millipede fauna (P. Sierwald, pers. comm.). Polydesmid millipedes are suggested to be semelparous (Blower 1985; Bhakat et al. 1989) and males in this group possess external gonopods on the seventh body ring modified from the 8th pair of walking legs (Simonsen 1990; Sierwald and Bond 2007).

In this study, I investigated the mating behaviour of an Australian polydesmid species, *Cladethosoma clarum*, by combining field observations and laboratory experiments. In laboratory experiments, I used sequential mating opportunities to determine whether female *Cladethosoma clarum* mate multiply and whether copulation duration differed between repeat copulations with the same male and additional copulations with novel males. I also examined morphological and behavioural differences between the sexes in order to identify potential mechanisms of sexual selection and describe the mating system of this species.

**Methods**

**Specimens collection and behavioural observation**

Specimens of *C. clarum* (Polydesmidia: Paradoxosomatidae) were collected from Barrenjoey Headlands in New South Wales, Australia (33°34′S, 151°19′E) between 26 April and 5 May 2000. Individuals were located on the surface, in leaf litter, and under stones and logs during random walks through the environment and collected by hand. Collection times were synchronised with periods of activity: typically between the hours of 0600 and 1200 hours, with maximum activity occurring during light rain or overcast conditions. For individuals visible from a distance, behaviour upon initial observation was assigned to one of four categories: feeding, walking, copulating, and resting. All behavioural observations were undertaken from a distance of ~1–2 m, reducing the risk of human interference while simultaneously allowing behaviour to be accurately categorised. When male–female encounters were observed, detailed descriptions of copulatory behaviour were recorded. Once the behaviour was recorded, individuals were handled to determine sex, placed in single-sex collection buckets and returned to the laboratory.

**Sexual dimorphism**

Once in the laboratory, individuals were weighed using an electronic balance (Mettler AE200, to nearest 0.001 g) and euthanised by gradual cooling. For all individuals (*n* = 72), several morphological variables were measured: body length (2nd body segment to terminal body segment) and width (at 10th segment) and femur length and width (right femur of 14th leg pair). Morphological measures were obtained using digital imaging techniques. Specifically, for each specimen, I captured digital images of each morphological feature, along with a scale, using an Olympus compound light microscope, Spot digital camera (Diagnostic Instruments, Inc.), and Auto-Montage (version 3.02.0005) image capture software (Synoptics, Ltd.). These images were then imported into Adobe Photoshop (Adobe Systems, Inc.) and measurements recorded to the nearest 0.001 mm.

**Mating experiments**

A subset of individuals (*n* = 19 females, 20 males) were held in the laboratory for approximately seven days before being used in mating experiments. Animals were housed in individual plastic pots with tight-fitting lids (9 cm diam × 10.5 cm high). Pots contained a substrate of Plaster of Paris and moist vermiculite, and above it a mixture of field-collected materials consisting of soil, leaf litter, and rotting wood. In order to document mating behaviour and investigate the mating system of *C. clarum*, two mating experiments were conducted. Females were randomly allocated to one of these two groups (*n* = 9 and 10, Experiments 1 and 2 respectively). For Experiment 1, females were provided three mating opportunities, each separated by a 24-h period, with the same male. For Experiment 2, females were also provided three mating opportunities, each separated by a 24-h period, but in this instance a novel male was used for each successive mating opportunity.

In order to generate copula pairs, females were placed in a small mating arena (aquaria, ~30 × 20 cm) containing a layer of vermiculite and moist soil and left for 15 min to acclimate. Once females appeared acclimated, the male was introduced to the arena and animal activity stimulated with a fine spray of water (if required). Copulation was assumed upon copula pair formation, specifically upon insertion of the gonopods, and considered complete when the pair physically separated. Males were removed from the mating arena immediately after copulation was complete. Copulation duration, measured from initial pair formation to separation, was recorded for each mating (to the second). In addition, a detailed description of mating behaviour was recorded.

**Statistical analysis**

To investigate the possibility of behavioural differences between the sexes, I compared the number of males and females observed in each behaviour category using Chi-square tests. For morphological variables, Shapiro–Wilk tests were used to test for normality throughout. Some morphological variables (body width, femur length and femur width) were not normally distributed and transformations failed to normalise distributions. I tested whether males and females differed in morphology using a two-sample *t*-test or, for non-normally distributed data, a Wilcoxon rank sum test. Mating behaviour was analysed in two ways. First, I compared copula duration across successive mating opportunities in the two mating experiments separately with a non-parametric Friedman repeated-measures ANOVA and Dunn’s multiple comparison test for *post hoc* pairwise comparisons. Next, using data from second and third matings only, I then tested whether copulation duration differed between females re-mating with the same male (*n* = 8) and those re-mating with novel males (*n* = 7) using a Wilcoxon rank sum test. Finally, data from all copulations in Experiments 1 and 2 were combined to determine the average duration of copulation in *C. clarum*. All statistical analyses were performed using the R (2.7.0) software package (R Development Core Team 2006).
Results

Behaviour

A total of 149 behavioural observations (52 male, 97 female) were recorded in the field. Feeding was the most frequently observed behaviour for both sexes (Table 1) and in these instances individuals appeared to be grazing on lichen. The sexes differed significantly with respect to frequency of observed behaviours. Specifically, females were more often observed feeding than were males, whereas males were more frequently observed walking than females (Table 1). Sexes did not differ significantly in frequency of copulation or resting (Table 1). Six observations of male–female copula pairs were recorded, with an additional copulatory attempt observed between two males. In this instance, one male appeared to initiate copulation by approaching the second male from behind and moving towards the head along the dorsal surface. However, upon reaching the head region, the first male disengaged and moved rapidly away from the second male. Resting individuals were rarely observed (Table 1).

Sexual dimorphism

Males and females differed in morphology. Specifically, body mass was greater in females than in males ($t = 2.47, P = 0.016$). Males and females also differed in body length and width, with males being significantly longer than females ($t = -10.64, P < 0.0001$) and females significantly wider than males ($W = 1051, P < 0.0001$). Similarly, males and females differed in femur dimensions, with males having significantly longer and wider femurs than females (length: $W = 0, P < 0.0001$; width: $W = 13, P < 0.0001$). Summary statistics of morphological variables are presented in Table 2.

<table>
<thead>
<tr>
<th>Behaviour</th>
<th>Males (52)</th>
<th>Females (97)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feeding</td>
<td>0.90 (87)</td>
<td>0.04 (4)</td>
<td>0.06 (6)</td>
</tr>
<tr>
<td>Walking</td>
<td>0.48 (25)</td>
<td>0.35 (18)</td>
<td>0.15 (8)</td>
</tr>
<tr>
<td>Copulating</td>
<td>34.3**</td>
<td>8.9*</td>
<td>0.29 NS</td>
</tr>
<tr>
<td>Resting</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 1. Observations of behaviour for male and female Cladethosoma clarum

Behaviours are expressed as the proportion of the total number of observations for each sex. No. of observations in each category is shown in parentheses. *, $P<0.05$; **, $P<0.001$; NS indicates non-significant result

<table>
<thead>
<tr>
<th>Behaviour</th>
<th>Males (31)</th>
<th>Females (41)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mass (g)</td>
<td>0.252 ± 0.007 (30)</td>
<td>0.276 ± 0.007 (40)</td>
<td></td>
</tr>
<tr>
<td>Body length (mm)</td>
<td>28.5 ± 0.3 (31)</td>
<td>24.04 ± 0.3 (41)</td>
<td></td>
</tr>
<tr>
<td>Body width (mm)</td>
<td>2.59 ± 0.05 (31)</td>
<td>2.83 ± 0.05 (41)</td>
<td></td>
</tr>
<tr>
<td>Femur length (mm)</td>
<td>0.822 ± 0.007 (31)</td>
<td>0.636 ± 0.008 (41)</td>
<td></td>
</tr>
<tr>
<td>Femur width (mm)</td>
<td>0.227 ± 0.003 (31)</td>
<td>0.182 ± 0.002 (41)</td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Body mass, length and width, and femur length and width of male and female Cladethosoma clarum

Means ± standard errors are shown, with sample sizes in parentheses

Copulatory behaviour and mating system

When placed in the same mating arena, neither males nor females appeared to be aware of the other sex until physical contact was made. In general, a male initiated mating activity immediately upon physical contact with the female by moving rapidly along her dorsal surface, from posterior to anterior, while tapping his antennae on her body. Upon reaching the anterior region of the female, the male then moved the anterior portion of his body into alignment with the female so that their ventral surfaces were contiguous (i.e. parallel copula position). Specifically, the male positioned the female’s head backward so that his seventh body segment lay opposite the third body segment of the female, allowing the gonopods and vulva to align. While grasping the female’s head with his anterior legs, the male arched his seventh body segment away from the female (presumably to allow ejaculation of sperm from genital openings on the second leg pair onto the gonopods: see Tanabe and Sota 2008). The male then repositioned his body so that the ventral surfaces were again contiguous, inserting his gonopods into the female’s vulva. The male then partially withdrew and reinserted his gonopod into the vulva approximately three times before maintaining continuous ventro–ventro contact for the duration of copulation. No obvious pre- or syn-copulatory courtship behaviour was observed. In contrast to the male, the female did not grasp or hold the male and appeared passive during copulation. Field observations of copulatory behaviour match the description provided here for laboratory experiments.

Overall (Experiments 1 and 2 combined), 19 females were given 56 mating opportunities, from which 30 successful copulations were observed, along with a single attempted copulation in which female avoidance behaviour was observed. In this instance, the male exhibited initial copulatory behaviour by moving along the dorsal surface of the female. In response, the female coiled and resisted any uncoiling attempts by the male, thus preventing copulation. Of the 19 females, 11 re-mated: eight females copulated twice and three females copulated three times. In these cases there was no observable difference in mating behaviour between the first and successive matings. Females re-mated whether they were paired with the same male or a series of different males. In Experiment 1, 55.6% of females mated two or more times with the same male. In Experiment 2, 60% of females re-mated with a second male. Similarly, both males paired with the same female and males paired with a novel female at successive mating opportunities re-mated: 55.6% of males mated with the same female two or more times, whereas 50% of males re-mated with a novel female two or more times. There was a significant difference in copula duration across successive matings when females were paired with the same male at each mating attempt ($\chi^2 = 7.75, P = 0.019$) (Fig. 1a). Specifically, copula duration was significantly longer at Mating Opportunity 1 compared with Mating Opportunity 3 ($P < 0.05$), but did not differ significantly between Opportunity 1 and 2 ($P > 0.05$), or Opportunity 2 and 3 ($P > 0.05$). Similarly, there was a significant difference in copula duration across successive matings when females were paired with a novel male at each mating attempt ($\chi^2 = 12.8, P = 0.0005$) (Fig. 1b). Again, copula duration was significantly longer at Mating Opportunity 1 compared with Mating Opportunity 3 ($P < 0.01$), but did not differ significantly between Opportunity 1 and 2 ($P > 0.05$), or Opportunity 2 and 3.
Since there was no difference in copula duration between Opportunity 2 and 3 within mating experiments (i.e. same versus novel male), I grouped these data and compared the duration of copulation between females copulating repeatedly with the same male and those copulating with a novel male and found no significant difference (mean copulation duration 3.83 min and 3.98 min, same and novel males respectively; W = 21.5, P = 0.80). Finally, the average duration of copulation was 4.7±2.7 min (mean ± s.d.) (n = 30, range = 2.6–17.2 min).

**Discussion**

This study provides the first report of behaviour and copulation in an Australian millipede – *Cladethosoma clarum*. In this species, the sexes exhibited differences in behaviour and morphology. Specifically, males were observed walking more frequently than females, whereas females were most often observed feeding and feed more than males. Additionally, males were significantly smaller in both body width and mass, but possessed longer legs relative to females. Finally, laboratory experiments demonstrated that female *C. clarum* both copulate repeatedly with the same male and mate multiply with novel males.

The distinct behavioural and morphological characteristics of female *C. clarum* observed in this study are presumably the result of selection for maximal fecundity. First, foraging determines an individual’s intake of resources, and variation in food acquisition is likely to affect resource allocation to reproductive investment. Indeed, fecundity has been shown to decrease with decreasing food or energy availability in several species (Wise 1975; Ballinger 1977; Boggs and Ross 1993). Thus female *C. clarum* are likely devoting most of their time budget to feeding in order to maximise gamete production. In addition, females were both heavier and wider than males. In the polydesmid millipede *Streptogonopus philsoni*, body width is positively correlated with the number of eggs present in the body cavity (Bhakat et al. 1989). A similar relationship between body size and egg production has been observed in a range of vertebrate and invertebrate taxa (e.g. Sand 1996; Berger et al. 2008) and is the basis of the fecundity advantage model (Darwin 1874). While this model has difficulty explaining the evolution of female-biased sexual size dimorphism in iteroparous species (Shine 1988), polydesmid millipedes are semelparous (Blower 1985; Bhakat et al. 1989). Consequently, an increase in body size would likely maximise lifetime reproductive success for female polydesmid species. Therefore, in *C. clarum*, the greater body mass and width of females likely reflects selection for increased body size via an increase in fecundity.

In contrast to females, male behaviour and morphology may be shaped by sexual selection. In *C. clarum*, males were highly mobile, which is consistent with observations of male behaviour in other millipede species (Blower 1985; Dangerfield et al. 1992) and likely represents a strategy of active mate location and acquisition (Telford and Dangerfield 1993). Additionally, males had significantly longer legs relative to females. In the Diplopoda, there is a prerequisite for slow strong gaits suitable for burrowing and pushing through leaf litter, and such strong movements and pushing power require a short leg to achieve maximum strength (Manton 1973). Consequently, long legs are likely to reduce the propulsive thrust used for burrowing. However, an increase in leg length results in an increase in speed (Manton 1973; Hopkin and Read 1992). More specifically, Manton (1973) showed that an increase in leg length results in a faster motion in Diplopoda because a longer leg can take a longer stride. Thus, male *C. clarum* appear to have a greater locomotory rate capacity compared with females. In Australia, millipede densities appear to be highly variable in space and time (Ord and Dangerfield 2001). If female *C. clarum* are widely scattered through the environment, males able to travel faster and

\[ P > 0.05 \]
quickly locate a series of females would be able to mate with relatively more females than their rivals. Furthermore, the occurrence of multiple mating by females suggests that male leg length in *C. clarum* may have evolved as an adaptation for rapid mate location under a mating system of scramble competition polygyny (Wells 1977; Alcock 1980; Thornhill and Alcock 1983).

Alternatively, the long legs of male *C. clarum* may function to control or stimulate the female during copulation. For example, in the parajulid millipede *Aniulus bolmani* males grasp and immobilise the female during copulation using their highly enlarged first leg pair (Mathews and Bultman 1993). However, given that female control can be achieved through the modification of just the first leg pair in that species, the increased length of all leg pairs observed in *C. clarum* may be unnecessary for female control. Furthermore, given the loss of burrowing power that accompanies longer legs, it seems most likely that leg length in male *C. clarum* reflects selection for increased speed to allow rapid location of females. Nonetheless, a female control or stimulation function cannot be ruled out at this stage.

Both female and male *C. clarum* mated multiply in the laboratory, establishing the potential for post-copulatory mechanisms of sexual selection, including cryptic female choice and sperm competition. In the spirostreptid millipedes, suggested mechanisms of sperm competition include sperm displacement, sperm mixing, and redistribution of ejaculates within the female spermathecae (Barnett *et al.* 1993, 1995; Barnett and Telford 1994). In this study, behavioural observations suggest that similar mechanisms of sperm competition may occur in *C. clarum*. Specifically, I observed males partially withdrawing and reinserting their gonopods into the female vulva multiple times at the beginning of copulation. If females had already copulated, this action may remove and/or reposition sperm from previous males. Alternatively, this behaviour may represent a form of copulatory courtship, and thus an attempt by males to influence cryptic female choice (Eberhard 1991), though additional studies are necessary to confirm these possibilities.

In *C. clarum*, copula duration may reflect a male tactic that emphasises mate-searching behaviour. In many millipede species, copulation is prolonged (e.g. >60 min: Telford and Dangerfield 1993; Cooper and Telford 2000), and this behaviour has been interpreted as a form of mate guarding whereby males attempt to assure paternity success by remaining physically connected to the female (Telford and Dangerfield 1990). In contrast, copula duration is relatively short in *C. clarum*. In addition, female copulation duration decreased with additional copulations in *C. clarum*: duration of Mating 3 was shorter than that of Matings 1 and 2. These results also contrast with findings in *A. uncinatus*, in which the second mating was shorter than the first (Telford and Dangerfield 1993). Though the exact reasons for these differences are unclear, it is possible that males of *C. clarum* experience selection pressures that oppose increased copula duration, such as a reduction in fitness via a reduction in the number of females inseminated, and thus a strategy of mate acquisition is favoured over mate guarding. Though this idea also remains to be tested, such a mate acquisition tactic aligns with the morphological and behavioural characteristics of males observed in this study: males are the mobile sex and have significantly longer legs that may function to increase female encounter rate as a result of an increase in speed.

In conclusion, the results of this study suggest that the mating system of *C. clarum* is scramble competition polygyny and that males and females of this species show several morphological and behavioural adaptations that may be associated with competition for mates in males and increased fecundity in females. Finally, these results highlight the need for further studies of behaviour and morphology in this diverse, but relatively understudied group. Such studies will undoubtedly uncover a wealth of new knowledge concerning millipede biology and, more generally, will help broaden our understanding of animal mating tactics and sexual strategies.

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